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(54) **Title:** BCL-XL INHIBITOR ANTIBODY-DRUG CONJUGATES AND METHODS OF USE THEREOF

(57) **Abstract:** Antibody-drug conjugates that bind to human oncology targets are disclosed. The antibody- drug conjugates comprise a Bcl-xL inhibitor drug moiety. The disclosure further relates to methods and compositions for use in the treatment of cancers by administering the antibody- drug conjugates provided herein. Linker-drug conjugates comprising Bcl-xL inhibitor drug moiety and methods of making same are also disclosed.

BCL-XL INHIBITOR ANTIBODY-DRUG CONJUGATES AND METHODS OF USE THEREOF

RELATED APPLICATON

[01] This application claims the benefit of and priority to the filing date under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/117,763, filed on November 24, 2020, the entire content of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[02] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 19, 2021, is named 132043-00420_SL.txt and is 550,925 bytes in size.

FIELD OF THE INVENTION

[03] The present disclosure relates to antibody-drug conjugates (ADCs) comprising a Bcl-xL inhibitor and an antibody or antigen-binding fragment thereof that binds an antigen target, e.g., an antigen expressed on a tumor or other cancer cell. The disclosure further relates to methods and compositions useful in the treatment and/or diagnosis of cancers that express a target antigen and/or are amenable to treatment by modulating Bcl-xL expression and/or activity, as well as methods of making those compositions. Linker-drug conjugates comprising an Bcl-xL inhibitor drug moiety and methods of making same are also disclosed.

BACKGROUND OF THE INVENTION

[04] Apoptosis (programmed cell death) is an evolutionarily conserved pathway essential for tissue homeostasis, development and removal of damaged cells. Deregulation of apoptosis contributes to human diseases, including malignancies, neurodegenerative disorders, diseases of the immune system and autoimmune diseases (Hanahan and Weinberg, *Cell.* 2011 Mar 4;144(5):646-74; Marsden and Strasser, *Annu Rev Immunol.* 2003;21:71-105; Vaux and Flavell, *Curr Opin Immunol.* 2000 Dec;12(6):719-24). Evasion of apoptosis is recognized as a hallmark of cancer, participating in the development as well as the sustained expansion of tumors and the resistance to anti-cancer treatments (Hanahan and Weinberg, *Cell.* 2000 Jan 7;100(1):57-70).

[05] The Bcl-2 protein family comprises key regulators of cell survival which can suppress

(*e.g.*, Bcl-2, Bcl-xL, Mcl-1) or promote (*e.g.*, Bad, Bax) apoptosis (Gross *et al.*, *Genes Dev.* 1999 Aug 1;13(15):1899-911, Youle and Strasser, *Nat. Rev. Mol. Cell Biol.* 2008 Jan;9(1):47-59).

[06] In the face of stress stimuli, whether a cell survives or undergoes apoptosis is dependent on the extent of pairing between the Bcl-2 family members that promote cell death with family members that promote cell survival. For the most part, these interactions involve the docking of the Bcl-2 homology 3 (BH3) domain of proapoptotic family members into a groove on the surface of pro-survival members. The presence of Bcl-2 homology (BH) domain defines the membership of the Bcl-2 family, which is divided into three main groups depending upon the particular BH domains present within the protein. The prosurvival members such as Bcl-2, Bcl-xL, and Mcl-1 contain BH domains 1–4, whereas Bax and Bak, the proapoptotic effectors of mitochondrial outer membrane permeabilization during apoptosis, contain BH domains 1–3 (Youle and Strasser, *Nat. Rev. Mol. Cell Biol.* 2008 Jan;9(1):47-59).

[07] Overexpression of the prosurvival members of the Bcl-2 family is a hallmark of cancer and it has been shown that these proteins play an important role in tumor development, maintenance and resistance to anticancer therapy (Czabotar *et al., Nat. Rev. Mol. Cell Biol.* 2014 Jan;15(1):49-63). Bcl-xL (also named BCL2L1, from BCL2-like 1) is frequently amplified in cancer (Beroukhim *et al.*, *Nature* 2010 Feb 18;463(7283):899-905) and it has been shown that its expression inversely correlates with sensitivity to more than 120 anti-cancer therapeutic molecules in a representative panel of cancer cell lines (NCI-60) (Amundson *et al.*, *Cancer Res.* 2000 Nov 1;60(21):6101-10).

[08] In addition, several studies using transgenic knockout mouse models and transgenic overexpression of Bcl-2 family members highlighted the importance of these proteins in the diseases of the immune system and autoimmune diseases (for a review, see Merino *et al.*, *Apoptosis* 2009 Apr;14(4):570-83. doi: 10.1007/s10495-008-0308-4.PMID: 19172396). Transgenic overexpression of Bcl-xL within the T-cell compartment resulted in resistance to apoptosis induced by glucocorticoid, g-radiation and CD3 crosslinking, suggesting that transgenic Bcl-xL overexpression can reduce apoptosis in resting and activated T-cells (Droin *et al.*, *Biochim Biophys Acta* 2004 Mar 1;1644(2-3):179-88. doi: 10.1016/j.bbamcr.2003.10.011.PMID: 14996502). In patient samples, persistent or high expression of antiapoptotic Bcl-2 family proteins has been observed (Pope *et al.*, *Nat Rev Immunol.* 2002 Jul;2(7):527-35. doi: 10.1038/nri846.PMID: 12094227). In particular, T-cells isolated from the joints of rheumatoid arthritis patients exhibited increased Bcl-xL expression and were resistant to spontaneous apoptosis (Salmon *et al.*, *J Clin Invest.* 1997 Feb 1;99(3):439-46. doi: 10.1172/JCI119178.PMID: 9022077).

[09] The findings indicated above motivated the discovery and development of a new class of drugs named BH3 mimetics. These molecules are able to disrupt the interaction between the proapoptotic and antiapoptotic members of the Bcl-2 family and are potent inducers of apoptosis. This new class of drugs includes inhibitors of Bcl-2, Bcl-xL, Bcl-w and Mcl-1. The first BH3 mimetics described were ABT-737 and ABT-263, targeting Bcl-2, Bcl-xL and Bcl-w (Park *et al.*, *J. Med. Chem.* 2008 Nov 13;51(21):6902-15; Roberts *et al.*, *J. Clin. Oncol.* 2012 Feb 10;30(5):488-96). After that, selective inhibitors of Bcl-2 (ABT-199 and S55746 – Souers *et al.*, *Nat Med.* 2013 Feb;19(2):202-8; Casara *et al.*, *Oncotarget* 2018 Apr 13;9(28):20075-20088), Bcl-xL (A-1155463 and A-1331852 - Tao *et al.*, *ACS Med Chem Lett.* 2014 Aug 26;5(10):1088-93; Leverson *et al.*, *Sci Transl Med.* 2015 Mar 18;7(279):279ra40) and Mcl-1 (A-1210477, S63845, S64315, AMG-176 and AZD-5991 - Leverson *et al.*, *Cell Death Dis.* 2015 Jan 15;6:e1590.; Kotschy *et al.*, *Nature* 2016, 538, 477-482; Maragno *et al.*, *AACR* 2019, Poster #4482; Kotschy *et al.*, WO 2015/097123; Caenepeel *et al.*, *Cancer Discov.* 2018 Dec;8(12):1582-1597; Tron *et al.*, *Nat. Commun.* 2018 Dec 17;9(1):5341) were also discovered. The selective Bcl-2 inhibitor ABT-199 is now approved for the treatment of patients with CLL and AML in combination therapy, while the other inhibitors are still under pre-clinical or clinical development. In pre-clinical models, ABT-263 has shown activity in several hematological malignancies and solid tumors (Shoemaker *et al.*, *Clin. Cancer Res.* 2008 Jun 1;14(11):3268-77; Ackler *et al.*, *Cancer Chemother. Pharmacol.* 2010 Oct;66(5):869-80; Chen *et al.*, *Mol. Cancer Ther.* 2011 Dec;10(12):2340-9). In clinical studies, ABT-263 exhibited objective antitumor activity in lymphoid malignancies (Wilson *et al.*, *Lancet Oncol.* 2010 Dec;11(12):1149-59; Roberts *et al.*, *J. Clin. Oncol.* 2012 Feb 10;30(5):488-96) and its activity is being investigated in combination with several therapies in solid tumors. The selective Bcl-xL inhibitors, A-1155463 or A-1331852, exhibited *in vivo* activity in pre-clinical models of T-ALL (T-cell Acute Lymphoblastic Leukemia) and different types of solid tumors (Tao *et al.*, *ACS Med. Chem. Lett.* 2014 Aug 26;5(10):1088-93; Leverson *et al.*, *Sci. Transl. Med.* 2015 Mar 18;7(279):279ra40). The use of BH3 mimetics has also shown benefit in pre-clinical models of diseases of the immune system and autoimmune diseases. Treatment with ABT-737 (Bcl-2, Bcl-xL, and Bcl-w inhibitor) resulted in potent inhibition of lymphocyte proliferation *in vitro*. Importantly, mice treated with ABT- 737 in animal models of arthritis and lupus showed a significant decrease in disease severity (Bardwell *et al.*, *J Clin Invest.* 1997 Feb 1;99(3):439-46. doi: 10.1172/JCI119178.PMID: 9022077). In addition, it has been shown that ABT-737 prevented allogeneic T-cell activation, proliferation, and cytotoxicity *in vitro* and inhibited allogeneic T- and B-cell responses after skin transplantation with high selectivity for lymphoid cells (Cippa *et al.*, .Transpl Int. 2011 Jul;24(7):722-32. doi: 10.1111/j.1432-2277.2011.01272.x. Epub 2011 May 25.PMID: 21615547). Therefore, therapeutically

targeting Bcl-xL or proteins upstream and/or downstream of it in an apoptotic signaling pathway represent a highly attractive approach for the development of novel therapies in oncology and in the field of immune and autoimmune diseases.

SUMMARY OF THE INVENTION

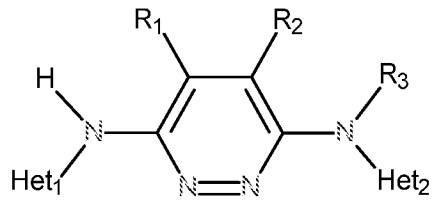
[10] In some embodiments, the present disclosure provides, in part, novel antibody-drug conjugate (ADC) compounds with biological activity against cancer cells. The compounds may slow, inhibit, and/or reverse tumor growth in mammals, and/or may be useful for treating human cancer patients. The present disclosure more specifically relates, in some embodiments, to ADC compounds that are capable of binding and killing cancer cells. In some embodiments, the ADC compounds disclosed herein comprise a linker that attaches a Bcl-xL inhibitor to a full-length antibody or an antigen-binding fragment. In some embodiments, the ADC compounds are also capable of internalizing into a target cell after binding.

[11] In some embodiments, ADC compounds may be represented by Formula (1):

$$Ab\text{-}(L\text{-}D)_p \qquad (1)$$

wherein Ab is an antibody or an antigen-binding fragment thereof;
D is a Bcl-xL inhibitor;
L is a linker that covalently attaches Ab to D; and
p is an integer from 1 to 16. In some embodiments, Ab is an antibody or an antigen-binding fragment thereof that targets a cancer cell.

[12] In some embodiments, for ADC compounds of Formula (I), D comprises a Bcl-xL inhibitor compound of Formula (I) or Formula (II) covalently attached to the linker L:



(I)

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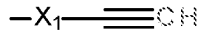
or an enantiomer, a diastereoisomer, and/or an addition salt thereof with a pharmaceutically acceptable acid or base (*i.e.*, a pharmaceutically acceptable salt) of any one of the foregoing, wherein:

- R_1 and R_2 independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally

substituted by a a linear or branched C_1-C_6alkyl group;
or R_1 and R_2 form with the carbon atoms carrying them a C_3-C_6cycloalkylene group,

- R_3 represents a group selected from: hydrogen; C_3-C_6cycloalkyl; linear or branched C_1-C_6alkyl; $-X_1-NR_aR_b$; $-X_1-N^+R_aR_bR_c$; $-X_1-O-R_c$; $-X_1-COOR_c$; $-X_1-PO(OH)_2$; $-X_1-SO_2(OH)$; $-X_1-N_3$ and :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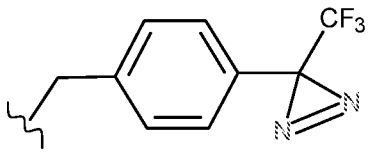
- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; $-SO_2$-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

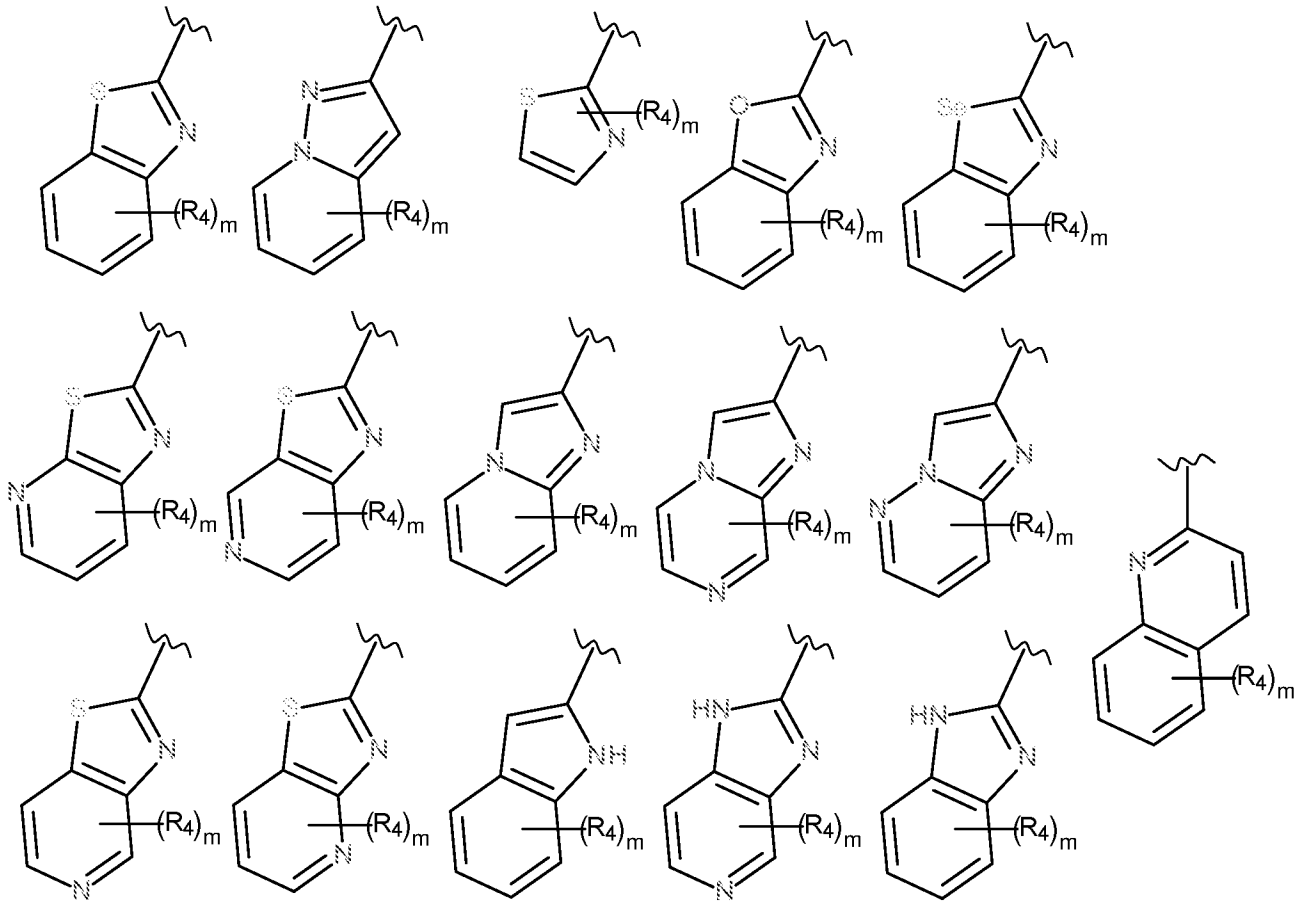
  the group:



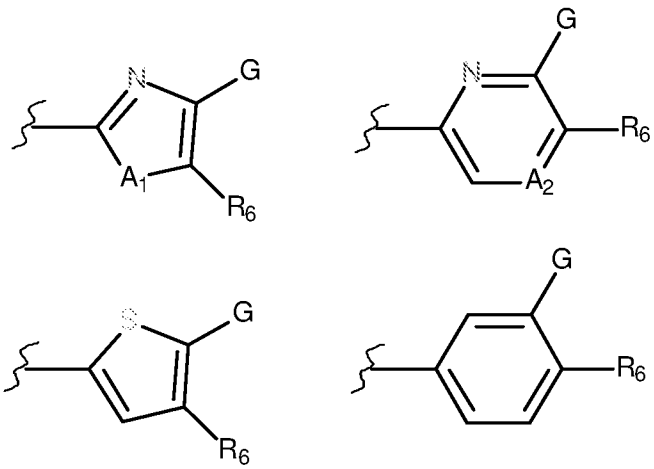
  or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;
  or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,
  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

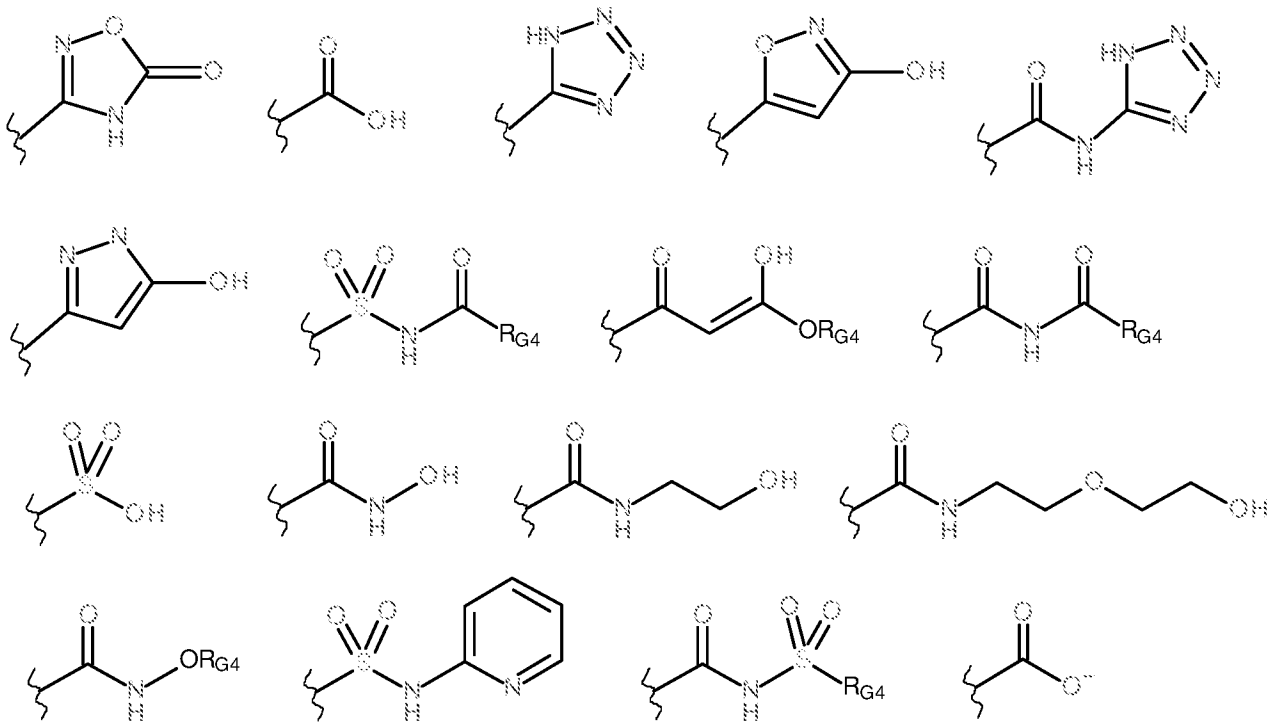
-C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$,

$-NR_{G1}C(=NCN)NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2NR_{G1}R_{G2}$, $-S(O)_2R_{G3}$, $-S(O)_2NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2R_{G2}$, $-NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, $-C(=S)NR_{G1}R_{G2}$, $-C(=NR_{G1})NR_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, $-NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



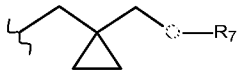
wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,
- R_6 represents a group selected from:

  hydrogen;

-C_2-C_6alkenyl;

-X_2-O-R_7;



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-X_2-NSO_2-R_7;

-C=C(R_9)-Y_1-O-R_7;

C_3-C_6cycloalkyl;

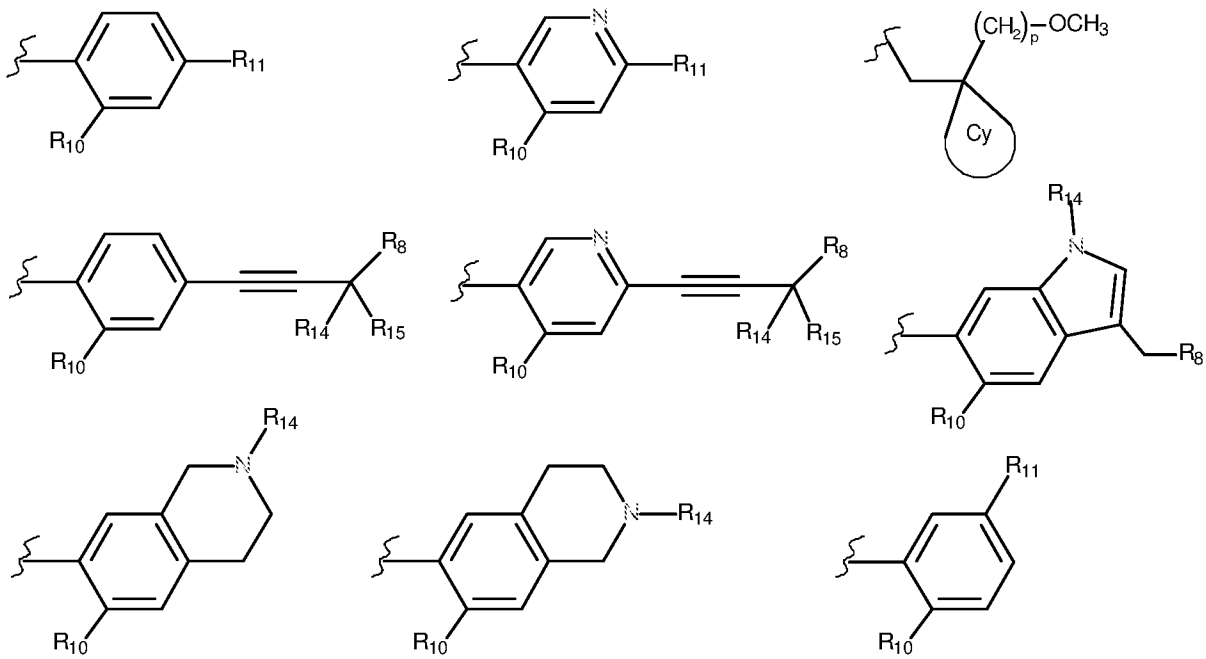
C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

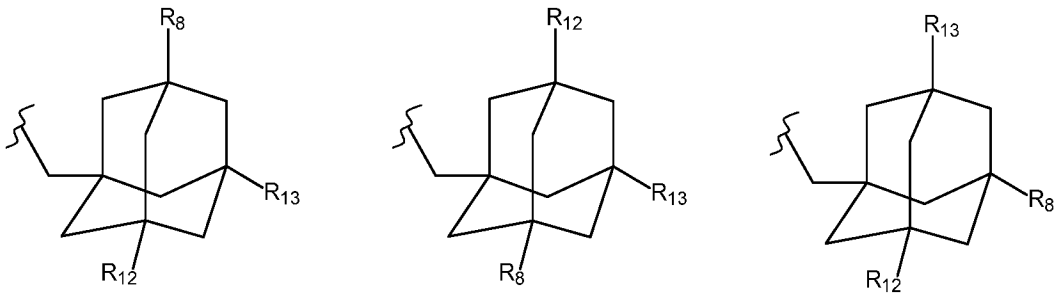
C_3-C_6cycloalkylene-Y_2-R_7 ;

C_3-C_6heterocycloalkylene-Y_2-R_7 group,

an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

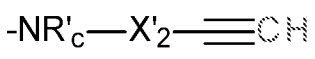
- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:





wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -$NR'_aR'_b$;
  -NR'_a-CO-OR'_c; -NR'_a-CO-R'_c; -$N^+R'_aR'_bR'_c$; -O-R'_c; -NH-X'_2-$N^+R'_aR'_bR'_c$; -O-X'_2-$NR'_aR'_b$, -X'_2-$NR'_aR'_b$, -NR'_c-X'_2-N_3 and :



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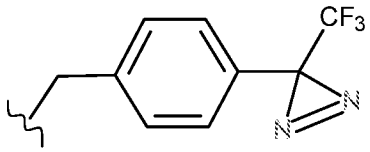
- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen;

heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH;

C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

the group:



or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,

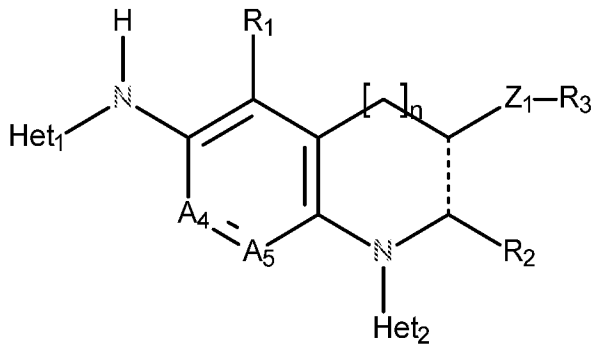
- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,

- m=0, 1 or 2,

- p=1, 2, 3 or 4,

- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine,

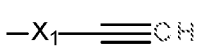
chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl, wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceed by virtue of one or more substituents bonded thereto; or



(II),

or an enantiomer, a diastereoisomer, and/or an addition salt thereof with a pharmaceutically acceptable acid or base (*i.e.*, a pharmaceutically acceptable salt) of the foregoing, wherein:

- n=0, 1 or 2,
- ------ represents a single or a double bond.
- A_4 and A_5 independently of one another represent a carbon or a nitrogen atom,
- Z_1 represents a bond, -N(R)-, or –O-, wherein R represents a hydrogen or a linear or branched C_1-C_6alkyl,
- R_1 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally substituted by a a linear or branched C_1-C_6alkyl group;
- R_2 represents a hydrogen or a methyl;
- R_3 represents a group selected from: hydrogen; linear or branched C_1-C_4alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; -X_1-O-R_c; -X_1-$COOR_c$; -X_1-$PO(OH)_2$; -X_1-$SO_2(OH)$; -X_1-N_3 and :

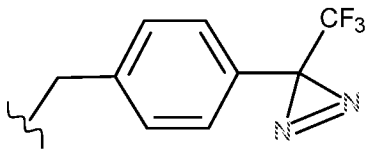


,

- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two

hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

the group:



or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;

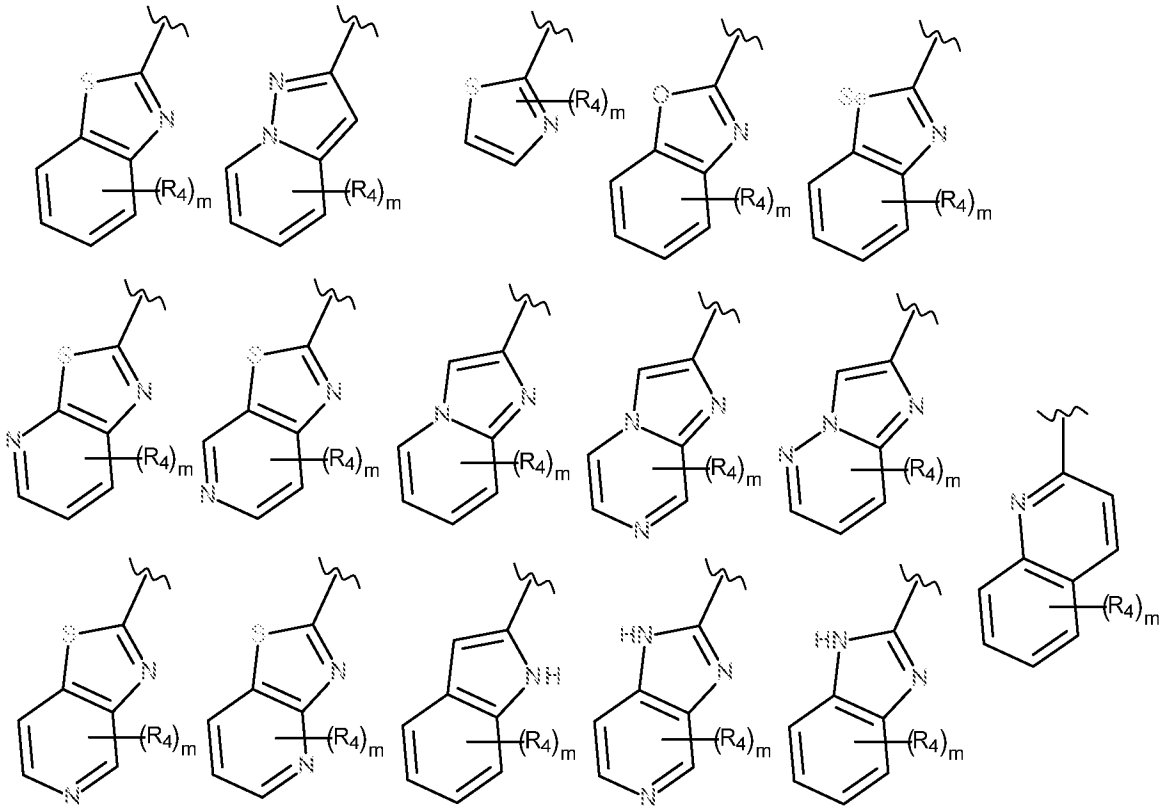
or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,

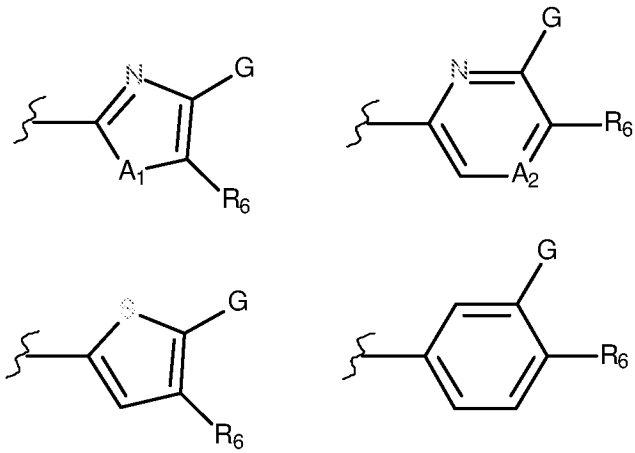
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,

  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

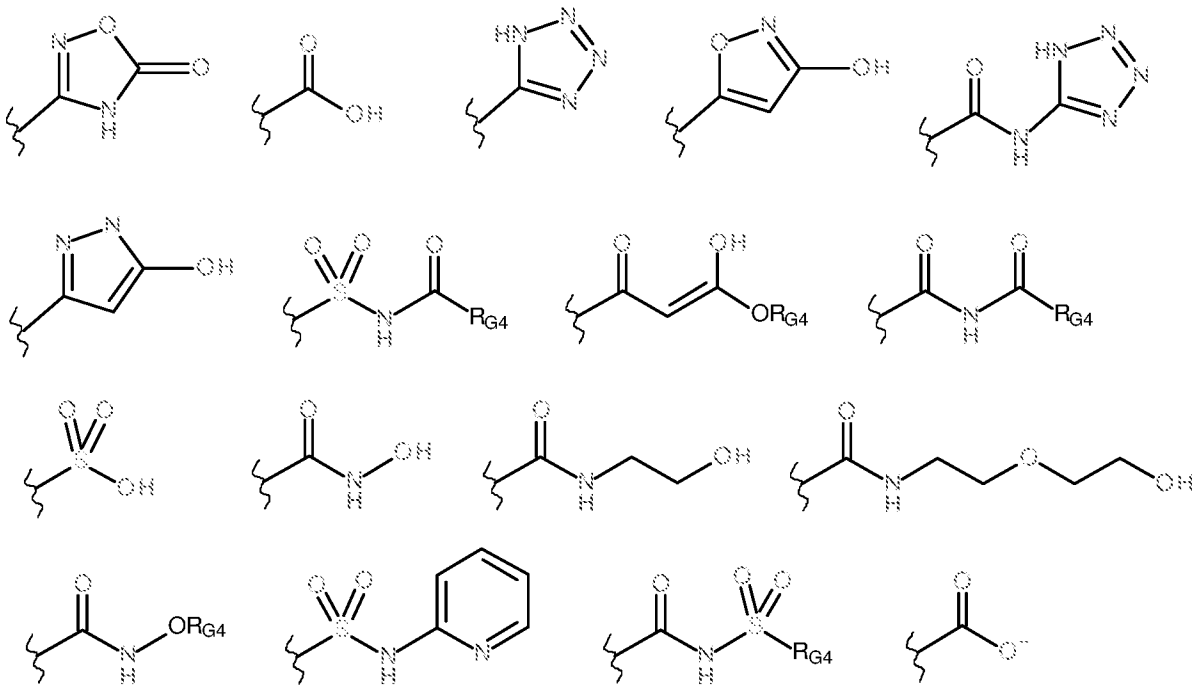
  -C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$, -NR_{G1}C(=NCN)$NR_{G1}R_{G2}$, -NR_{G1}S(O)$_2NR_{G1}R_{G2}$, -S(O)$_2R_{G3}$, -S(O)$_2NR_{G1}R_{G2}$, -NR_{G1}S(O)$_2R_{G2}$, -NR_{G1}C(=NR_{G2})$NR_{G1}R_{G2}$, -C(=S)$NR_{G1}R_{G2}$, -C(=NR_{G1})$NR_{G1}R_{G2}$, C_1-

C_6alkyl optionally substituted by a hydroxyl group, halogen, -NO_2, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



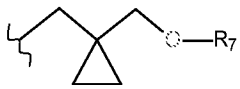
wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,
- R_6 represents a group selected from:

hydrogen;

-C_2-C_6alkenyl;

$-X_2-O-R_7$;



;

$-X_2-NSO_2-R_7$;

$-C=C(R_9)-Y_1-O-R_7$;

C_3-C_6cycloalkyl;

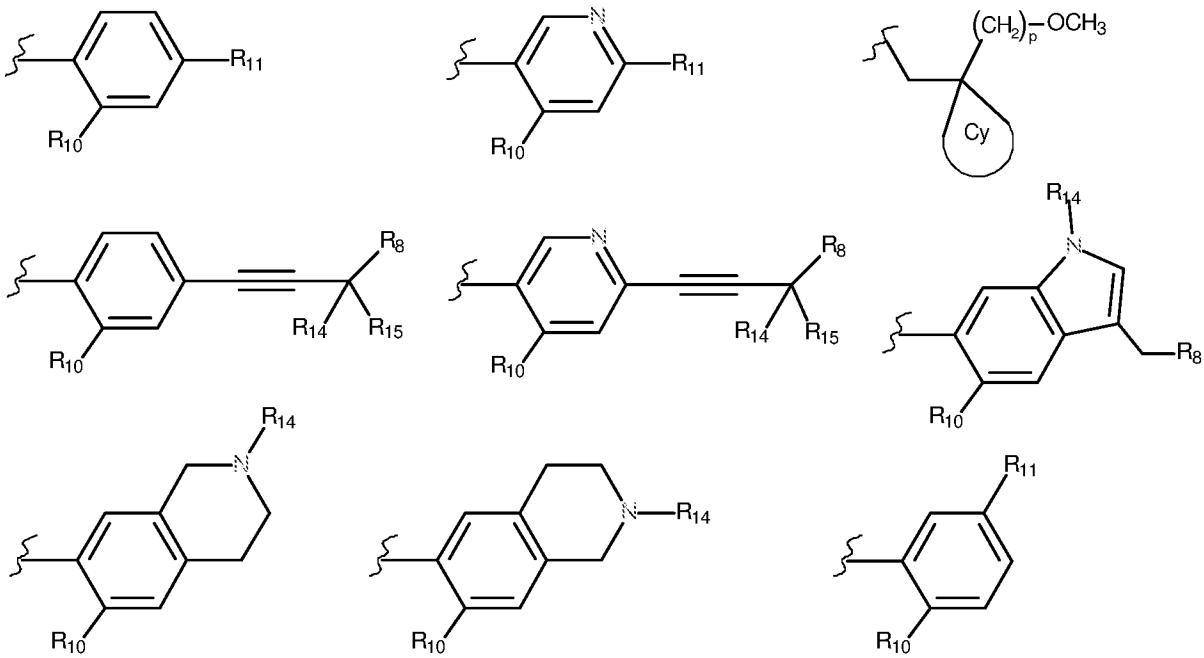
C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

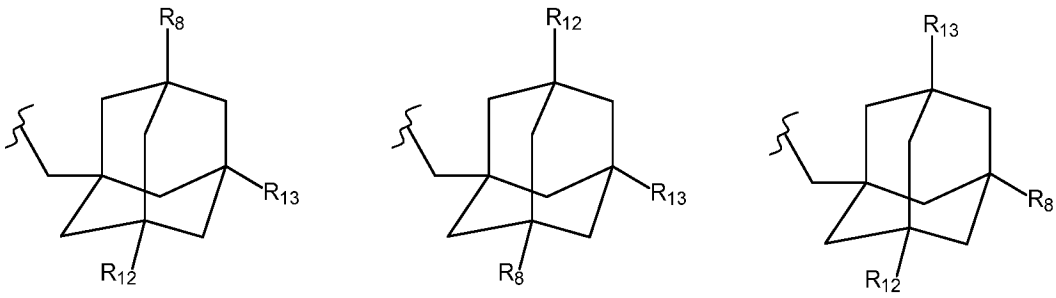
C_3-C_6cycloalkylene-Y_2-R_7 ;

C_3-C_6heterocycloalkylene-Y_2-R_7 group,

an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

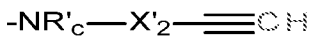
- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:





wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -$NR'_aR'_b$; -NR'_a-CO-OR'_c; -NR'_a-CO-R'_c; -$N^+R'_aR'_bR'_c$; -O-R'c; -NH-X'_2-$N^+R'_aR'_bR'_c$; -O-X'_2-$NR'_aR'_b$, -X'_2-$NR'_aR'_b$, -NR'_c-X'_2-N_3 and :

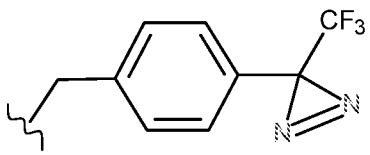


,

- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, halogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 represents a linear or branched C_1-C_4alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X_2 represents a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,

- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,
- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,
- m=0, 1 or 2,
- p=1, 2, 3 or 4,
- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen

atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl,

- wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceed by virtue of one or more substituents bonded thereto.

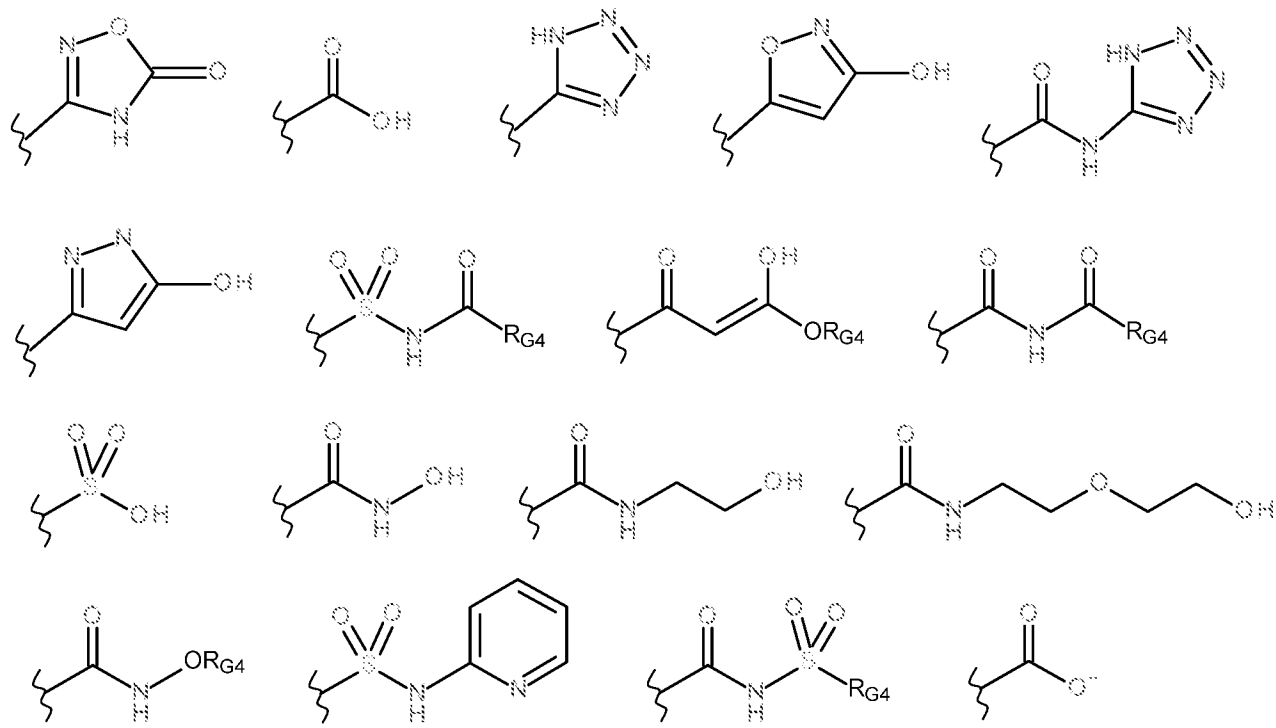
In some embodiments, for Formula (I) or Formula (II), G is selected from the group consisting of:

$-C(O)OR_{G3}$, $-C(O)NR_{G1}R_{G2}$, $-C(O)R_{G2}$, $-NR_{G1}C(O)R_{G2}$, $-NR_{G1}C(O)NR_{G1}R_{G2}$, $-OC(O)NR_{G1}R_{G2}$, $-NR_{G1}C(O)OR_{G3}$, $-C(=NOR_{G1})NR_{G1}R_{G2}$, $-NR_{G1}C(=NCN)NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2NR_{G1}R_{G2}$, $-S(O)_2R_{G3}$, $-S(O)_2NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2R_{G2}$, $-NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, $-C(=S)NR_{G1}R_{G2}$, $-C(=NR_{G1})NR_{G1}R_{G2}$, halogen, $-NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:

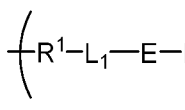


wherein R_{G4} is selected from C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl.

[13] In some embodiments, *p* is an integer from 1 to 8. In some embodiments, *p* is an integer from 1 to 5. In some embodiments, *p* is an integer from 2 to 4. In some embodiments, *p* is 2. In some embodiments, *p* is 4. In some embodiments, *p* is determined by liquid chromatography-mass spectrometry (LC-MS).

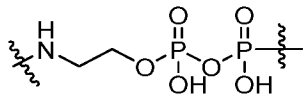
[14] In some embodiments, the linker (L) comprises an attachment group, at least one spacer group, and at least one cleavable group. In some cases, the cleavable group comprises a pyrophosphate group and/or a self-immolative group. In specific embodiments, L comprises an attachment group; at least one bridging spacer group; and at least one cleavable group comprising a pyrophosphate group and/or a self-immolative group.

[15] In some embodiments, the antibody-drug conjugate comprises a linker-drug (or "linker-payload") moiety -(L-D) is of the formula (A):



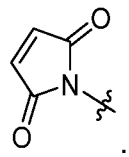
wherein R^1 is an attachment group, L_1 is a bridging spacer group, and E is a cleavable group.

[16] In some embodiments, the cleavable group comprises a pyrophosphate group. In some embodiments, the cleavable group comprises:



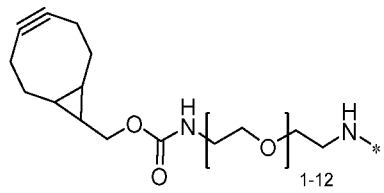
[17] In some embodiments, the bridging spacer group comprises a polyoxyethylene (PEG) group. In some cases, the PEG group may be selected from PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, and PEG15. In some embodiments, the bridging spacer group may comprise: -CO-CH_2-CH_2-PEG12-. In other embodiments, the bridging spacer group comprises a butanoyl, pentanoyl, hexanoyl, heptanoyl, or octanoyl group. In some embodiments, the bridging spacer group comprises a hexanoyl group.

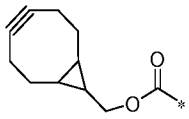
[18] In some embodiments the attachment group is formed from at least one reactive group selected from a maleimide group, thiol group, cyclooctyne group, and an azido group. For example, maleimide group may have the structure:



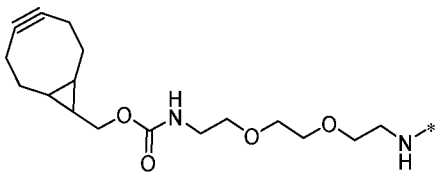
[19] The azido group may have the structure: -N=N^+=N^-.

[20] The cyclooctyne group may have the structure:



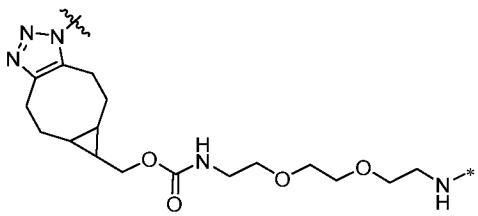
or  , and wherein —* is a bond to the antibody.

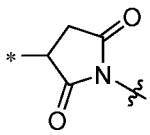
[21] In some cases, the cyclooctyne group has the structure:



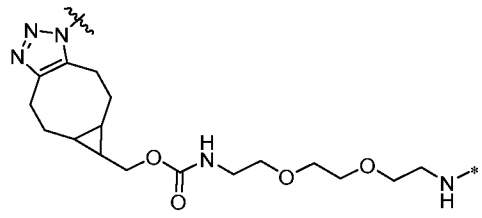
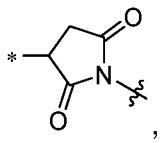
, and wherein —* is a bond to the antibody.

[22] In some embodiments, the attachment group has a formula comprising



or  , and wherein —* is a bond to the antibody.

[23] In some embodiments, the antibody is joined to the linker (L) by an attachment group selected from:

 and  ,

wherein —* is a bond to the antibody, and wherein  is a bond to the bridging spacer group. As used herein, the term "joined" refers to covalently attached to or covalently linked.

[24] In some embodiments, the bridging spacer group is joined or covalently linked to a cleavable group.

[25] In some embodiments, the bridging spacer group is -CO-CH_2-CH_2-PEG12-.

[26] In some embodiments, the cleavable group is -pyrophosphate-CH_2-CH_2-NH_2-.

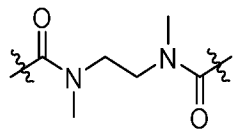
[27] In some embodiments, the cleavable group is joined or covalently linked to the Bcl-xL inhibitor (D).

[28] In some embodiments, the linker comprises: an attachment group, at least one bridging spacer group, a peptide group, and at least one cleavable group.

[29] In some embodiments, the antibody-drug conjugate comprises a linker-drug moiety, -(L-D), is of the formula (B):

$$\left(-R^1\text{-}L_1\text{—}Lp\text{—}E\text{-}\left(L_2\right)_m\text{—}D\right) \quad \text{(B)},$$

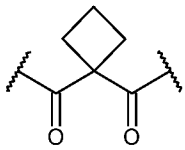
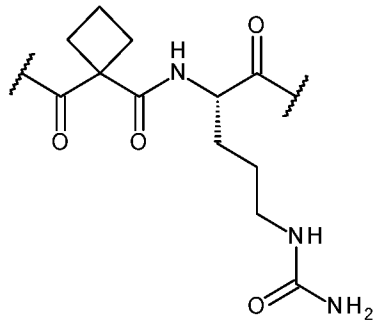
wherein R^1 is an attachment group, L_1 is a bridging spacer, Lp is a peptide group comprising 1 to 6 amino acid residues, E is a cleavable group, L_2 is a bridging spacer, m is 0 or 1; and D is a Bcl-xL inhibitor. In some cases, m is 1 and the bridging spacer comprises:

 .

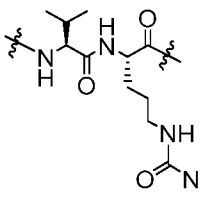
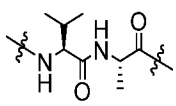
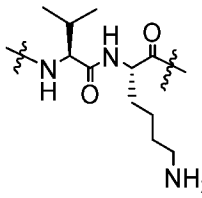
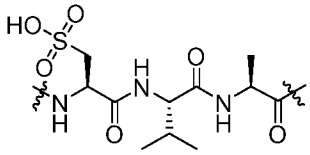
[30] In some embodiments, the at least one bridging spacer comprises a PEG group. In some cases, the PEG group is selected from, PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, and PEG15. In some cases, the at least one bridging spacer is selected from *-C(O)-CH_2-CH_2-PEG1-**, *-C(O)-CH_2-PEG3-**, *-C(O)-CH_2-CH_2-PEG12**, *-NH-CH_2-CH_2-PEG1-**, a polyhydroxyalkyl group, *-C(O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(O)-**, *-C(O)-CH_2-CH_2-PEG12-NH-C(O)CH_2-CH_2-**, and wherein ** indicates the point of direct or indirect attachment of the at least one bridging spacer to the attachment group and * indicates the point of direct or indirect attachment of the at least one bridging spacer to the peptide group.

[31] In some embodiments, L_1 is selected from *-C(O)-CH_2-CH_2-PEG1-**, *-C(O)-CH_2-PEG3-**, *-C(O)-CH_2-CH_2-PEG12**, *-NH-CH_2-CH_2-PEG1-**, and a polyhydroxyalkyl group, wherein ** indicates the point of direct or indirect attachment of L_1 to R^1 and * indicates the point of direct or indirect attachment of L_1 to Lp.

[32] In some embodiments, m is 1 and L_2 is -C(O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(O)-.

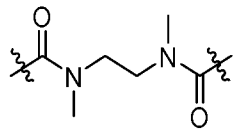
[33] In some embodiments, the peptide group comprises 1 to 12 amino acid residues. In some embodiments, the peptide group (Lp) comprises 1 to 10 amino acid residues. In some embodiments, the peptide group (Lp) comprises 1 to 8 amino acid residues. In some embodiments, the peptide group (Lp) comprises 1 to 6 amino acid residues. In some embodiments, the peptide group comprises 1 to 4 amino acid residues. In some embodiments, the peptide group comprises 1 to 3 amino acid residues. In some embodiments the peptide group comprises 1 to 2 amino acid residues. In some cases, the amino acid residues are selected from L-glycine (Gly), L-valine (Val), L-citrulline (Cit), L-cysteic acid (sulfo-Ala), L-lysine (Lys), L-isoleucine (Ile), L-phenylalanine (Phe), L-methionine (Met), L-asparagine (Asn), L-proline (Pro), L-alanine (Ala), L-leucine (Leu), L-tryptophan (Trp), and L-tyrosine (Tyr). For example, the peptide group may comprise Val-Cit, Val-Ala, Val-Lys, and/or sulfo-Ala-Val-Ala. In some embodiments, the peptide group (Lp) comprises 1 amino acid residue linked to a  group. In some embodiments, the peptide group (Lp) comprises a group :  .

[34] In some cases, the peptide group comprises a group selected from:

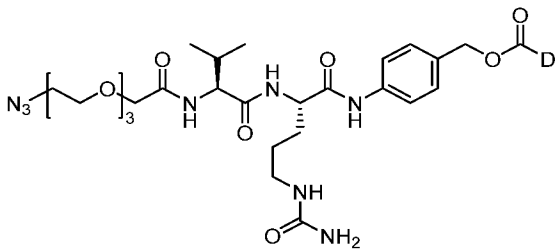
 ,  ,  , and  .

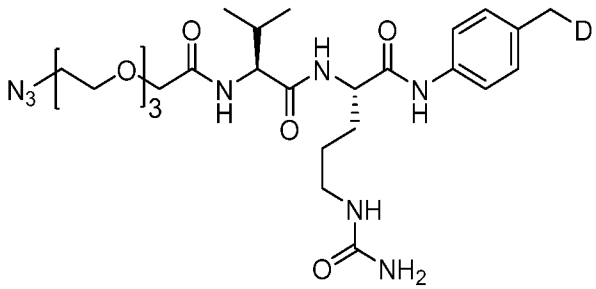
[35] In some embodiments, the self-immolative group comprises para-aminobenzyl-carbamate, para-aminobenzyl-ammonium, para-amino-(sulfo)benzyl-ammonium, para-

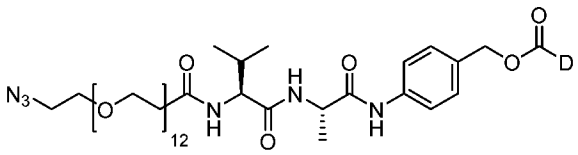
amino-(sulfo)benzyl-carbamate, para-amino-(alkoxy-PEG-alkyl)benzyl-carbamate, para-amino-(polyhydroxycarboxytetrahydropyranyl)alkyl-benzyl-carbamate, or para-amino-(polyhydroxycarboxytetrahydropyranyl)alkyl-benzyl-ammonium.

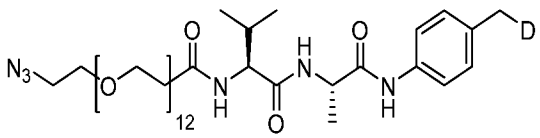
[36] In some embodiments, m is 1 and the bridging spacer comprises  .

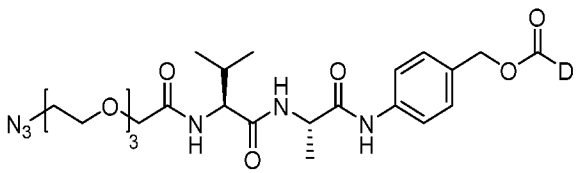
[37] In some embodiments, the linker-drug moiety, -(L-D), is formed from a compound selected from:

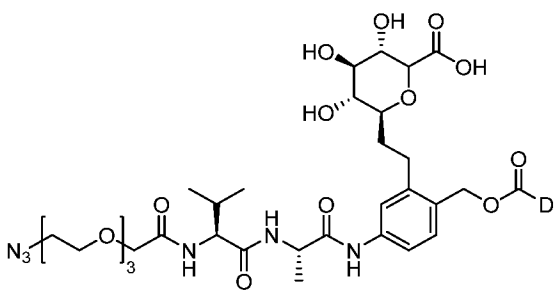
 ,

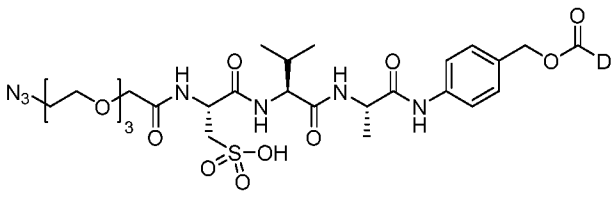
 ,

 ,

 ,

 ,

 ,



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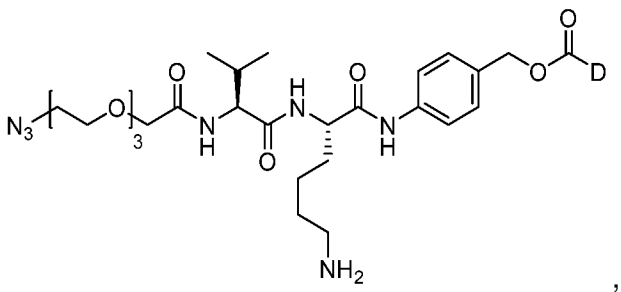

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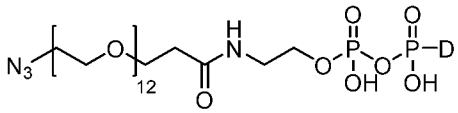

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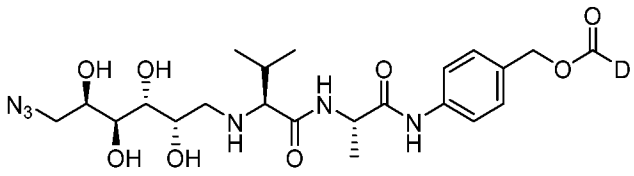

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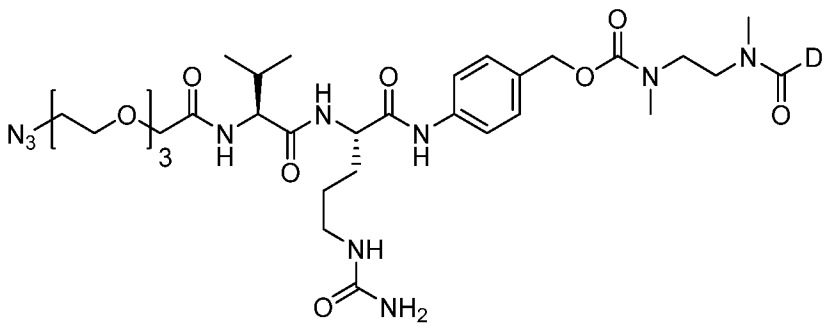

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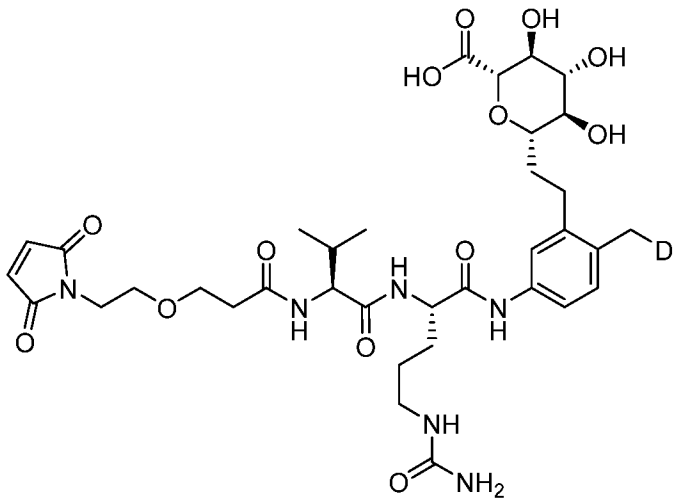

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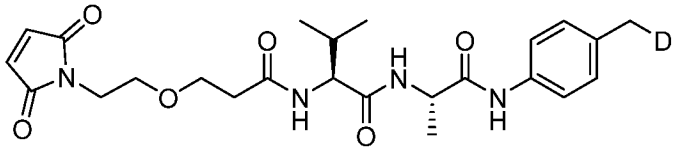
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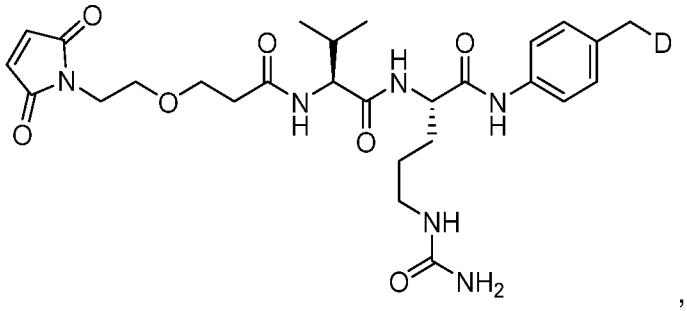
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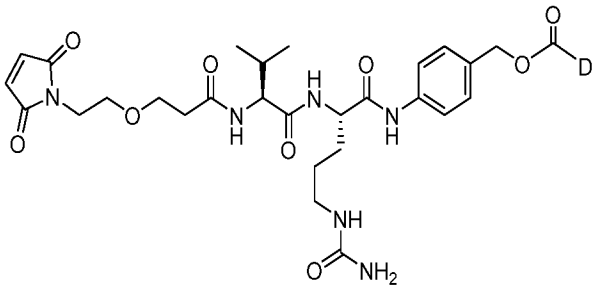
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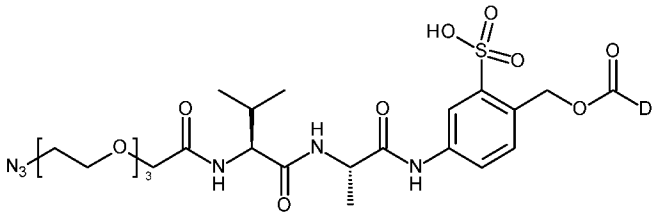
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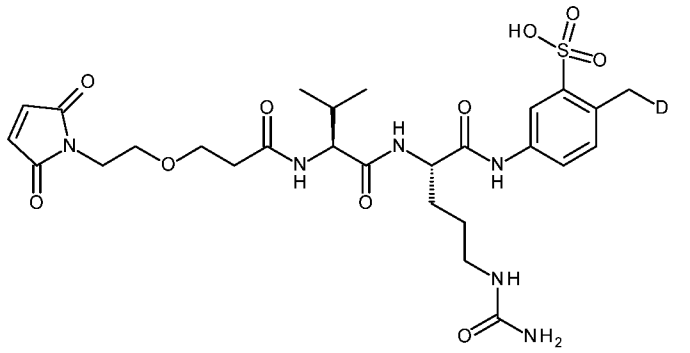
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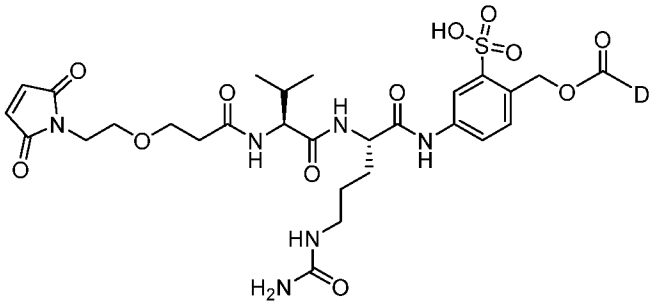
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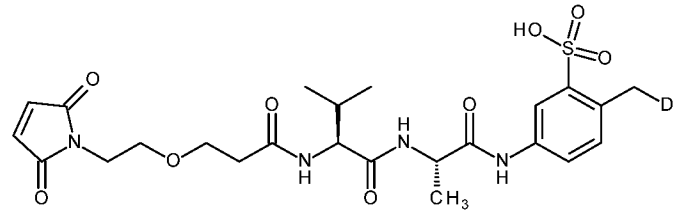

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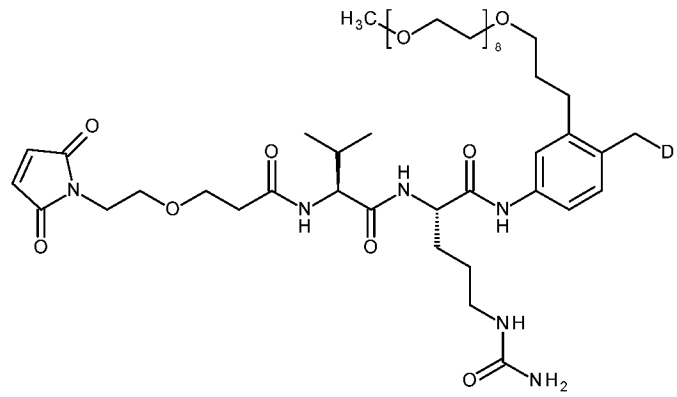

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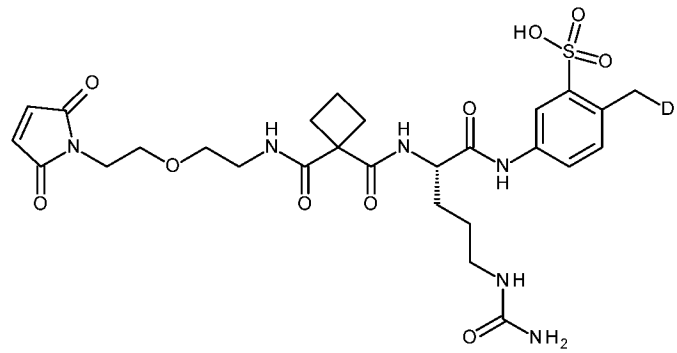

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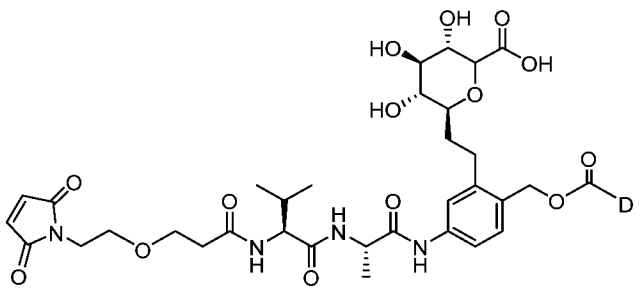

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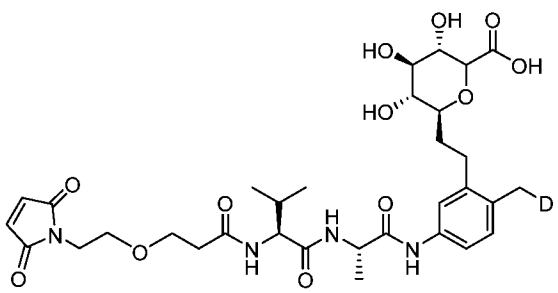

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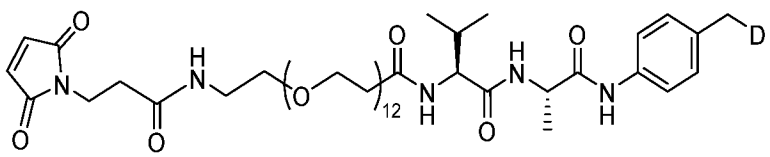

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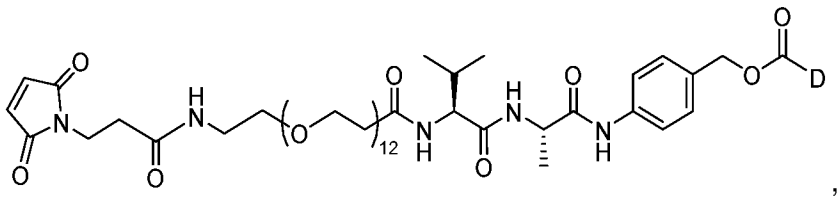

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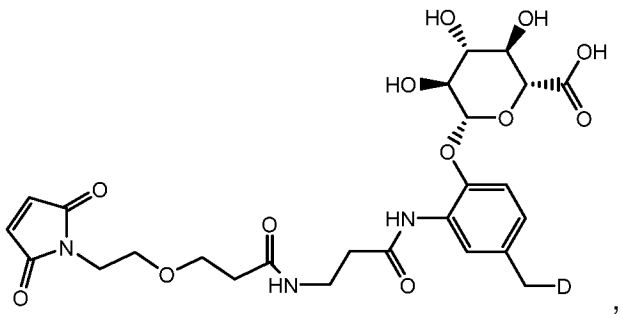

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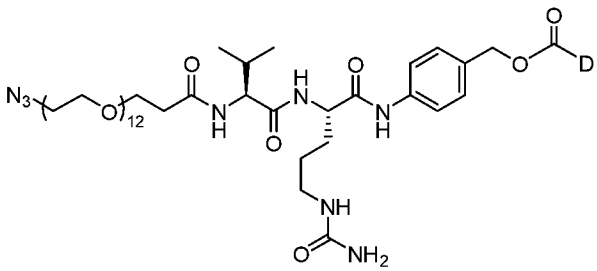

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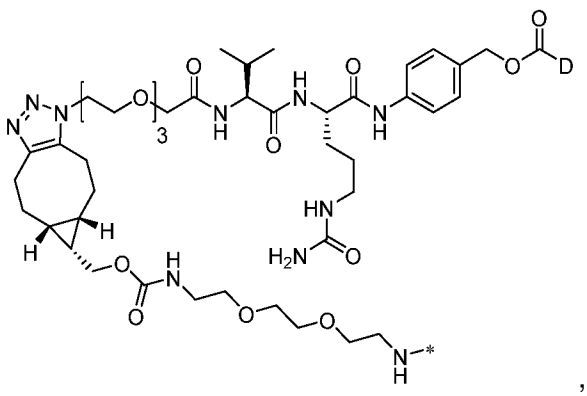
[38] In some embodiments, the antibody-drug conjugate comprises the linker-drug group, -(L-D), which comprises a formula selected from:



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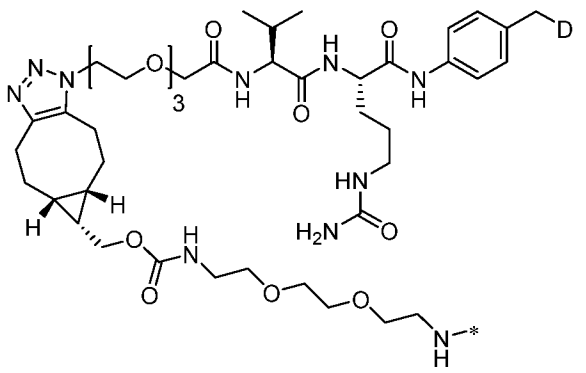

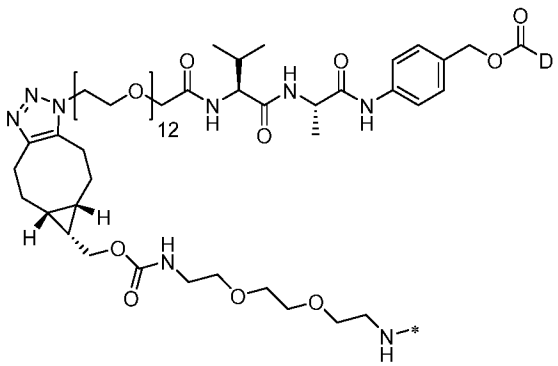

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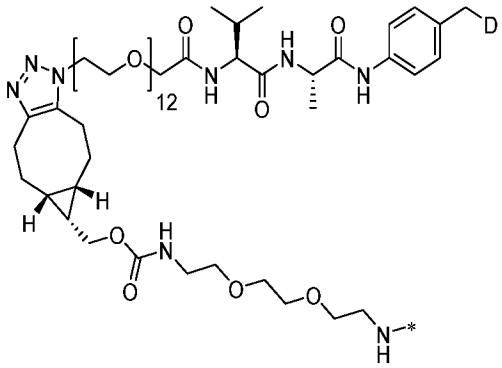

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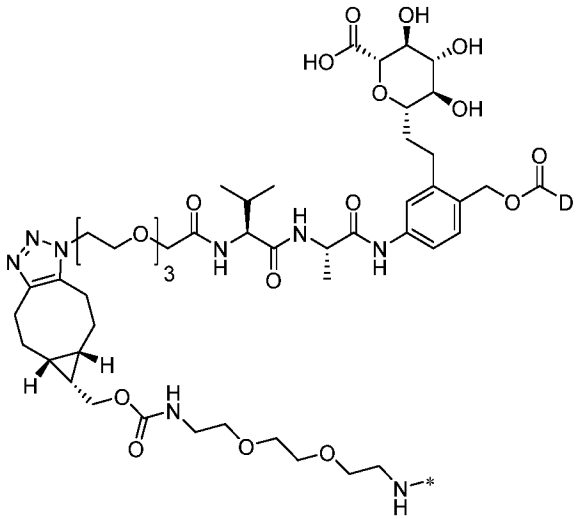

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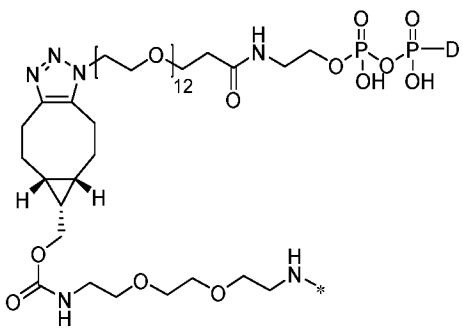

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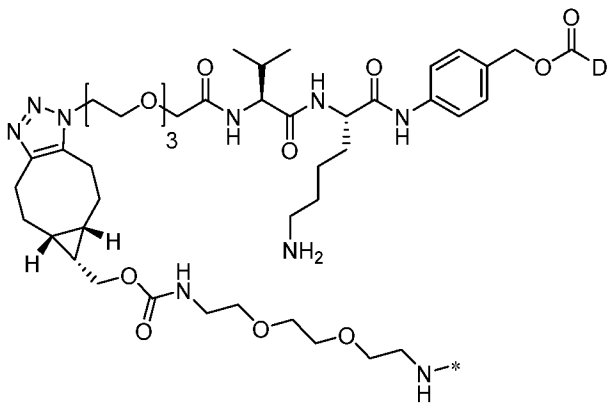

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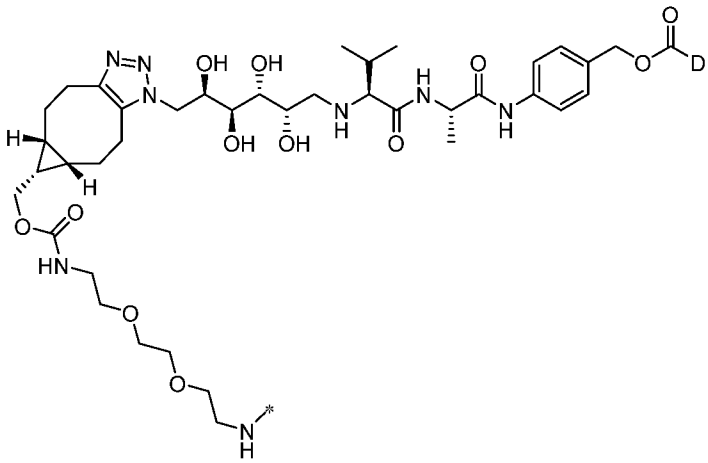

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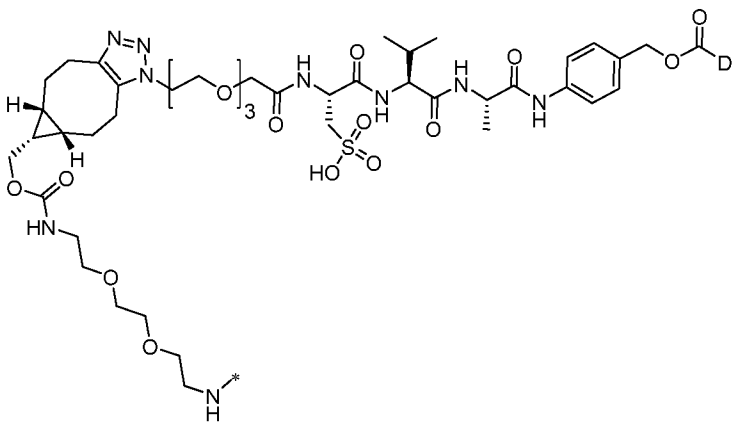

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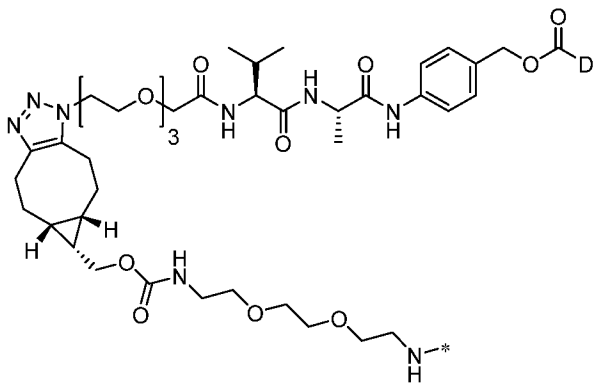

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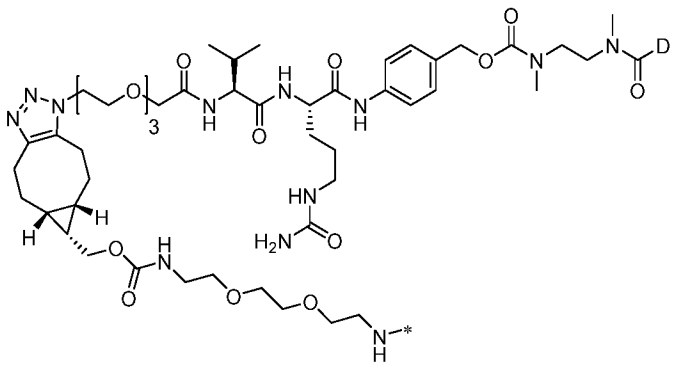

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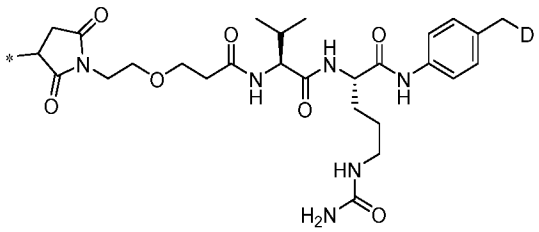

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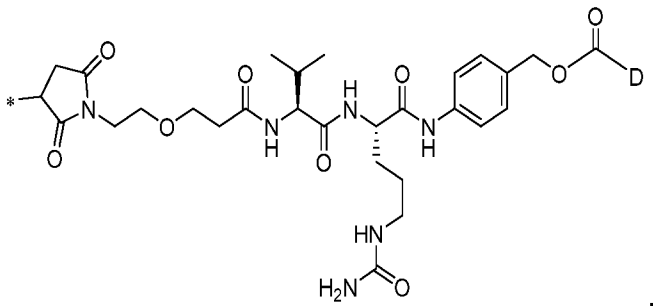

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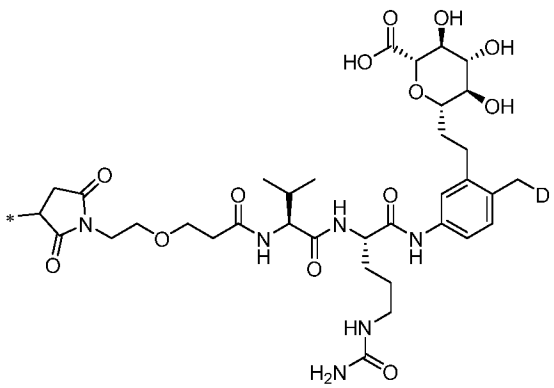

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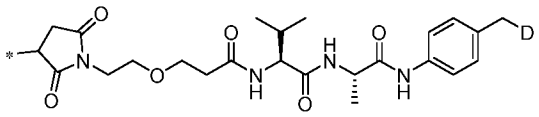

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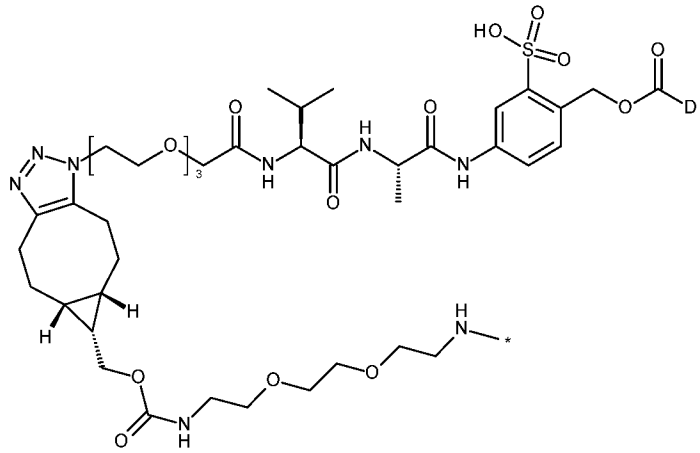

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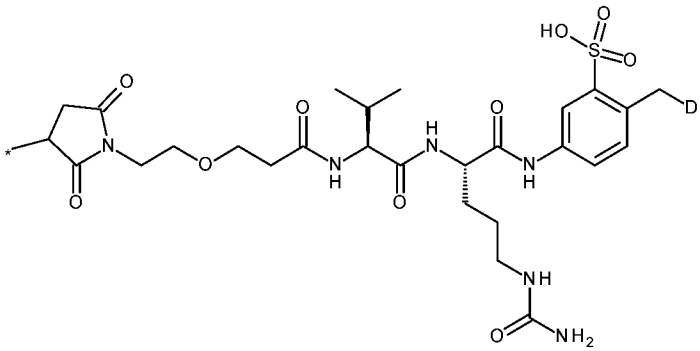

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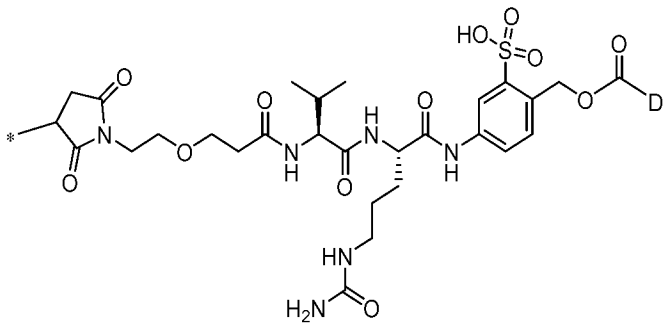

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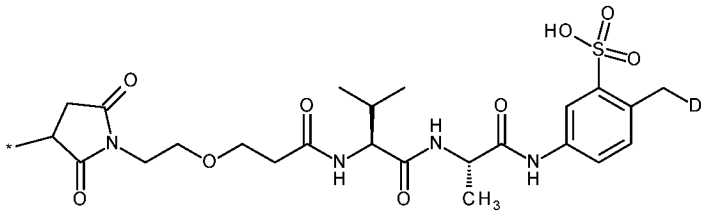

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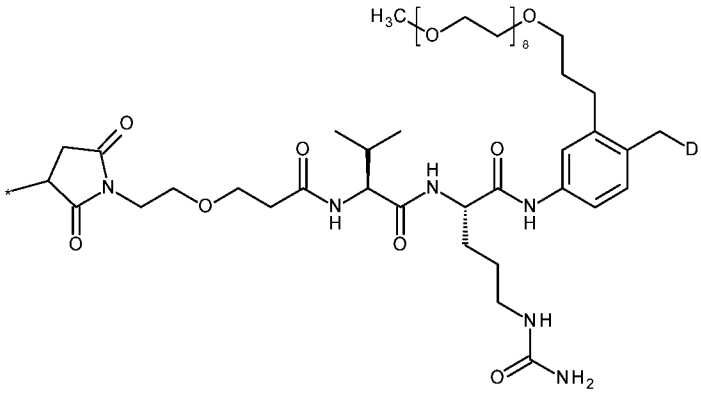

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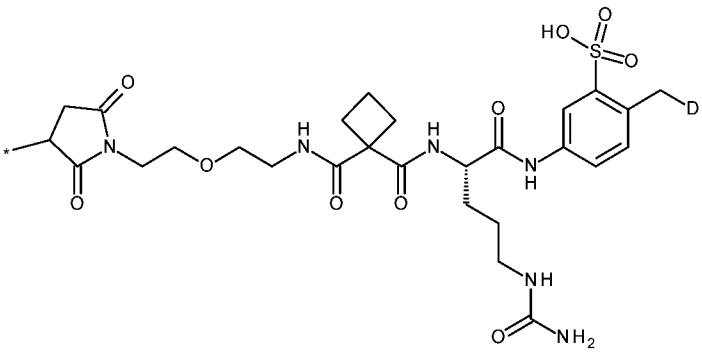

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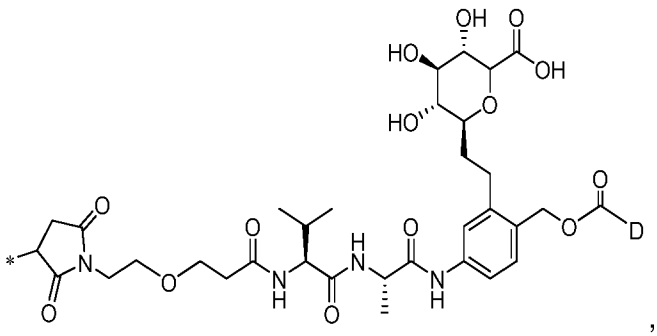

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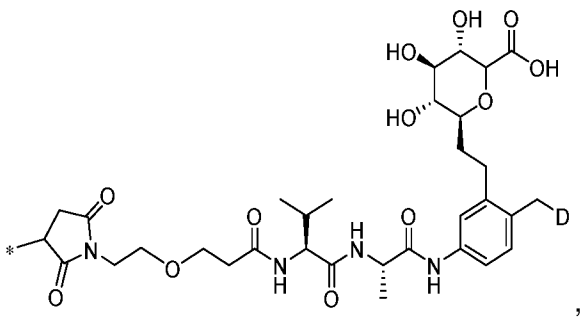

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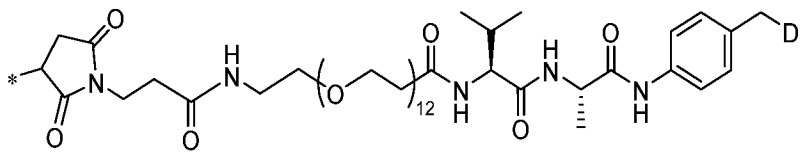

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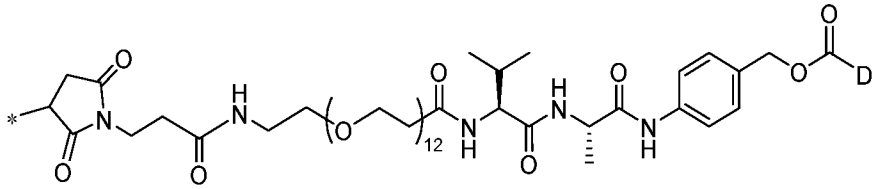

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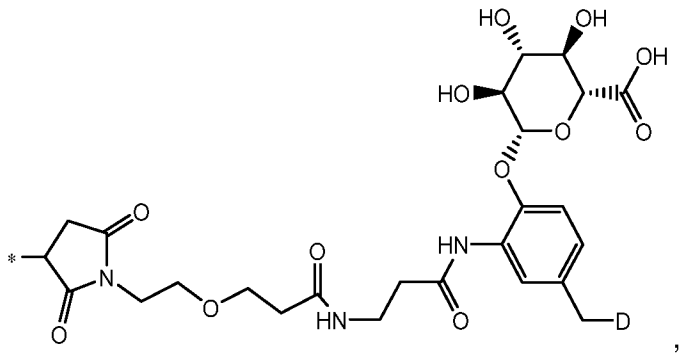

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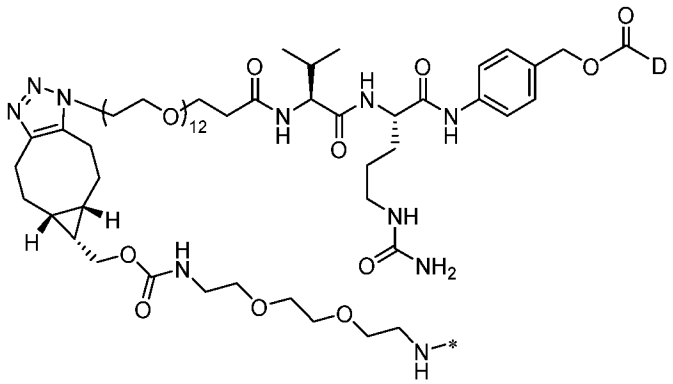
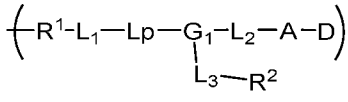


,

and

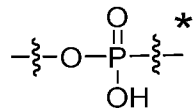
and wherein —* is a bond to the antibody.

[39] In some embodiments, the antibody-drug conjugate comprises the linker drug group, -(L-D), which is of the formula (C):



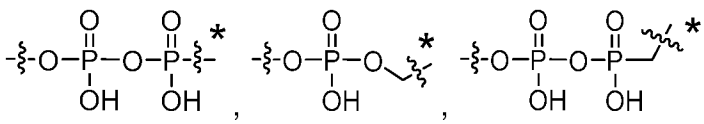
(C),

wherein: R^1 is an attachment group, L_1 is a bridging spacer; L_p is a peptide group comprising 1 to 6 amino acids; D is a Bcl-xL inhibitor; G_1-L_2-A is a self-immolative spacer; L_2 is a bond, a



methylene, a neopentylene or a C_2-C_3 alkenylene; A is a bond, -OC(=O)-*,

,

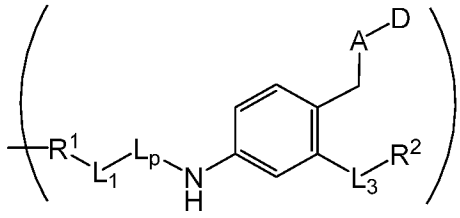


, -

OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the

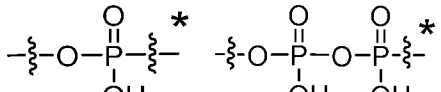
* of A indicates the point of attachment to D; L_3 is a spacer moiety; and R^2 is a hydrophilic moiety.

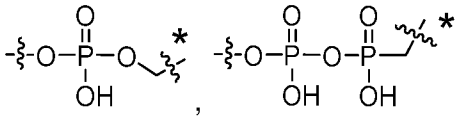
[40] In some embodiments, the antibody-drug conjugate comprises the linker drug group, -(L-D), which is of the formula (D):



(D),

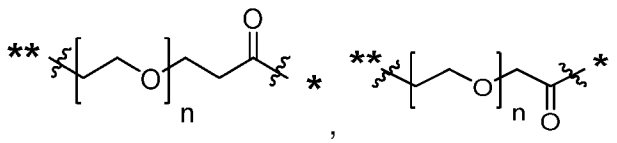
wherein: R^1 is an attachment group; L_1 is a bridging spacer; Lp is a peptide group comprising 1 to 6 amino acids; A is a bond, -OC(=O)-*,





-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; L_3 is a spacer moiety; and R^2 is a hydrophilic moiety.

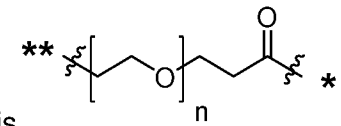
[41] In some embodiments, L_1 comprises:



or

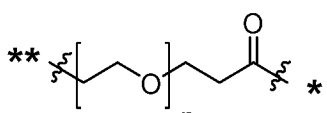
*-CH(OH)CH(OH)CH(OH)CH(OH)-**,wherein each n is an integer from 1 to 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.

[42] In some embodiments, L_1 is

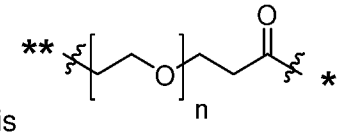


, and n is an integer from 1 to 12 wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.

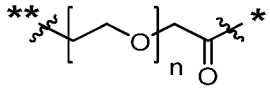
[43] In some embodiments, L_1 is



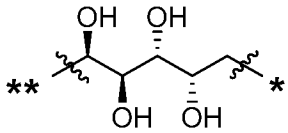
, and n is 1, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.



[44] In some embodiments, L_1 is , and n is 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.



[45] In some embodiments, L_1 is , and n is an integer from 1 to 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.



[46] In some embodiments, L_1 comprises , wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1.

[47] In some embodiments, L_1 is a bridging spacer comprising:

*-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**;

*-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-**;

*-C(=O)O$(CH_2)_m$SSC(R^3)$_2$$(CH_2)_m$C(=O)N$R^3$$(CH_2)_mNR^3$C(=O)$(CH_2)_m$-**;

*-C(=O)O$(CH_2)_m$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**;

*-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**; *-C(=O)$(CH_2)_m$$X_1$$(CH_2)_m$-**;

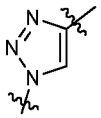
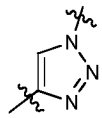
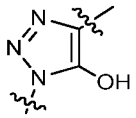
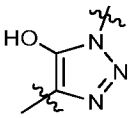
*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$C($R^3$)$_2$-** or

*-C(=O)$(CH_2)_m$C(=O)NH$(CH_2)_m$-**, where the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1,

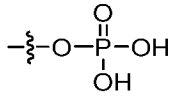
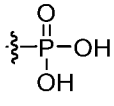
wherein X_1 is  ,  ,  or  ; and

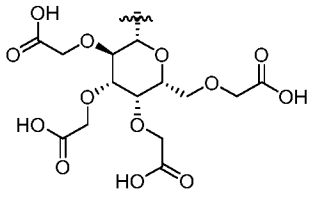
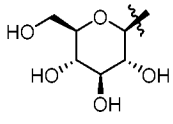
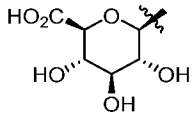
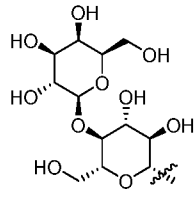
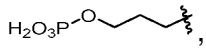
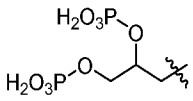
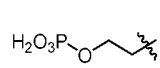
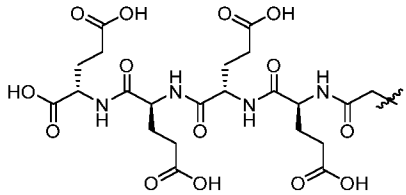
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

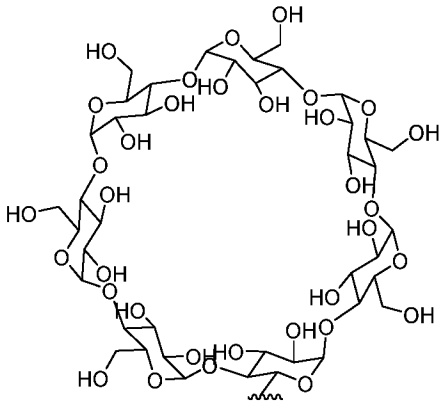
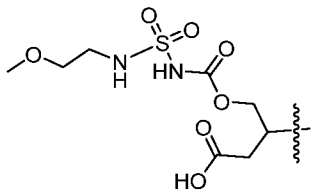
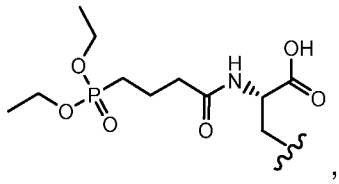
each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and

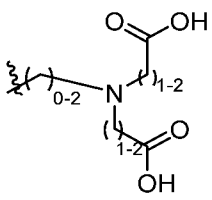
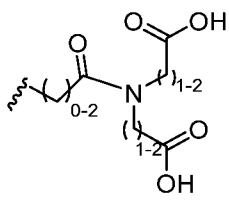
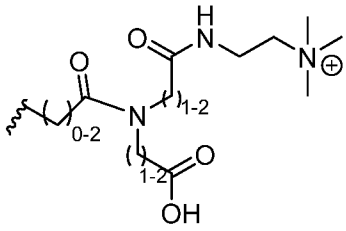
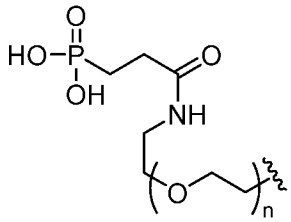
each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30.

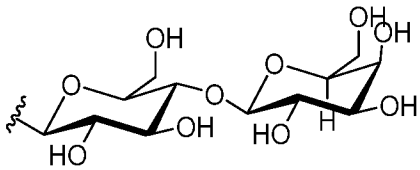
[48] In some embodiments, R^2 is a hydrophilic moiety comprising polyethylene glycol, polyalkylene glycol, a polyol, a polysarcosine, a sugar, an oligosaccharide, a polypeptide,

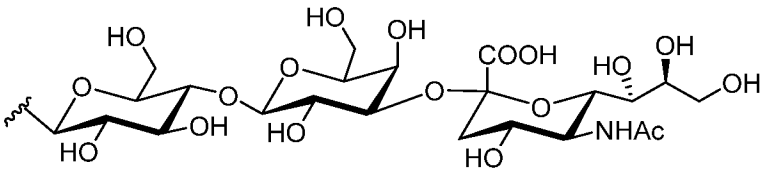
C_2-C_6 alkyl substituted with 1 to 3  or  groups, or C_2-C_6alkyl substituted with 1 to 2 substituents independently selected from -OC(=O)NHS(O)$_2$NHCH$_2$CH$_2$OCH$_3$, -NHC(=O)C_{1-4}alkylene-P(O)(OCH$_2$CH$_3$)$_2$ and -COOH groups . In some embodiments, R^2 is

 ,  ,  ,  ,  ,  ,  ,  or

 ,  ,  ,

 ,  ,  , 

wherein n is an integer between 1 and 6,  ,



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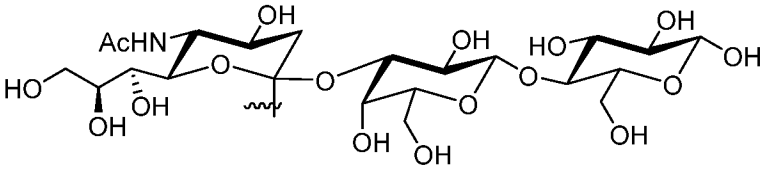
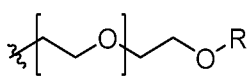
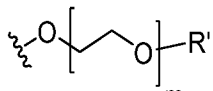
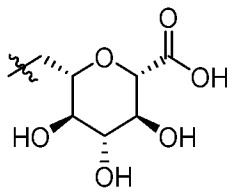
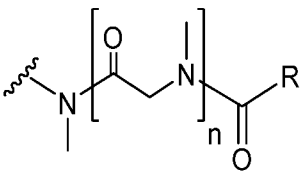


.

[49] In some embodiments, the hydrophilic moiety comprises a polyethylene glycol of formula:  or  , wherein R is H, $-CH_3$ $CH_2CH_2NHC(=O)OR_a$, $-CH_2CH_2NHC(=O)R_a$, or or $-CH_2CH_2C(=O)OR_a$, R' is OH, $-OCH_3$, $CH_2CH_2NHC(=O)OR_a$, $-CH_2CH_2NHC(=O)R_a$, or $-OCH_2CH_2C(=O)OR_a$, and each of m and n is an integer between 2 and 25 (*e.g.*, between 3 and 25).

[50] In some embodiments, the hydrophilic moiety comprises  .

[51] In some embodiments, the hydrophilic moiety comprises a polysarcosin, e.g., with the following moiety



, wherein n is an integer between 3 and 25; and R is H, $–CH_3$ or $-CH_2CH_2C(=O)OH$.

[52] In some embodiments, L_3 is a spacer moiety having the structure , wherein:

W is $-CH_2-$, $-CH_2O-$, $-CH_2N(R^b)C(=O)O-$, $-NHC(=O)C(R^b)_2NHC(=O)O-$, $-NHC(=O)C(R^b)_2NH-$, $-NHC(=O)C(R^b)_2NHC(=O)-$, $-CH_2N(X\text{-}R^2)C(=O)O-$, $-C(=O)N(X\text{-}R^2)-$, $-CH_2N(X\text{-}R^2)C(=O)-$, $-C(=O)NR^b-$, $-C(=O)NH-$, $-CH_2NR^bC(=O)-$, $-CH_2NR^bC(=O)NH-$, $-CH_2NR^bC(=O)NR^b-$, $-NHC(=O)-$, $-NHC(=O)O-$, $-NHC(=O)NH-$, $-OC(=O)NH-$, $-S(O)_2NH-$, $-NHS(O)_2-$, $-C(=O)-$, $-C(=O)O-$ or $-NH-$, wherein each R^b is independently selected from H, C_1-C_6alkyl, and C_3-C_8 cycloalkyl; and

X is a bond, triazolyl or -CH_2-triazolyl-, wherein X is connected to R^2.

[53] In some embodiments, L_3 is a spacer moiety having the structure  , wherein:

W is -CH_2-, -CH_2O-, -$CH_2N(R^b)C(=O)O$-, -$NHC(=O)C(R^b)_2NHC(=O)O$-, -$NHC(=O)C(R^b)_2NH$-, -$NHC(=O)C(R^b)_2NHC(=O)$-, -$CH_2N(X\text{-}R^2)C(=O)O$-, -$C(=O)N(X\text{-}R^2)$-, -$CH_2N(X\text{-}R^2)C(=O)$-, -$C(=O)NR^b$-, -$C(=O)NH$-, -$CH_2NR^bC(=O)$-, -$CH_2NR^bC(=O)NH$-, -$CH_2NR^bC(=O)NR^b$-, -$NHC(=O)$-, -$NHC(=O)O$-, -$NHC(=O)NH$-, -$OC(=O)NH$-, -$S(O)_2NH$-, -$NHS(O)_2$-, -$C(=O)$-, -$C(=O)O$- or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl, and C_3-C_8 cycloalkyl; and

[54] X is -CH_2-triazolyl-$C_{1\text{-}4}$ alkylene-$OC(O)NHS(O)_2NH$-, -$C_{4\text{-}6}$ cycloalkylene-$OC(O)NHS(O)_2NH$-, -$(CH_2CH_2O)_n$-$C(O)NHS(O)_2NH$-, -$(CH_2CH_2O)_n$-$C(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-, -CH_2-triazolyl-$C_{1\text{-}4}$ alkylene-$OC(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-, or -$C_{4\text{-}6}$ cycloalkylene-$OC(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-,wherein each n independently is 1, 2, or 3 and wherein X is connected to R^2.

[55] In some embodiments, the attachment group is formed by a reaction comprising at least one reactive group. In some cases, the attachment group is formed by reacting: a first reactive group that is attached to the linker, and a second reactive group that is attached to the antibody or is an amino acid residue of the antibody.

[56] In some embodiments, at least one of the reactive groups comprises:

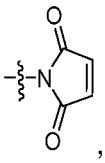
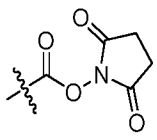
a thiol,
a maleimide,
a haloacetamide,
an azide,
an alkyne,
a cyclcooctene,
a triaryl phosphine,
an oxanobornadiene,
a cyclooctyne,
a diaryl tetrazine,
a monoaryl tetrazine,
a norbornene,
an aldehyde,
a hydroxylamine,
a hydrazine,
NH_2-NH-C(=O)-,

a ketone,

a vinyl sulfone,

an aziridine,

an amino acid residue,


, -ONH_2, -NH_2,

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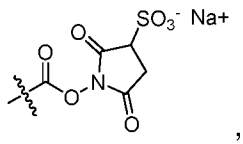
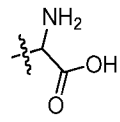
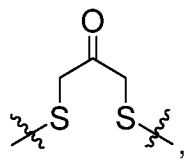

,

,

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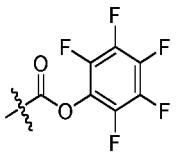
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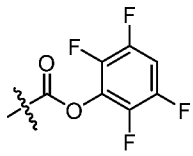
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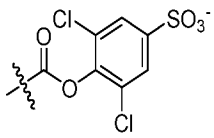
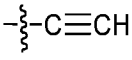
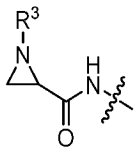
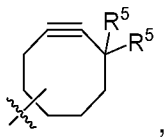

, -N_3,

, -SH, -SR^3,

-SSR^4, -$S(=O)_2(CH=CH_2)$, -$(CH_2)_2S(=O)_2(CH=CH_2)$, -$NHS(=O)_2(CH=CH_2)$,

-$NHC(=O)CH_2Br$, -$NHC(=O)CH_2I$,

, -$C(O)NHNH_2$,

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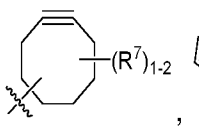
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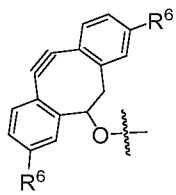
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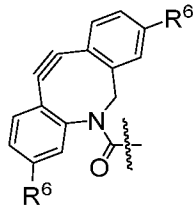
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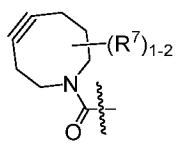
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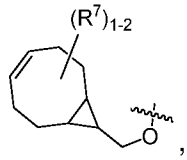
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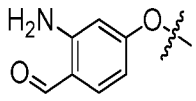
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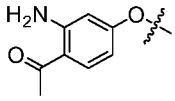
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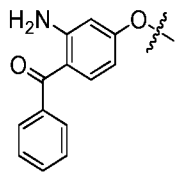
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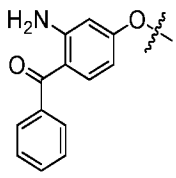
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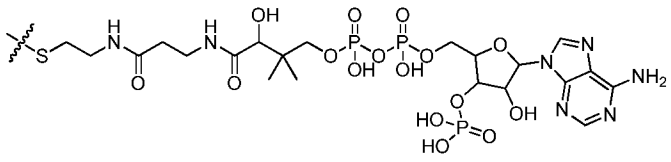
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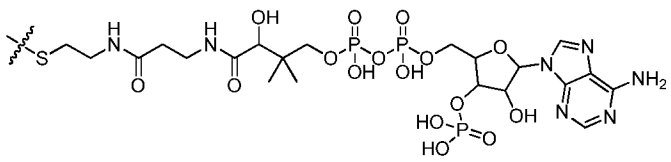
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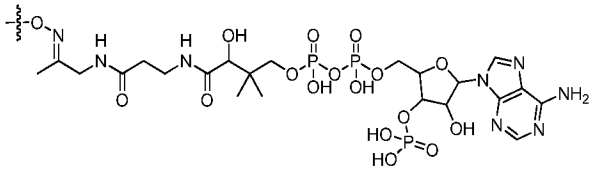
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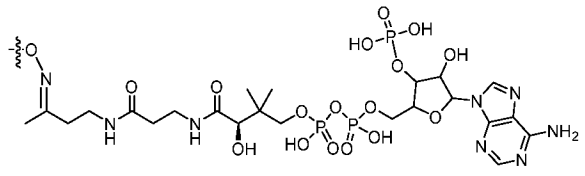
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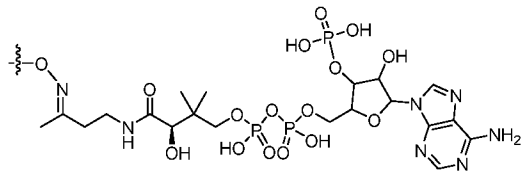
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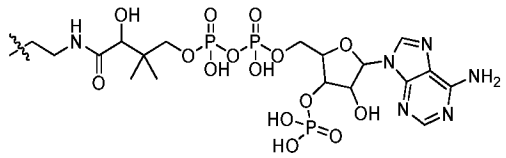
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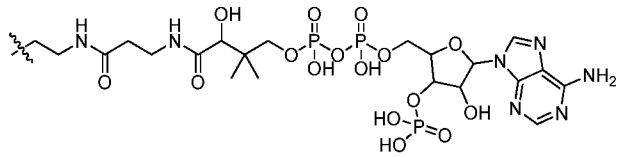
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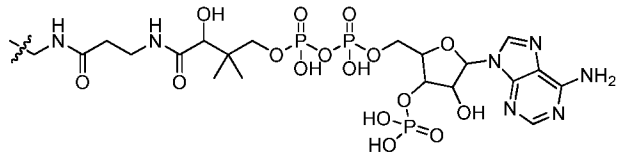

;

wherein:

each R^3 is independently selected from H and C_1-C_6alkyl;

each R^4 is 2-pyridyl or 4-pyridyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and -OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and-OH;

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH.

[57] In some embodiments, the first reactive group and second reactive group comprise:

a thiol and a maleimide,

a thiol and a haloacetamide,

a thiol and a vinyl sulfone,

a thiol and an aziridine,

an azide and an alkyne,

an azide and a cyclooctyne,

an azide and a cyclooctene,

an azide and a triaryl phosphine,

an azide and an oxanobornadiene,

a diaryl tetrazine and a cyclooctene,

a monoaryl tetrazine and a nonbornene,

an aldehyde and a hydroxylamine,

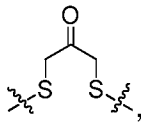
an aldehyde and a hydrazine,

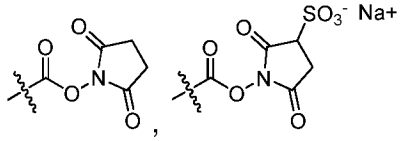
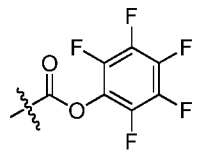
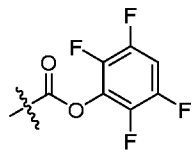
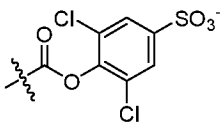
an aldehyde and NH_2-NH-C(=O)-,

a ketone and a hydroxylamine,

a ketone and a hydrazine,

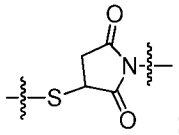
a ketone and NH2-NH-C(=O)-,

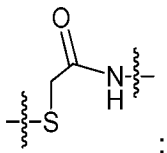
a hydroxylamine and  ,

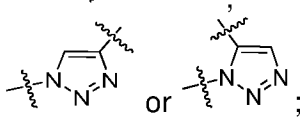
an amine and  ,  ,  , or  , or

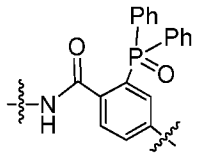
a CoA or CoA analogue and a serine residue.

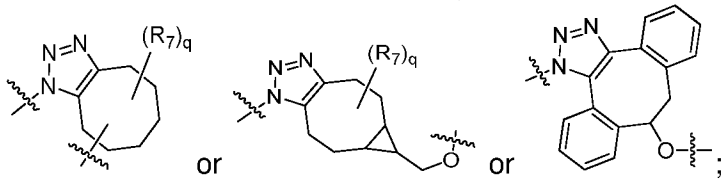
[58] In some embodiments, the attachment group comprises a group selected from:

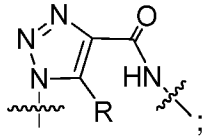
 ;

 ;

 ;

 ;

 .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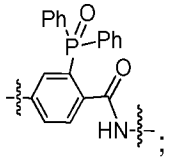
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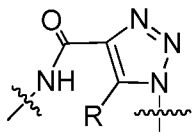
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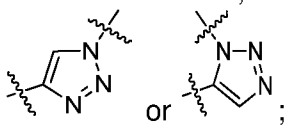
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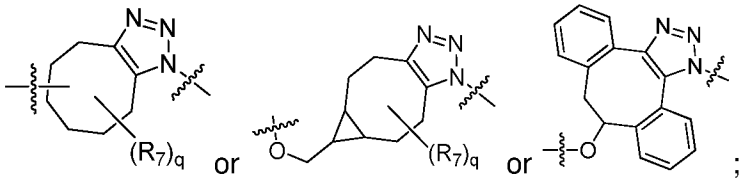
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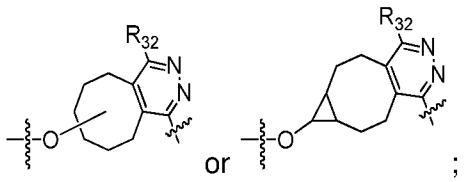
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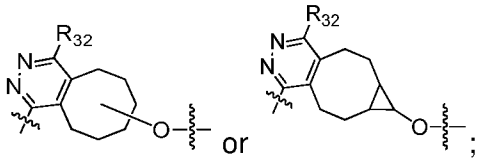
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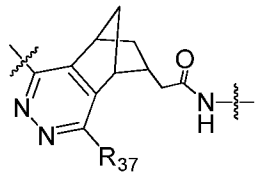
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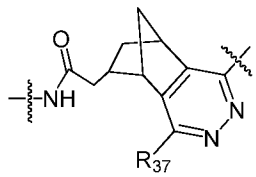
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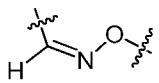
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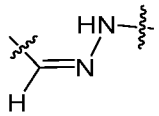
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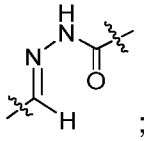
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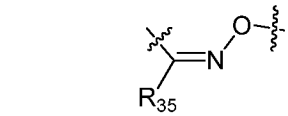
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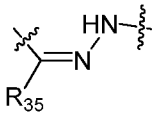
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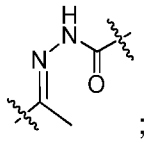
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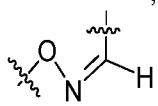
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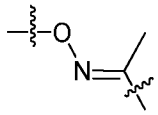
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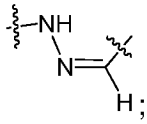
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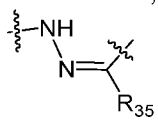
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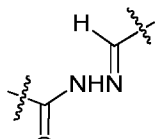
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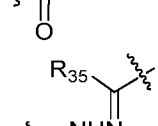
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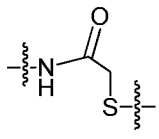
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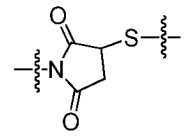
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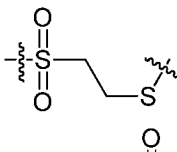
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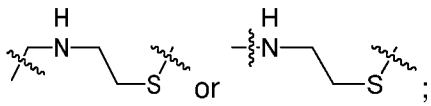
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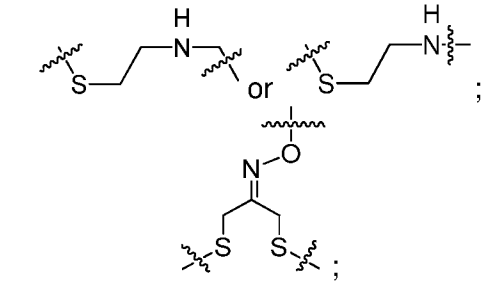
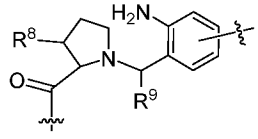
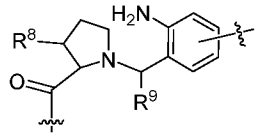
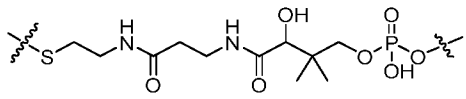
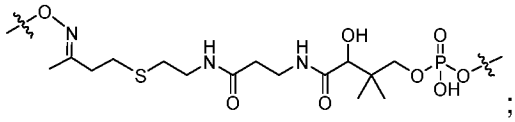


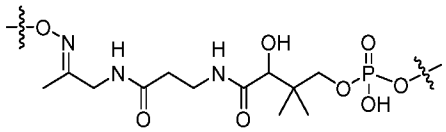
amide;

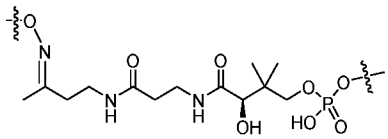


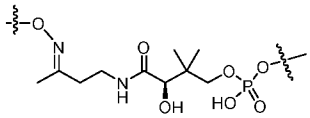


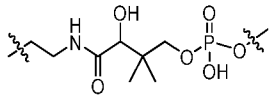


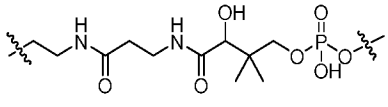


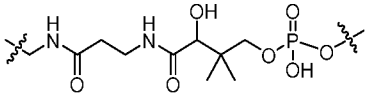












disulfide,

wherein:

R^{32} is H, C_{1-4} alkyl, phenyl, pyrimidine or pyridine;

R^{35} is H, C_{1-6} alkyl, phenyl or C_{1-4} alkyl substituted with 1 to 3 –OH groups;

each R^7 is independently selected from H, C_{1-6} alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4} alkoxy substituted with –C(=O)OH and C_{1-4} alkyl substituted with –C(=O)OH;

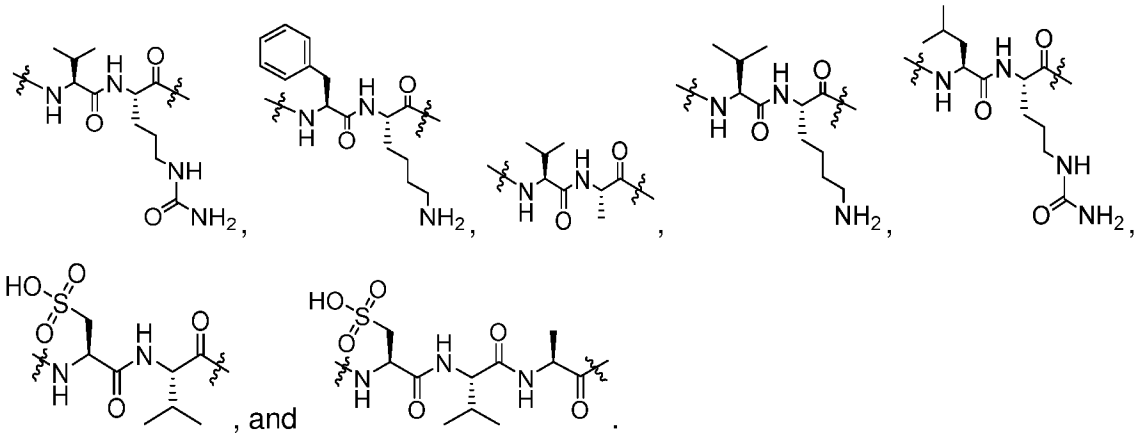
R^{37} is independently selected from H, phenyl and pyridine;

q is 0, 1, 2 or 3;

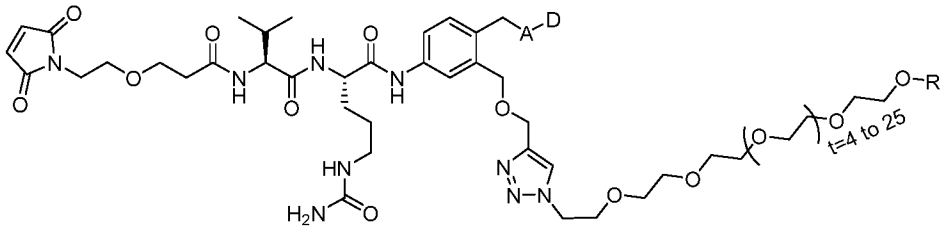
R^8 is H or methyl; and

R^9 is H, $-CH_3$ or phenyl.

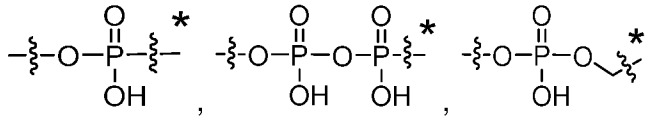
[59] In some embodiments, the peptide group (Lp) comprises 1 to 6 amino acid residues. In some embodiments, the peptide group (Lp) comprises 1 to 4 amino acid residues. In some embodiments, the peptide group comprises 1 to 3 amino acid residues. In some embodiments, the peptide group comprises 1 to 2 amino acid residues. In some embodiments, the amino acid residues are selected from L-glycine (Gly), L-valine (Val), L-citrulline (Cit), L-cysteic acid (sulfo-Ala), L-lysine (Lys), L-isoleucine (Ile), L-phenylalanine (Phe), L-methionine (Met), L-asparagine (Asn), L-proline (Pro), L-alanine (Ala), L-leucine (Leu), L-tryptophan (Trp), and L-tyrosine (Tyr). In some embodiments, the peptide group comprises Val-Cit, Phe-Lys, Val-Ala, Val-Lys, Leu-Cit, sulfo-Ala-Val, and/or sulfo-Ala-Val-Ala. In some embodiments, Lp is selected from:

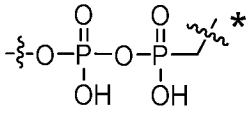

, and .

[60] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:


, wherein:

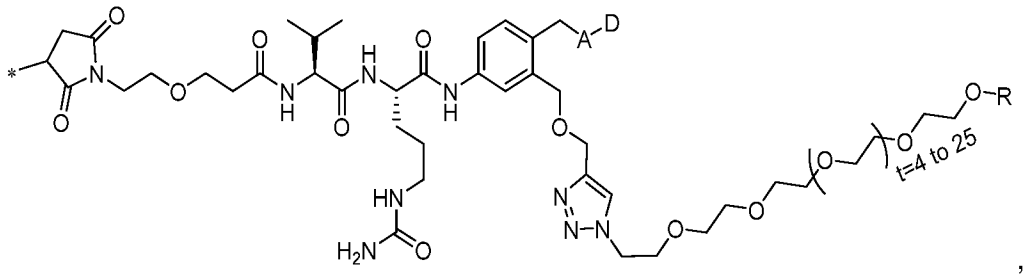
R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,

 ,

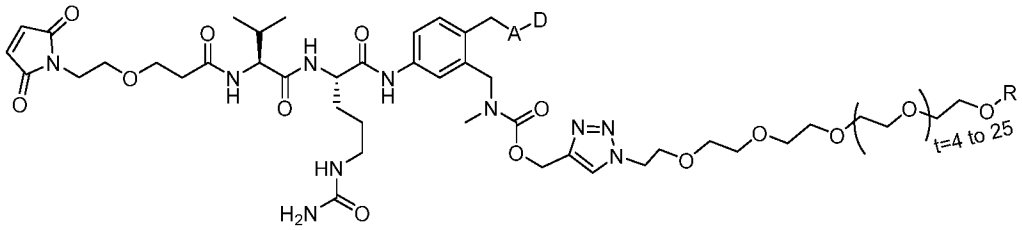
-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

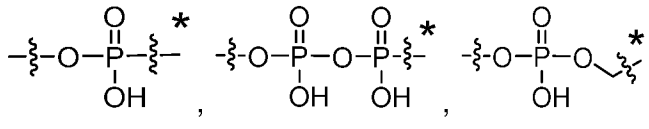
 ,

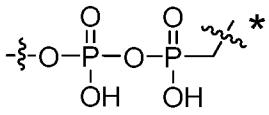
wherein: —* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[61] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

 , wherein:

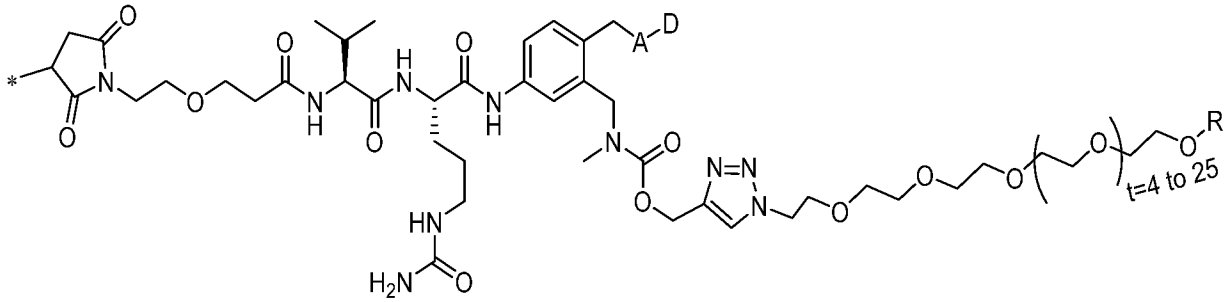
R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,

 ,

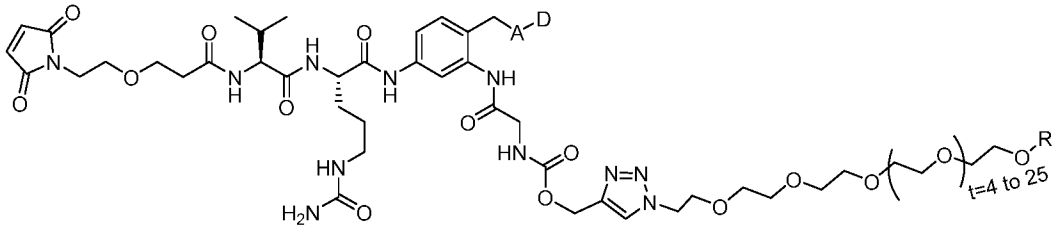
-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



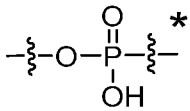
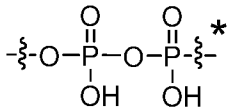
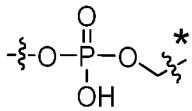
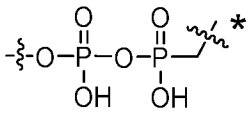
, wherein: —* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[62] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



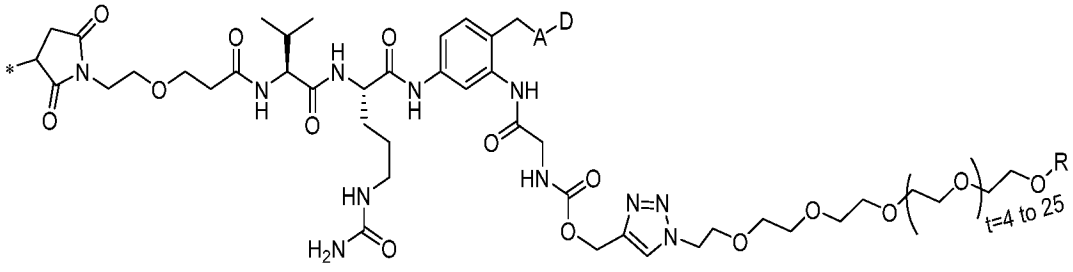
, wherein:

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

$-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-$* or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-$*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

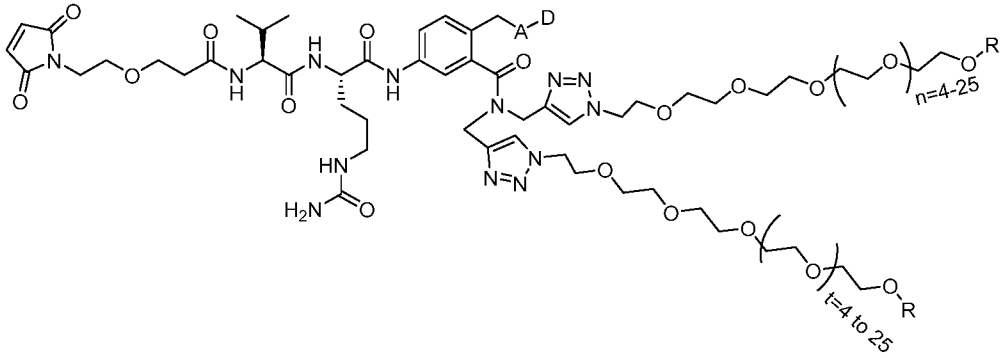
D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



, wherein:

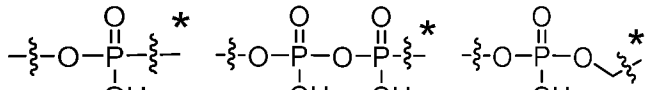
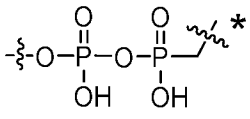
—* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[63] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

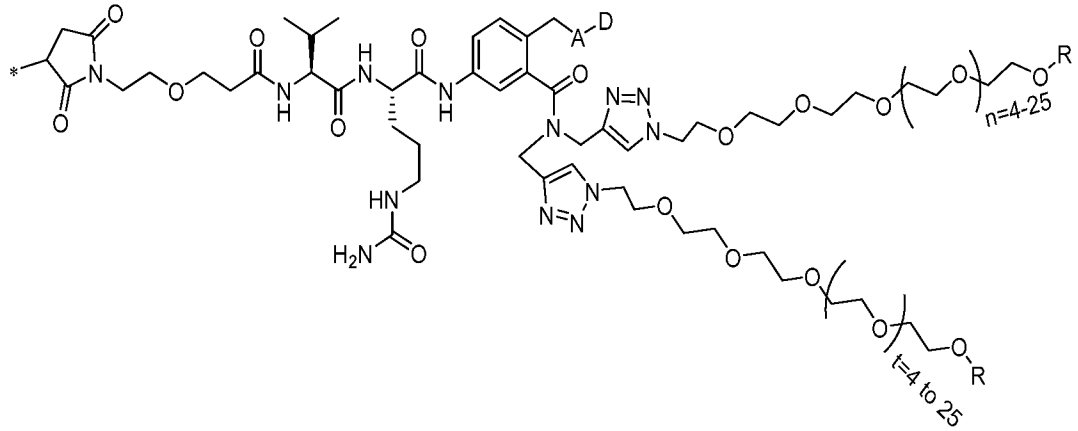


, wherein:

each R is independently selected from H, $-CH_3$, and $-CH_2CH_2C(=O)OH$;

A is a bond, $-OC(=O)-*$,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

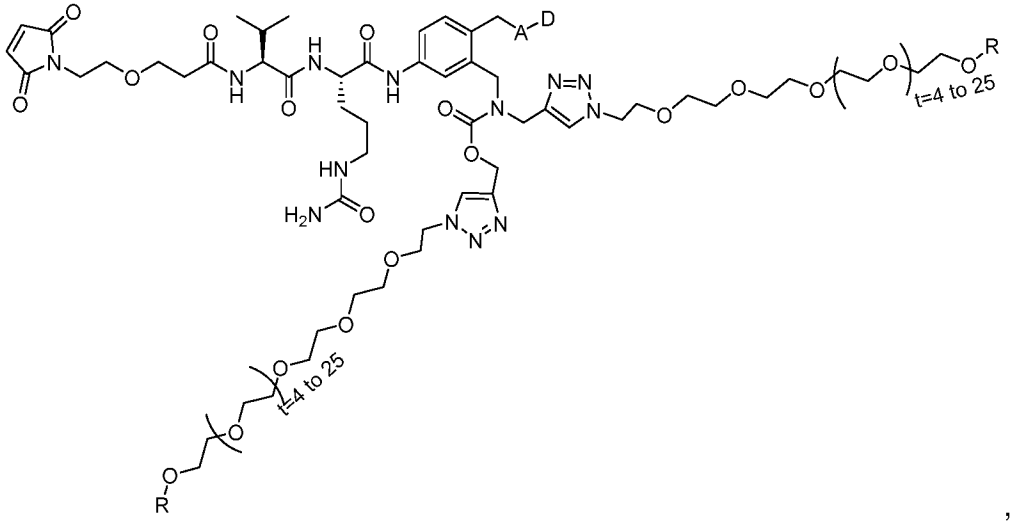
D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



,

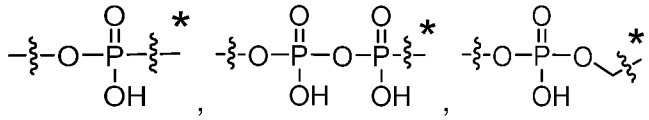
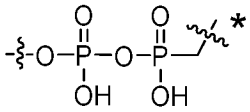
wherein: $—*$ is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or $-OC(=O)-*$; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[64] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

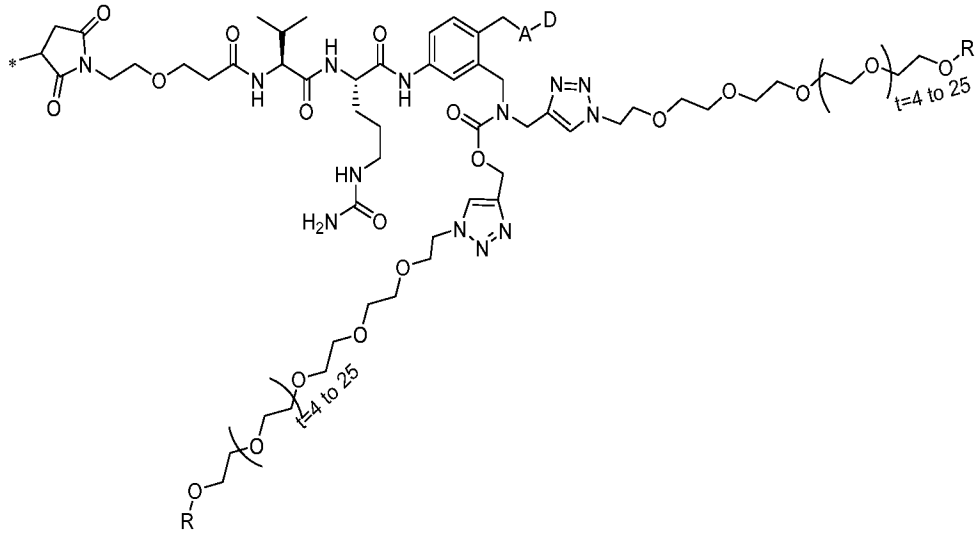


, wherein:

each R is independently selected from H, $-CH_3$, and $-CH_2CH_2C(=O)OH$;

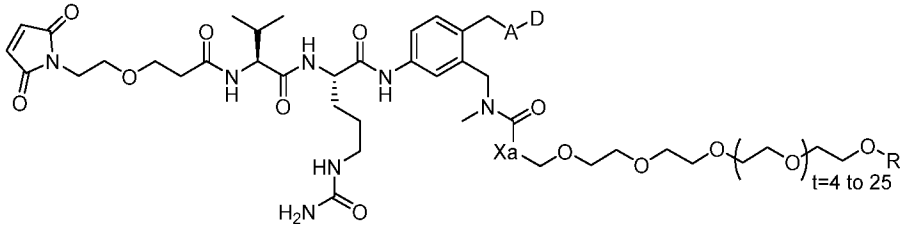
A is a bond, $-OC(=O)-*$,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



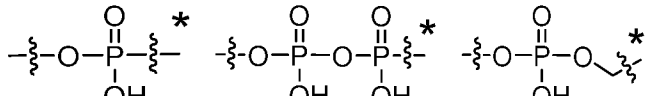
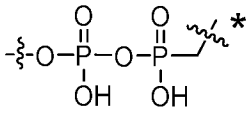
, wherein: $-*$ is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or $-OC(=O)-*$; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[65] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

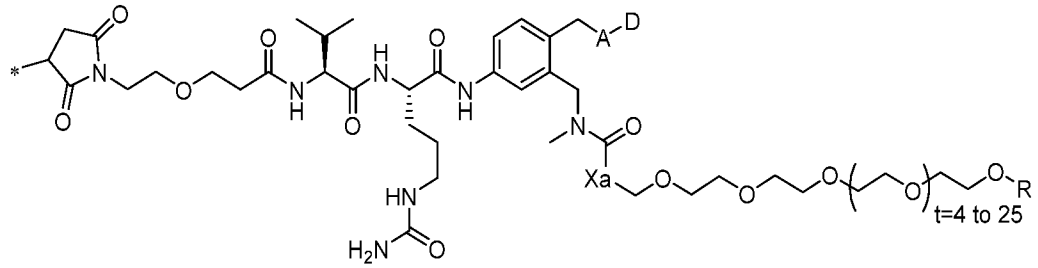


, wherein:

Xa is -CH_2-, -OCH_2-, -$NHCH_2$- or -$NRCH_2$- and each R independently is H, -CH_3 or -$CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  , -OC(=O)$N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -OC(=O)$N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

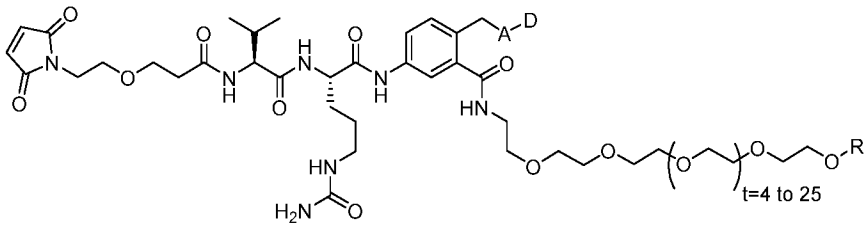
D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



, wherein:

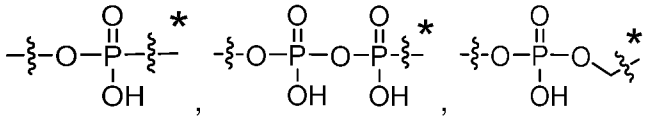
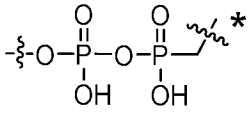
—* is a bond to the antibody; and Xa, A, D and R are as defined above. In some embodiments, Xa is -CH_2- or -$NHCH_2$-; A is a bond or -OC(=O)-*; and R is -CH_3 or -$CH_2CH_2C(=O)OH$.

[66] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

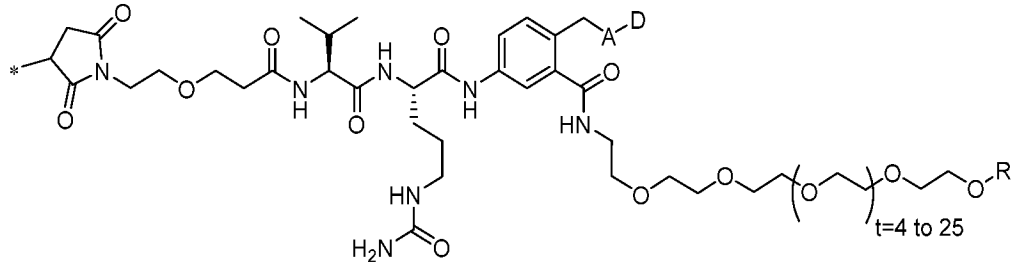


, wherein:

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$;

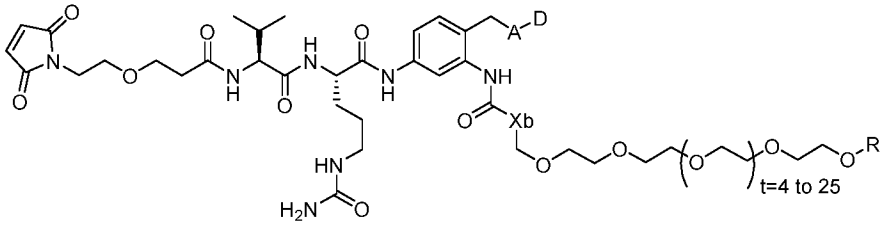
A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

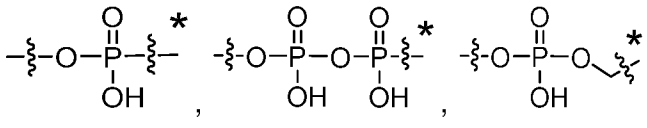
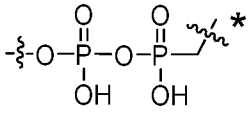
 , wherein:

—* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

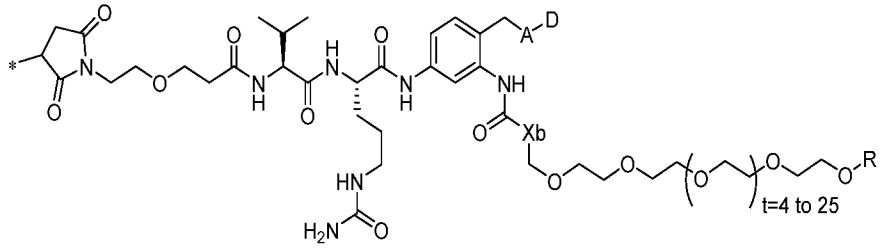
[67] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

 , wherein:

Xb is $-CH_2-$, $-OCH_2-$, $-NHCH_2-$ or $-NRCH_2-$ and each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

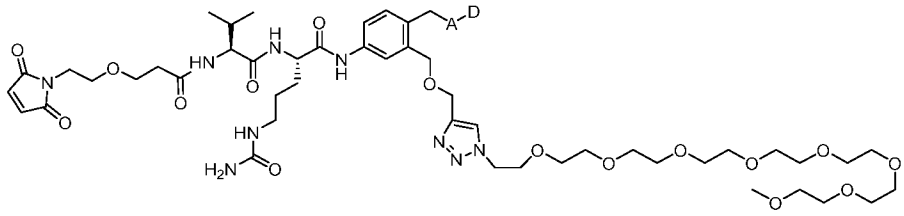
A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

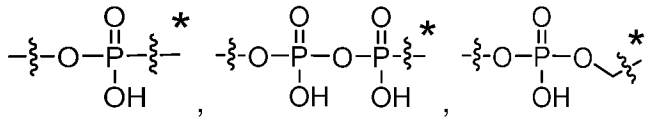
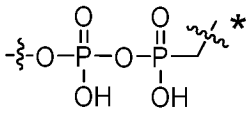


, wherein: —* is a bond to the antibody; and Xb, A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

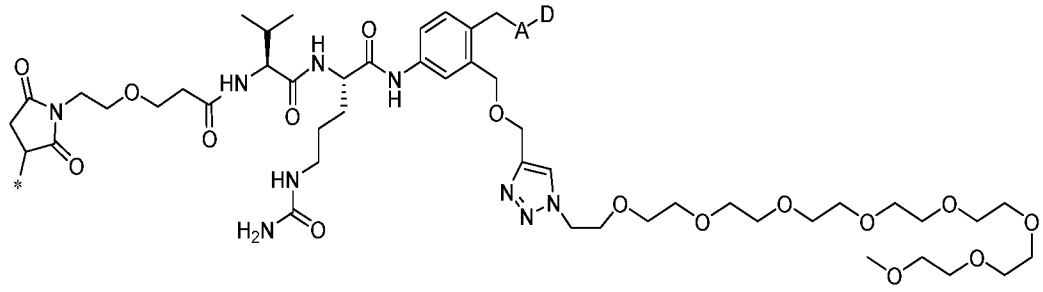
[68] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

A is a bond, -OC(=O)-*,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -$OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

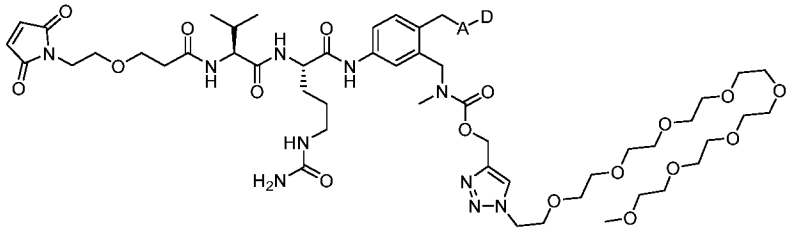
D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



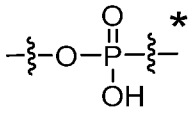
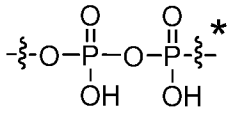
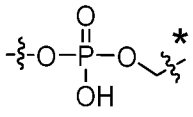
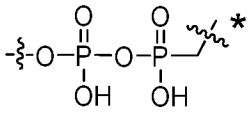
, wherein:

—* is a bond to the antibody; and A and are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

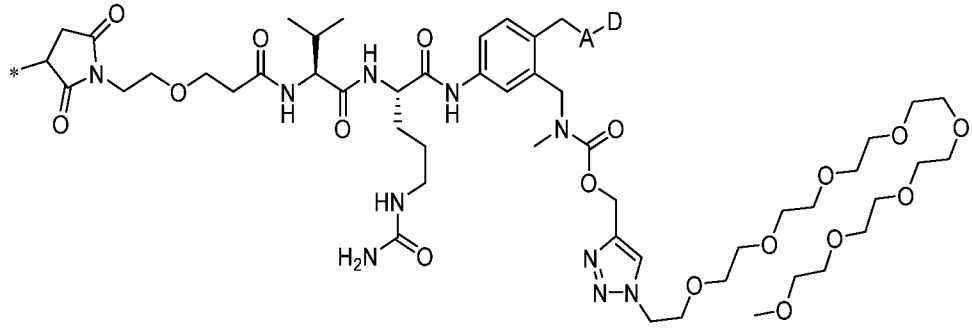
[69] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

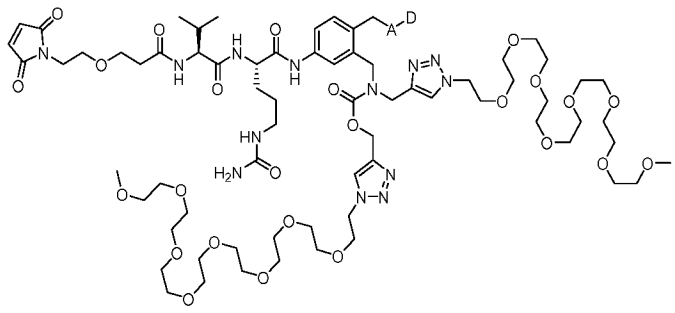
A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

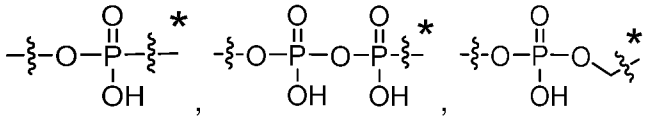
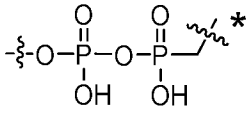


, wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

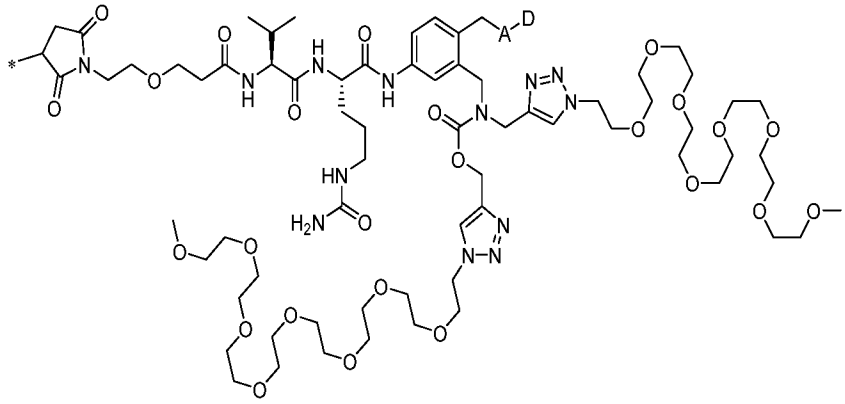
[70] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



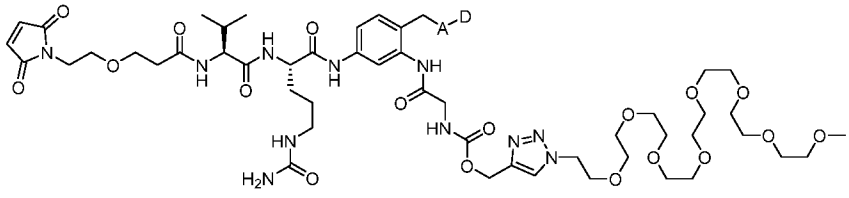
, wherein:

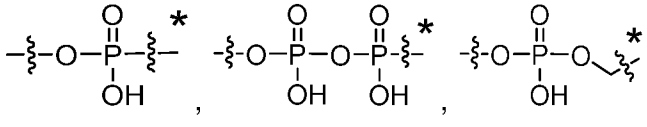
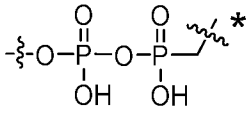
A is a bond, -OC(=O)-*,   , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

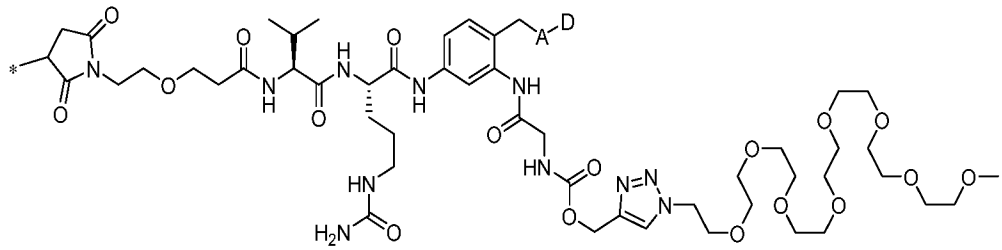
 , wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

[71] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:

 , wherein:

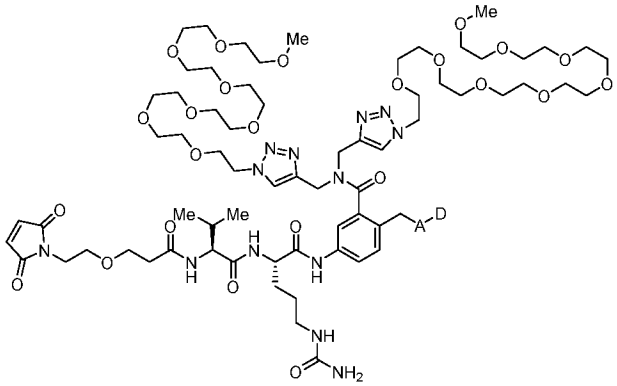
A is a bond, -OC(=O)-*,   , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

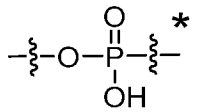
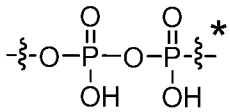
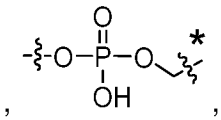
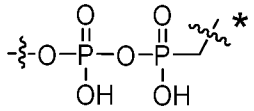


, wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

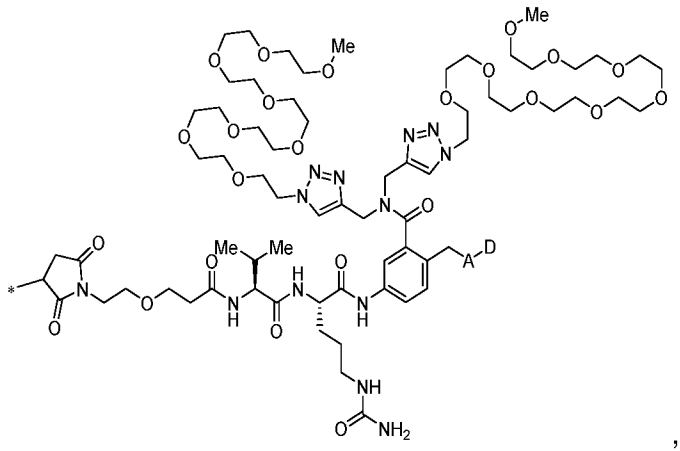
[72] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

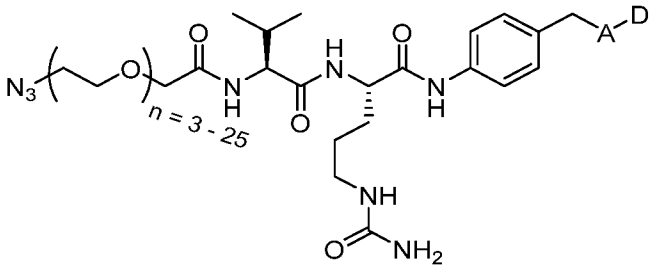
A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

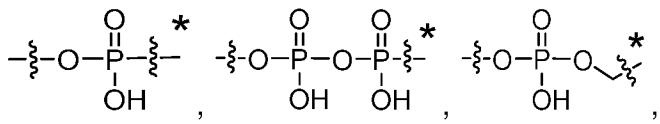
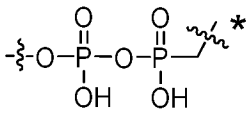


, wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

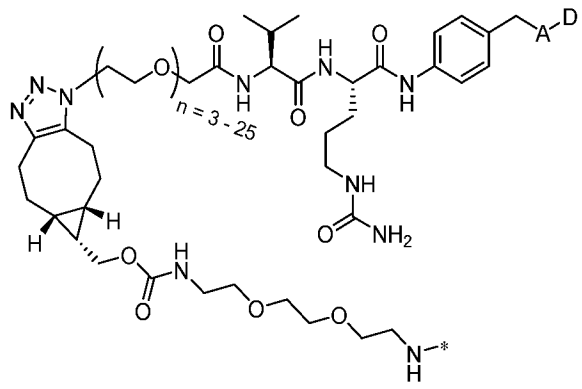
[73] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

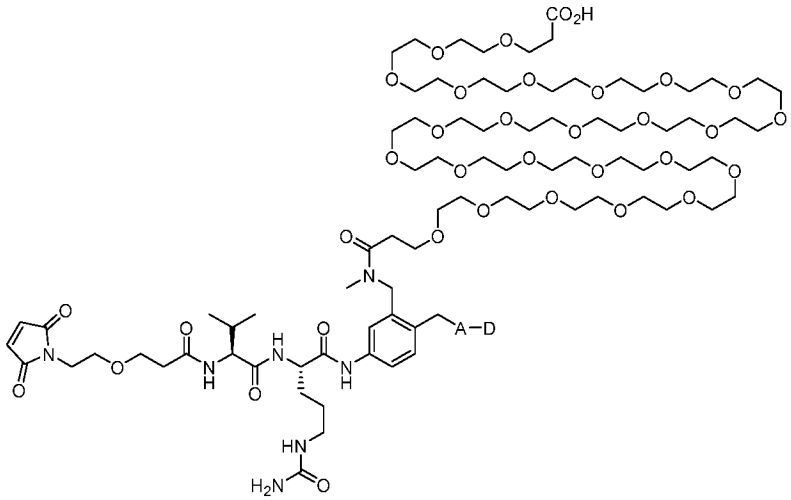
A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

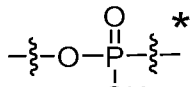
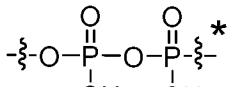
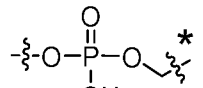
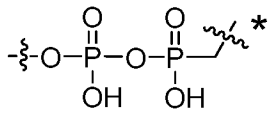


, wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

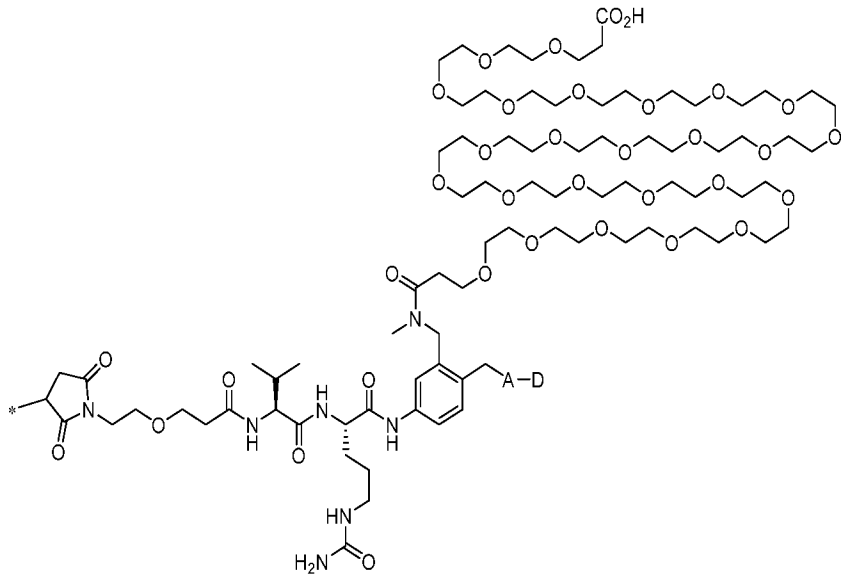
[74] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

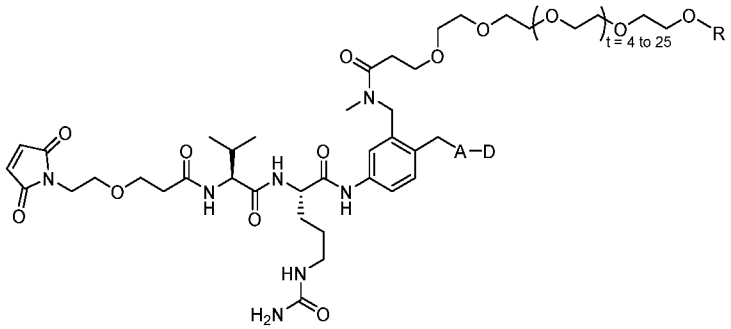
A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



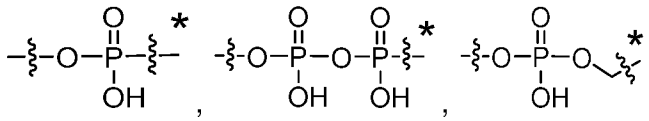
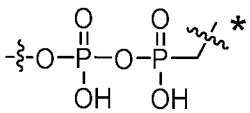
, wherein: —* is a bond to the antibody; and A and D are as defined above. In some embodiments, A is a bond or -OC(=O)-*.

[75] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



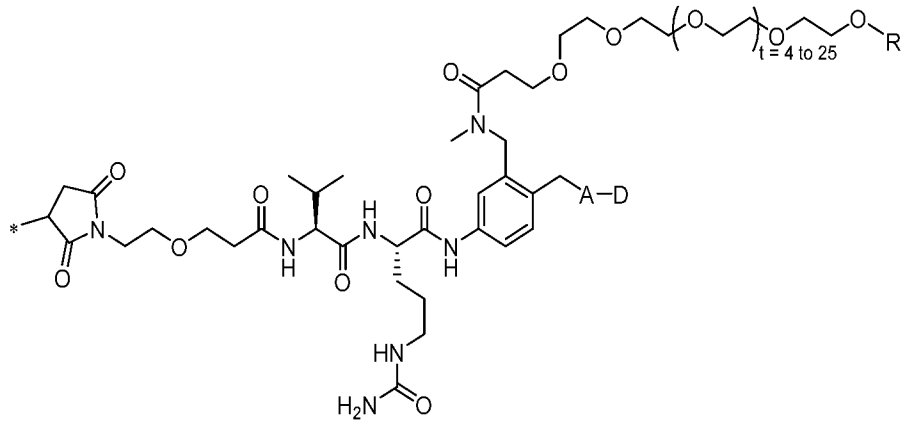
, wherein:

each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

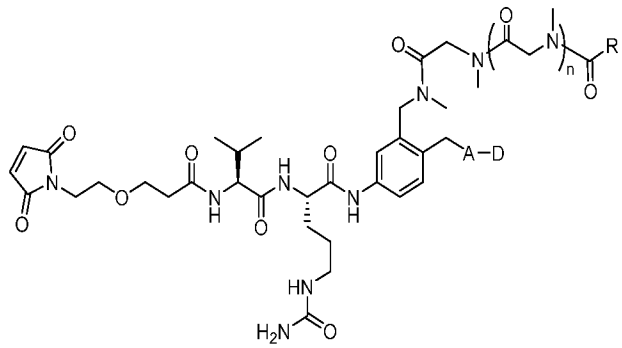
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:



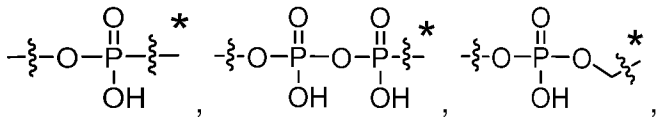
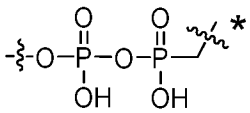
, wherein: —* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[76] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



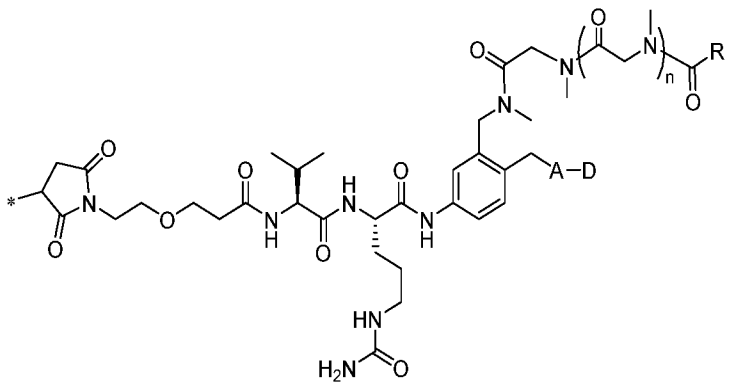
,wherein:

each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  , -OC(=O)$N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -OC(=O)$N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*,

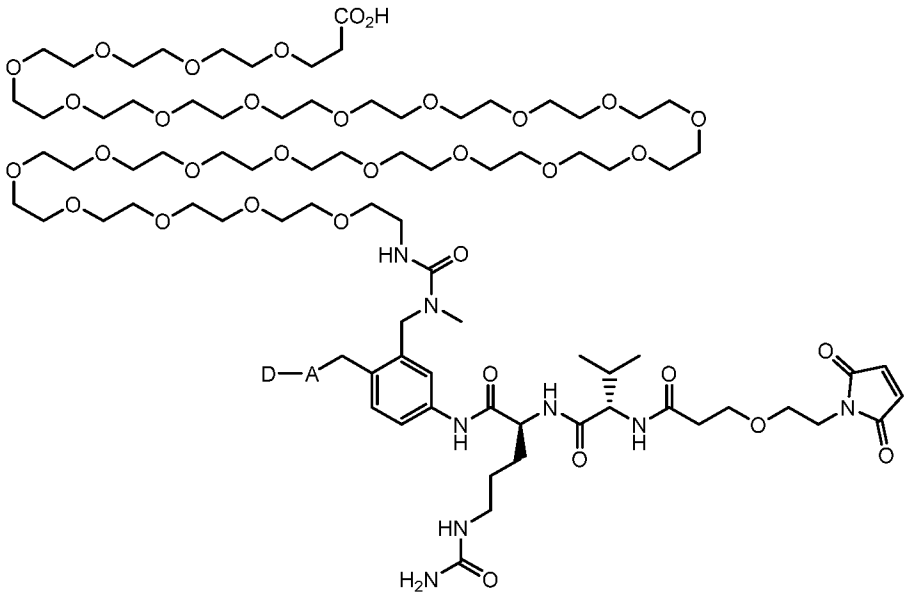
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor. In some embodiments, the linker-drug group -(L-D) comprises the following formula:

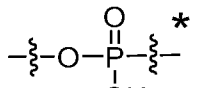
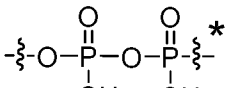
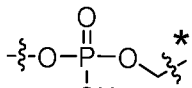
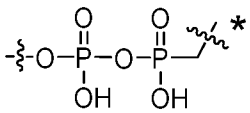


, wherein: —* is a bond to the antibody; and A, D and R are as defined above. In some embodiments, A is a bond or -OC(=O)-*; and R is $-CH_3$ or $-CH_2CH_2C(=O)OH$.

[77] In some embodiments, the linker-drug group -(L-D) comprises or is formed from a compound of formula:



, wherein:

A is a bond, -OC(=O)-*,  ,  ,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor.

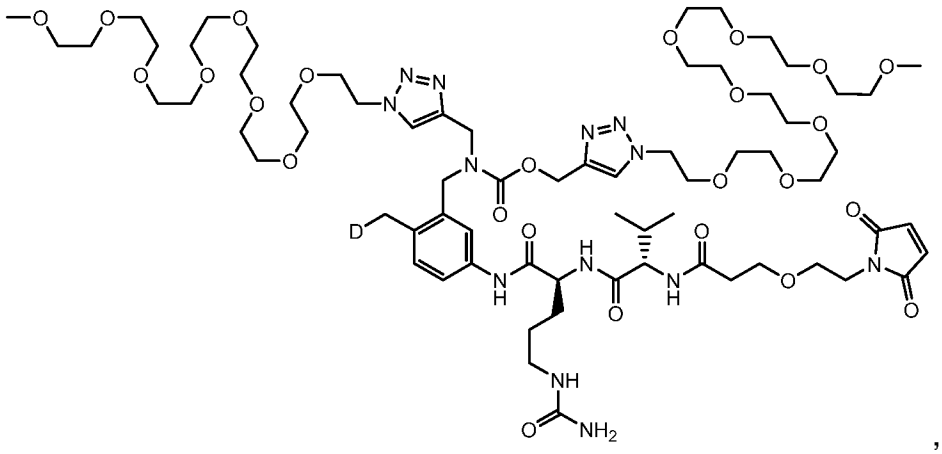
[78] In some embodiments, A is a bond.

[79] In some embodiments, A is -OC(=O)-*.

[80] In some embodiments, R is $–CH_3$.

[81] In some embodiments, R is $–CH_2CH_2COOH$.

[82] In some embodiments, the antibody-drug conjugate comprises the linker-drug group, -(L-D), which is formed from a compound selected from:



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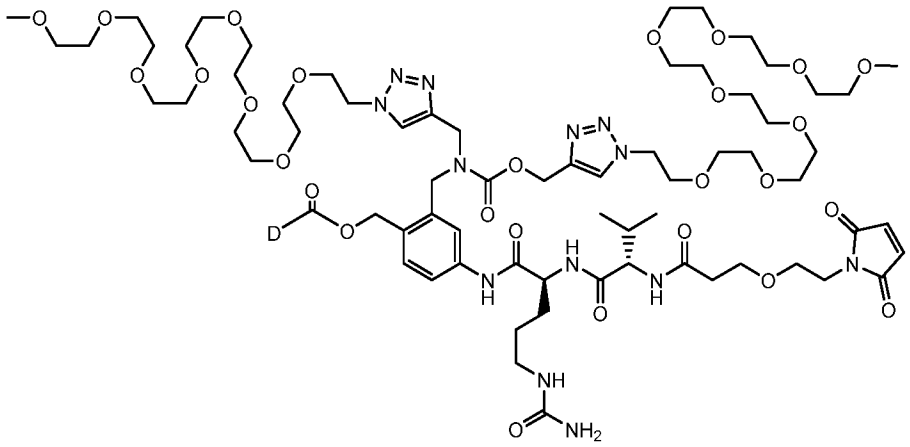

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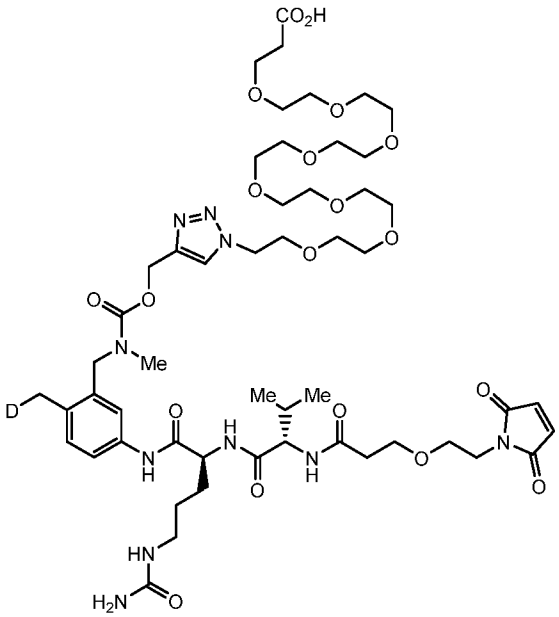

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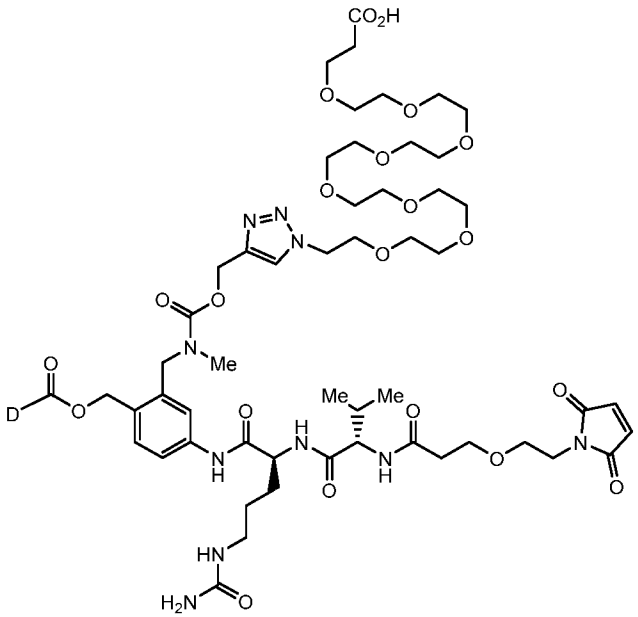

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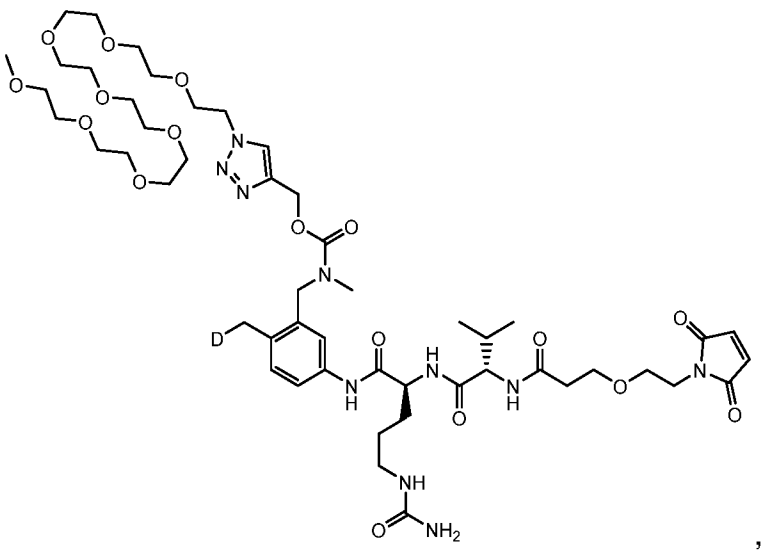

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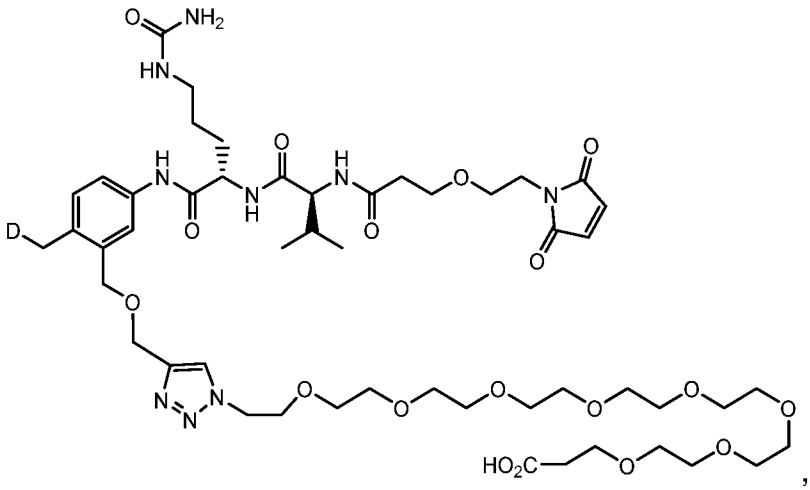

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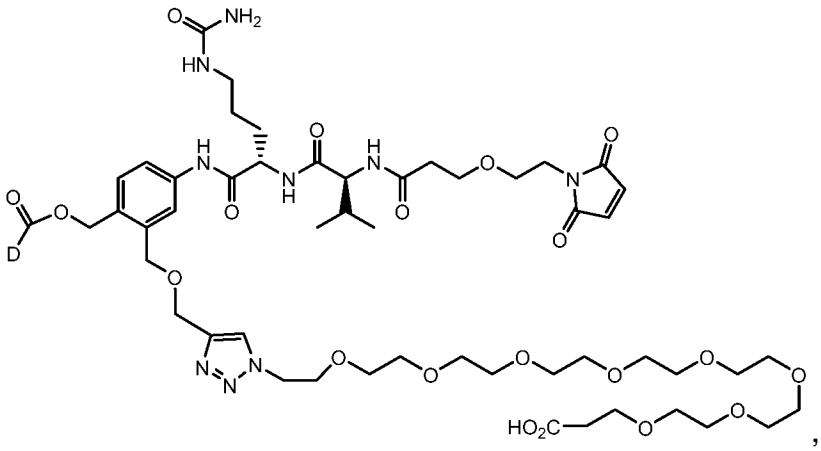

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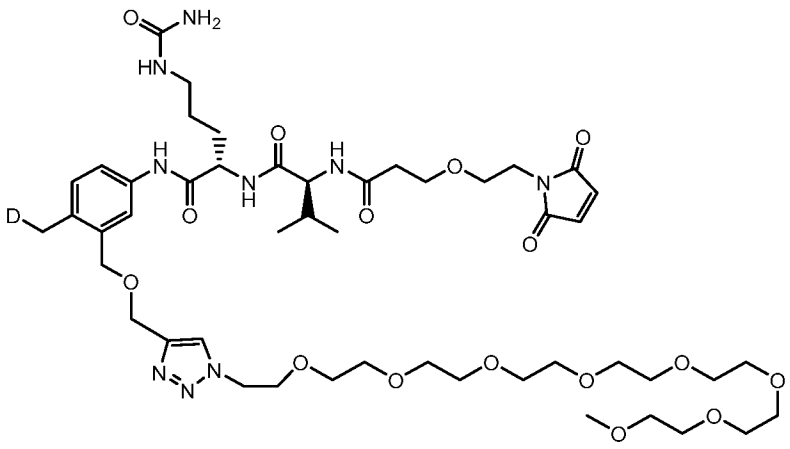

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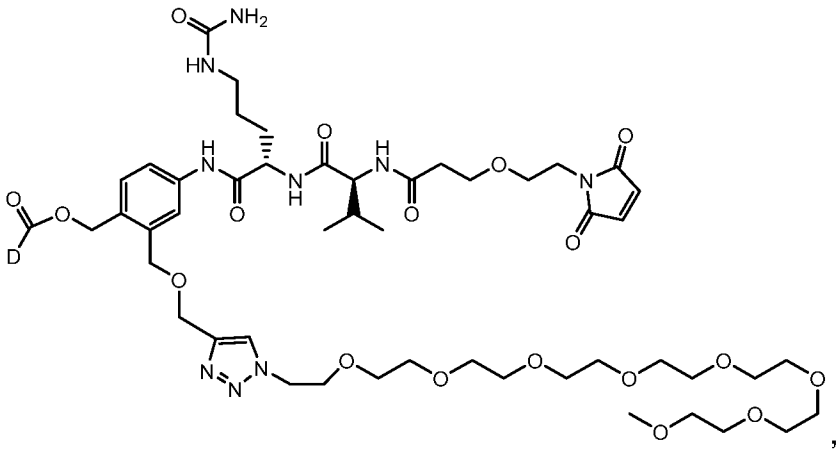

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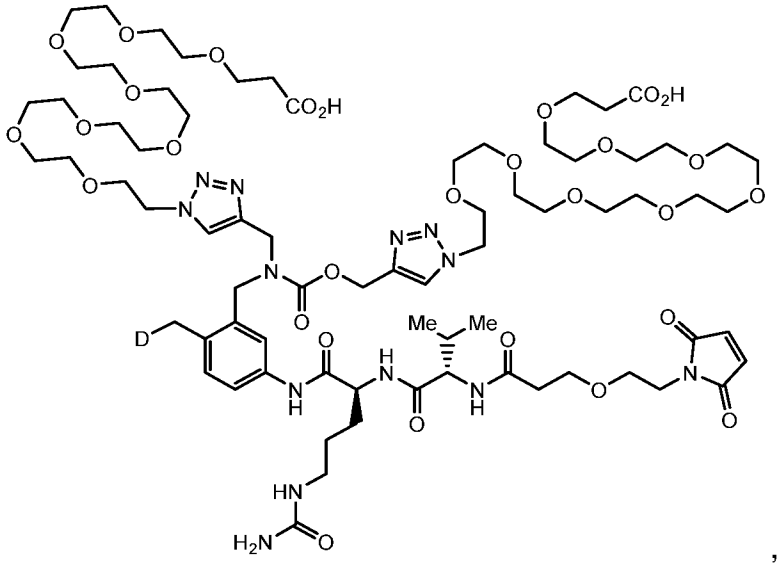

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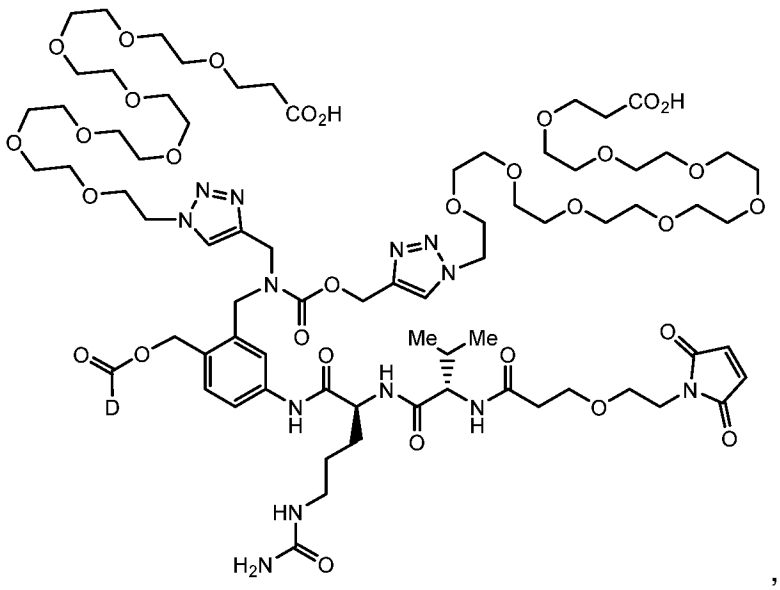

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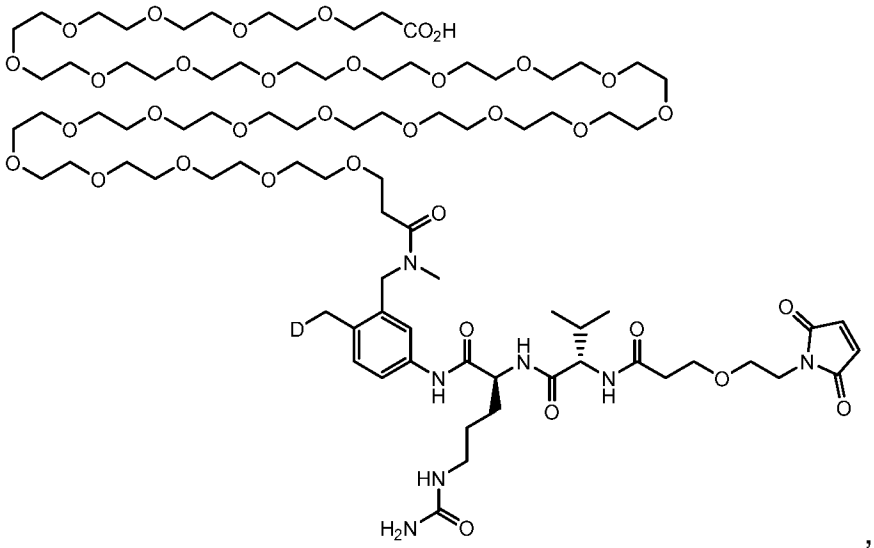

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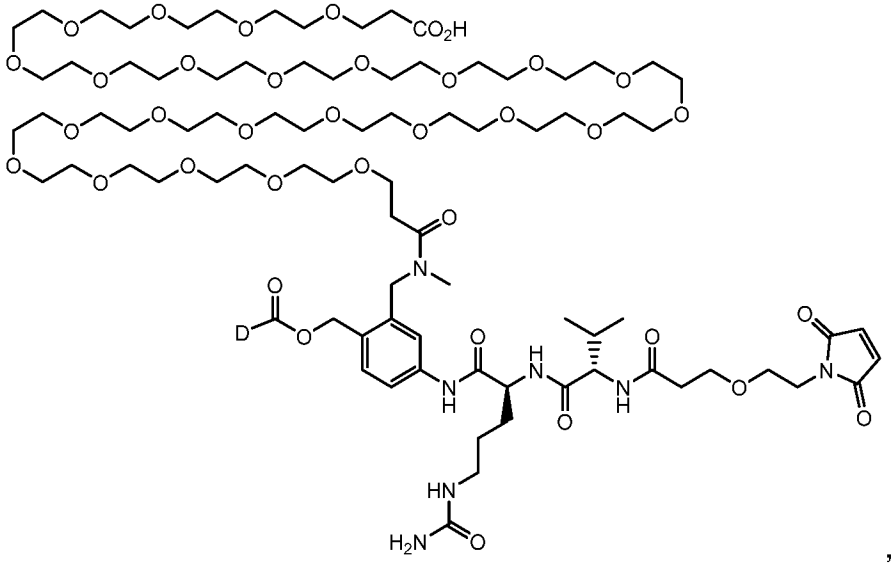

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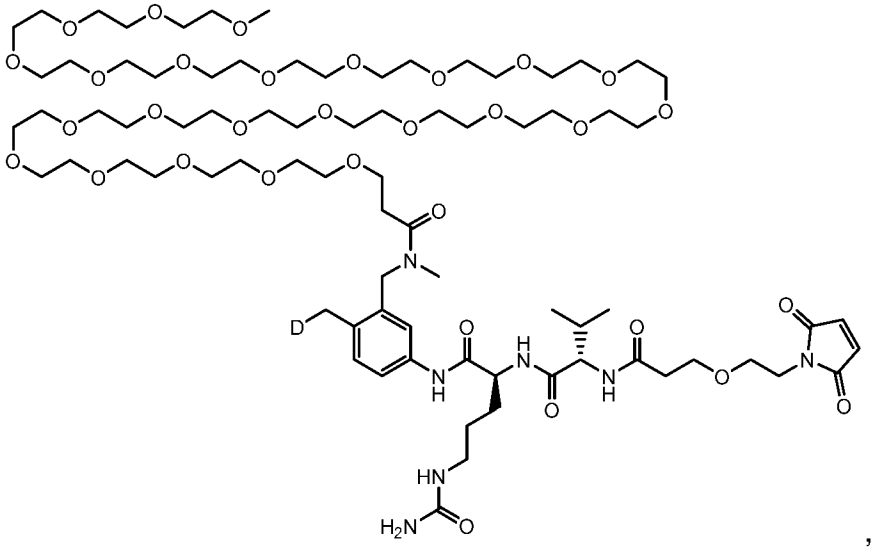

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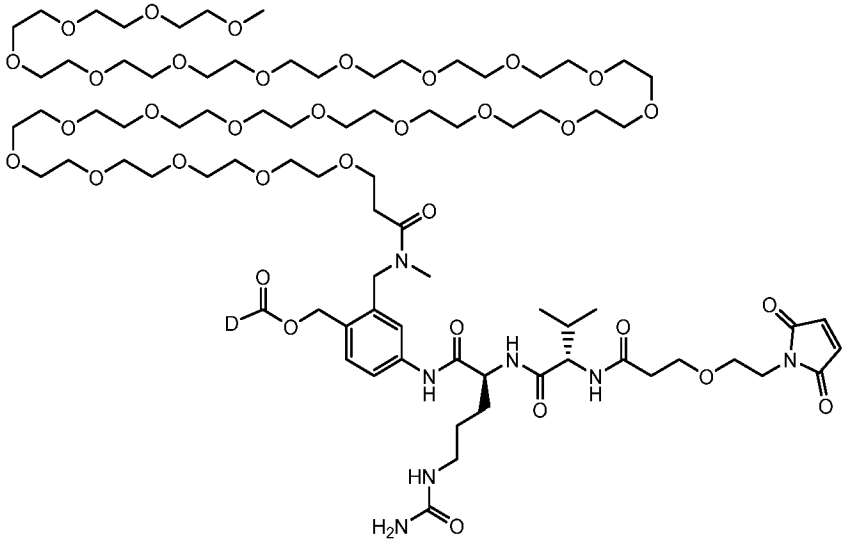

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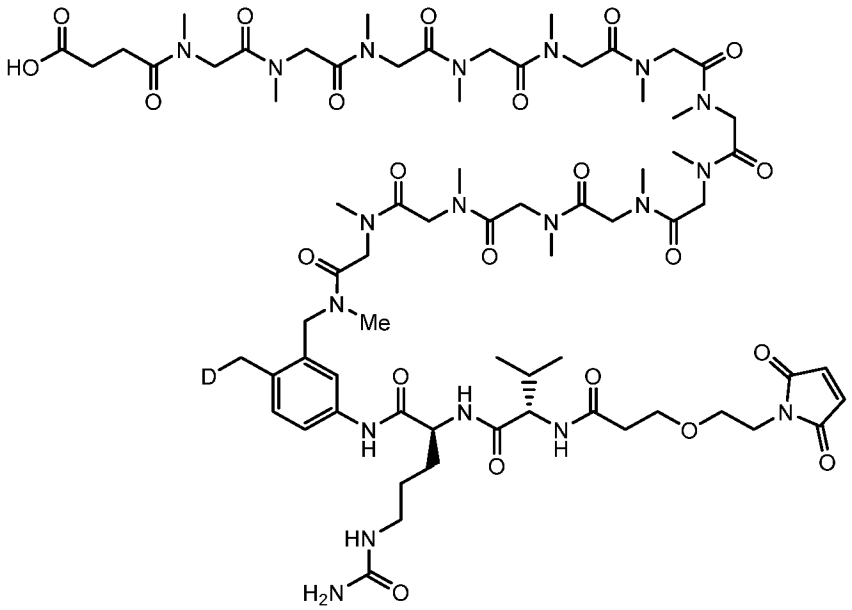

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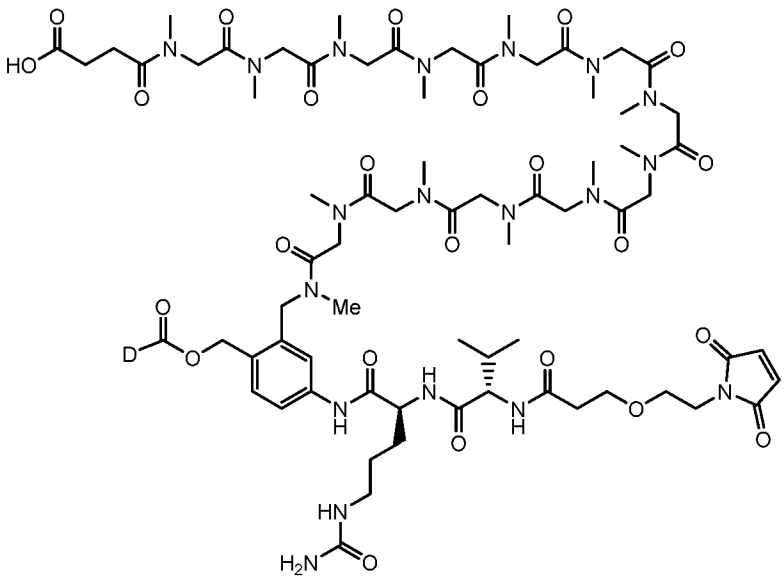

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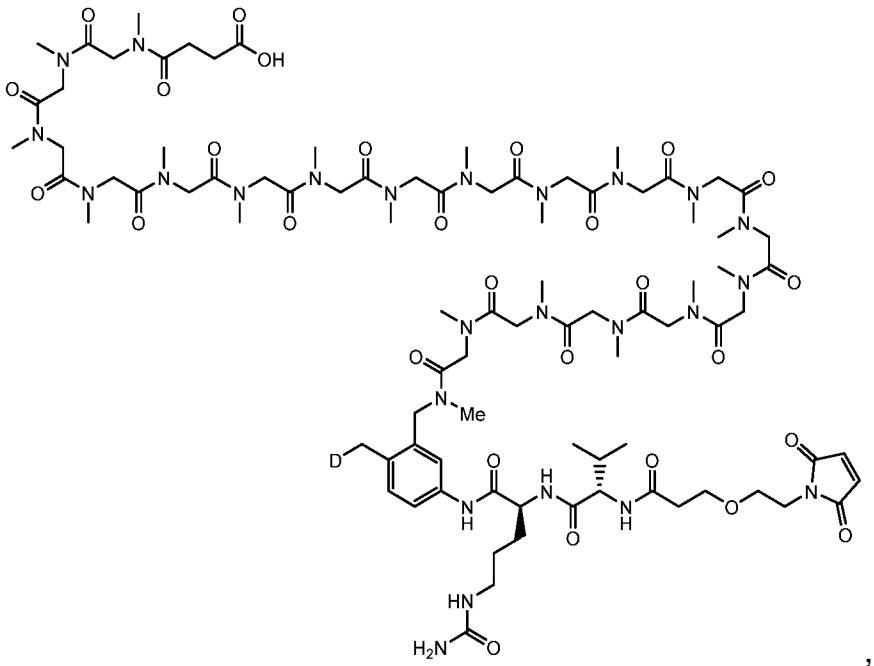

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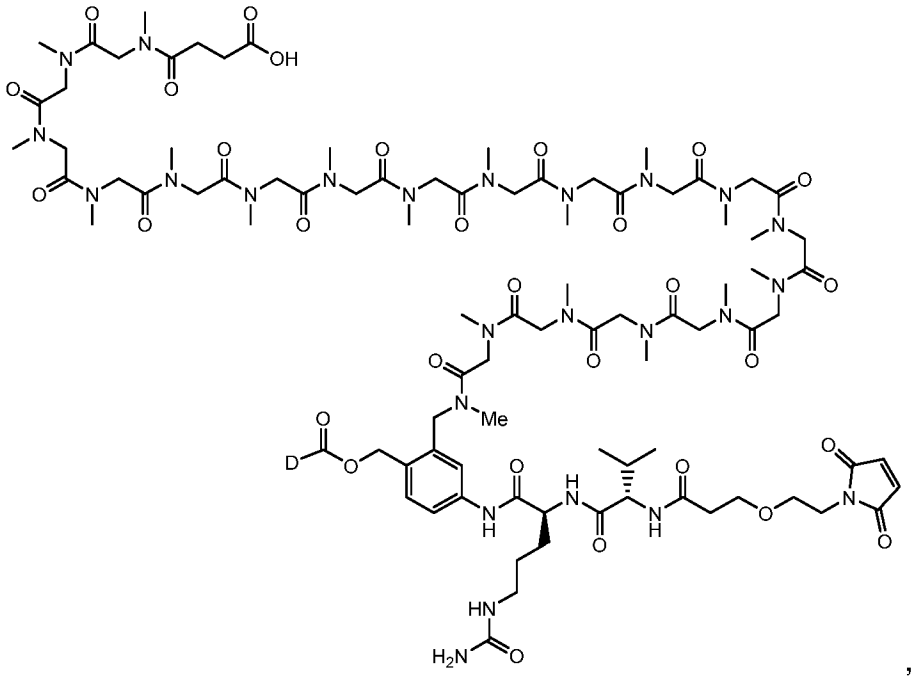

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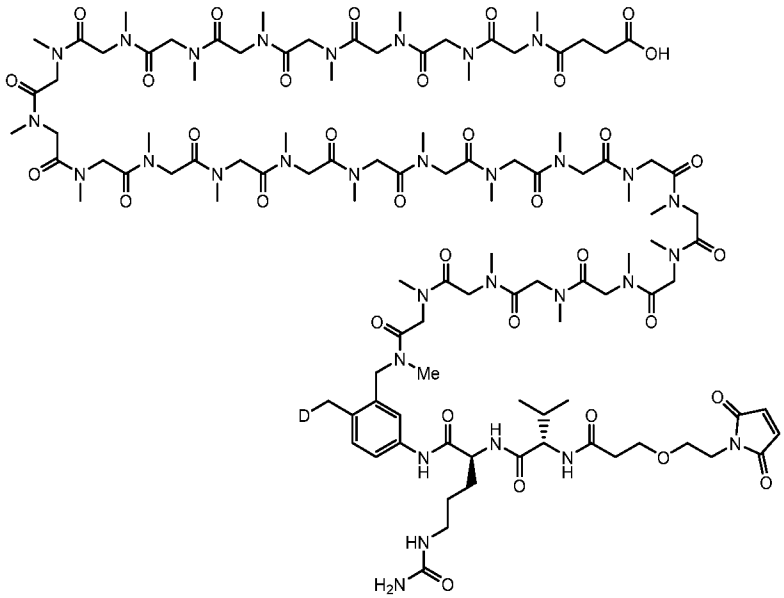

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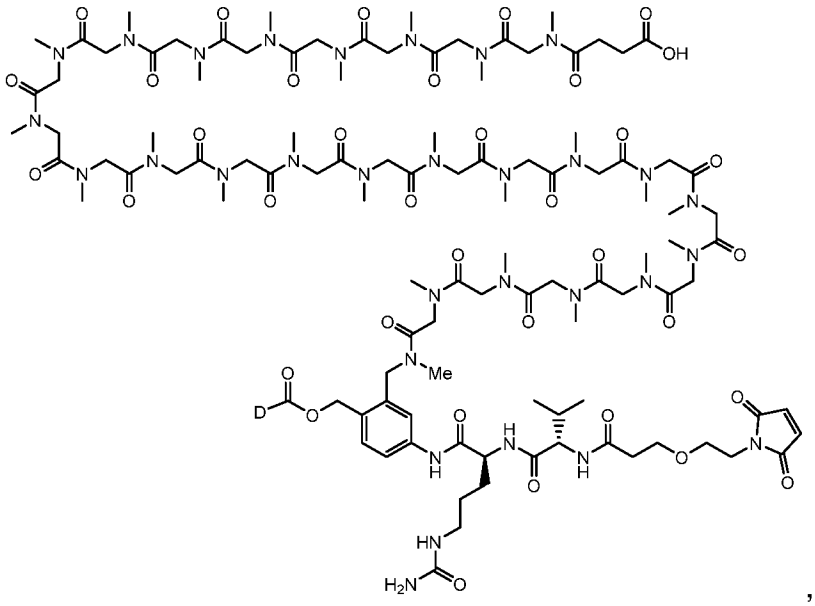

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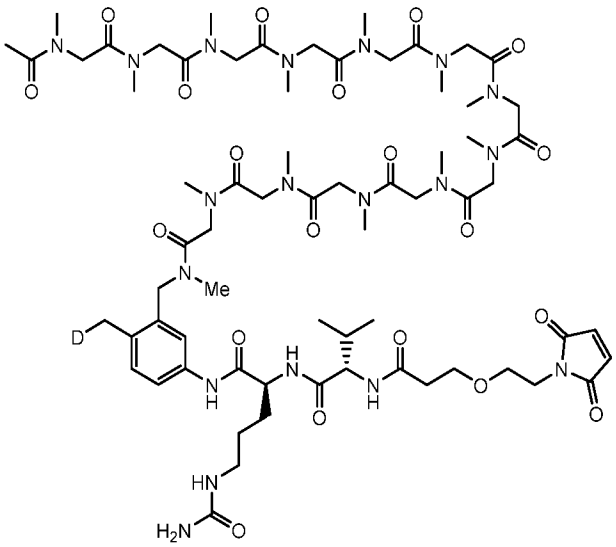

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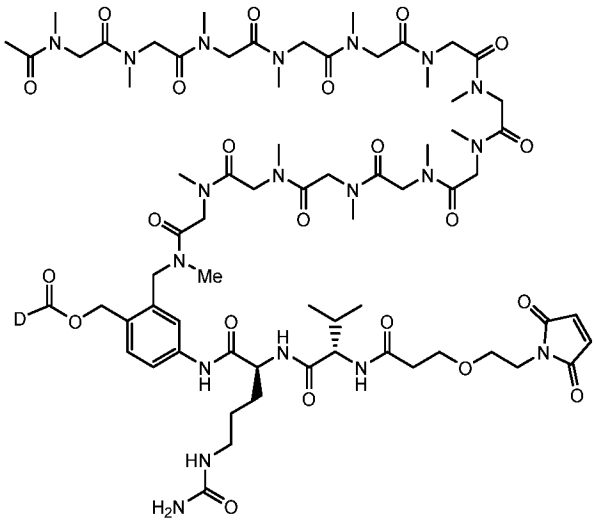

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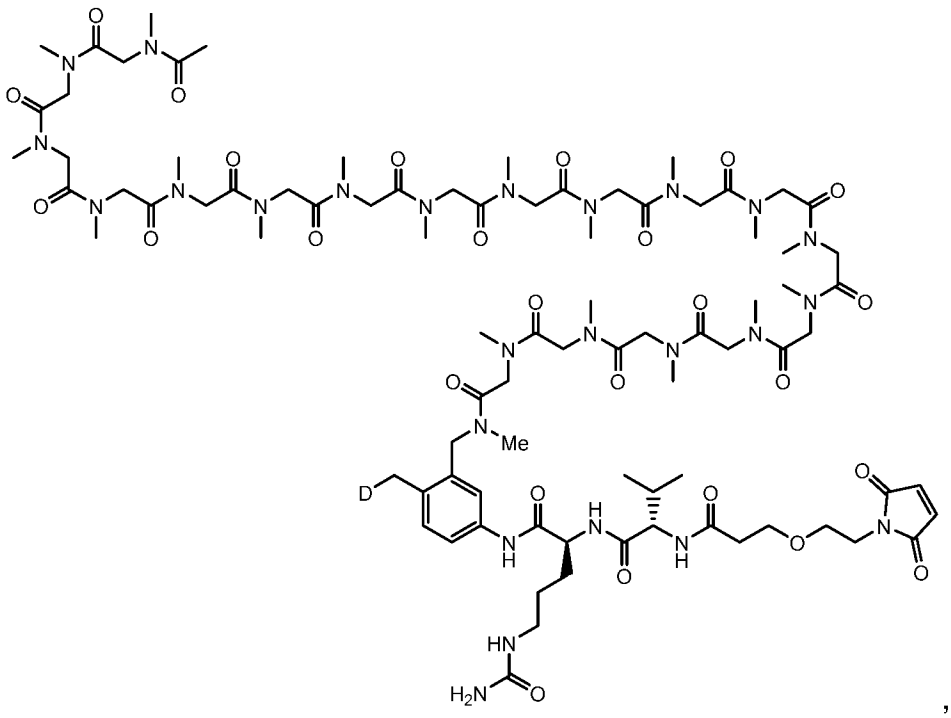

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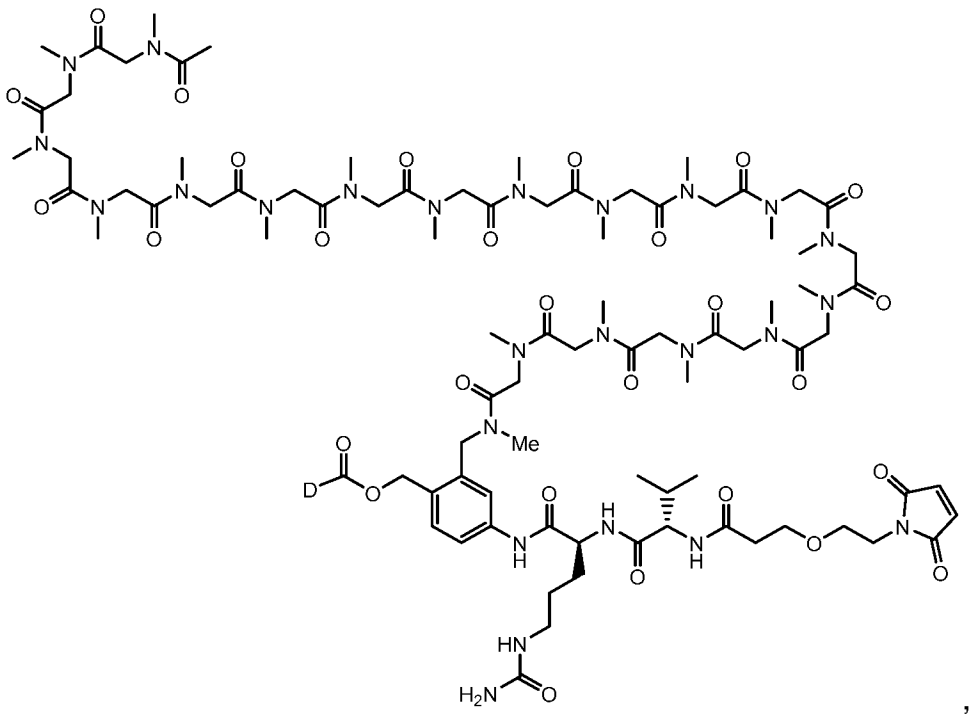

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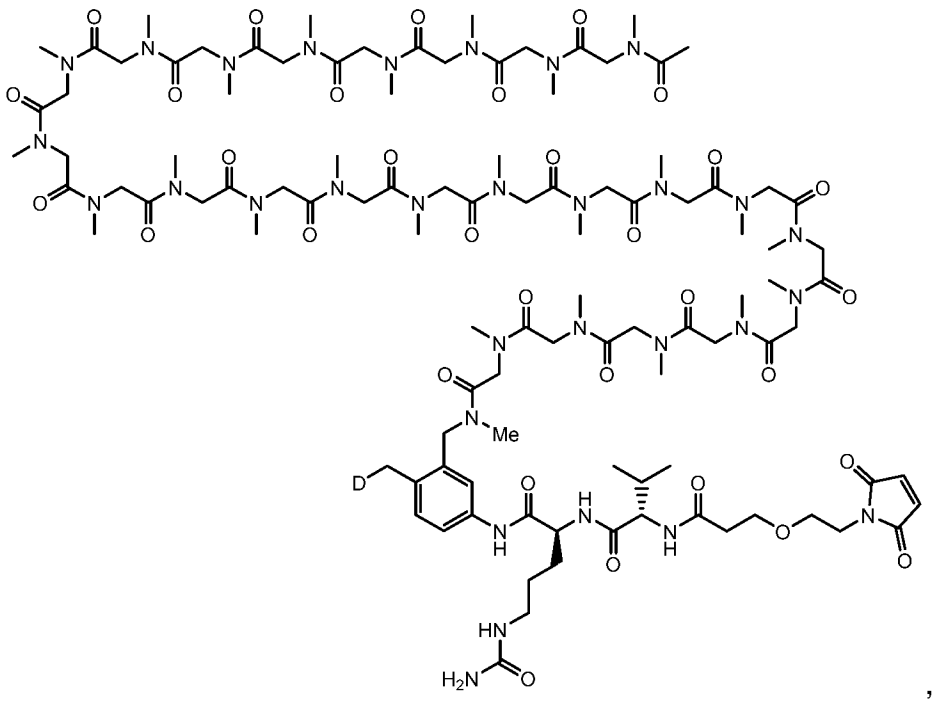

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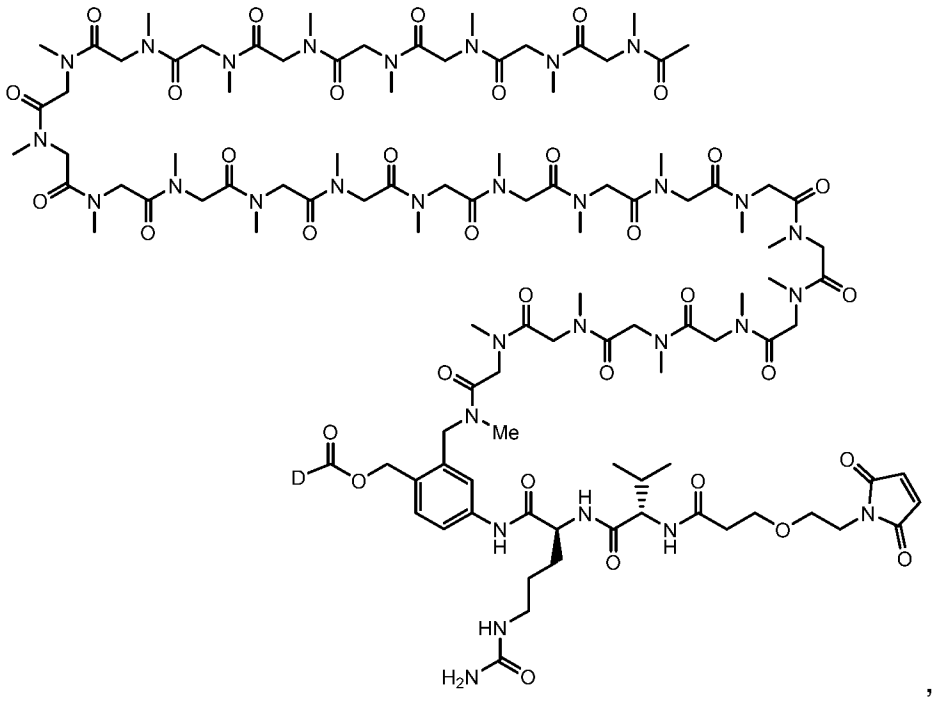

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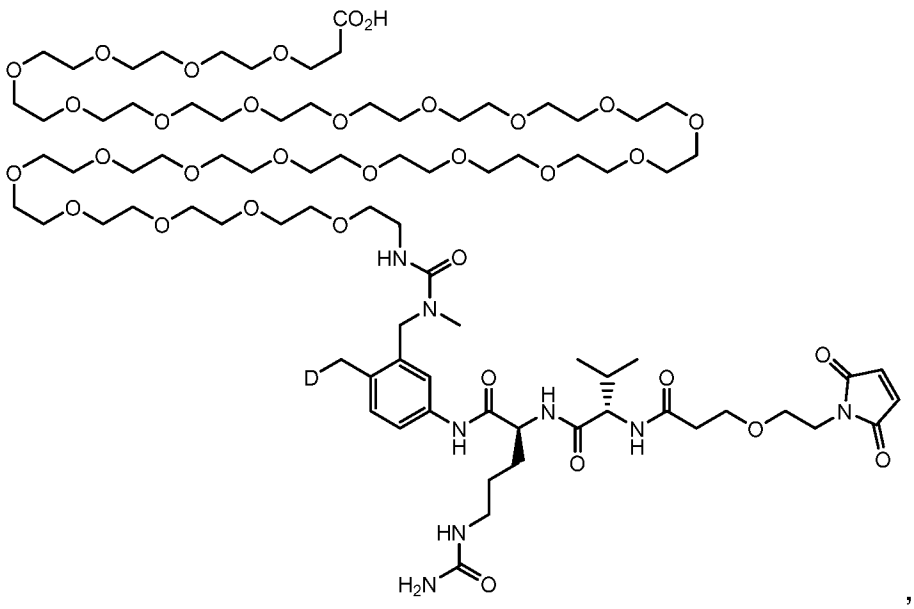

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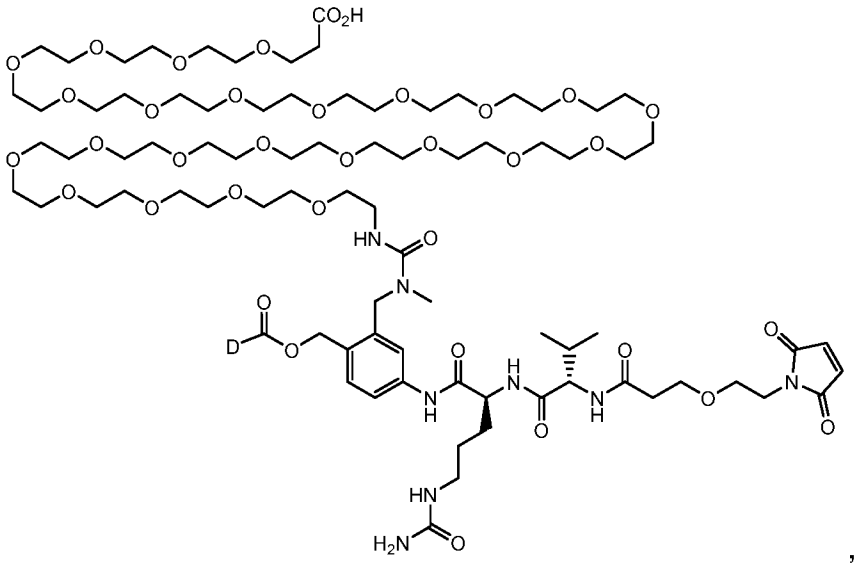

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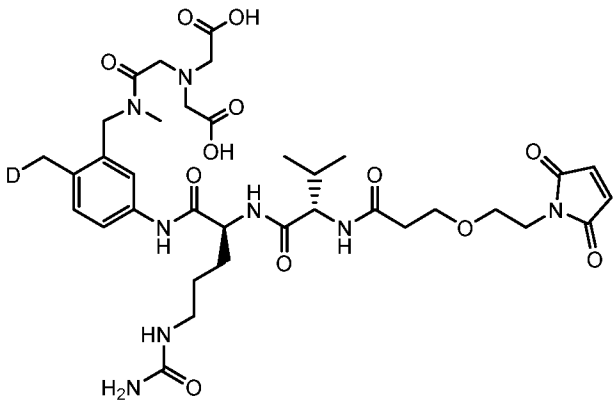

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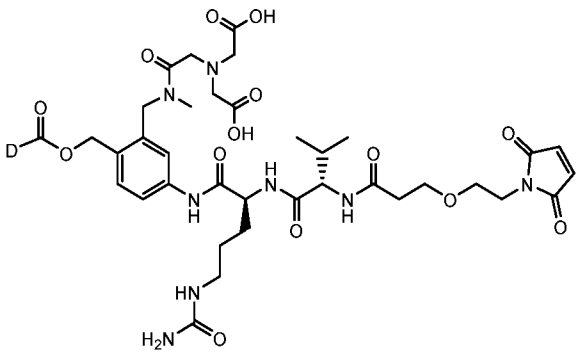

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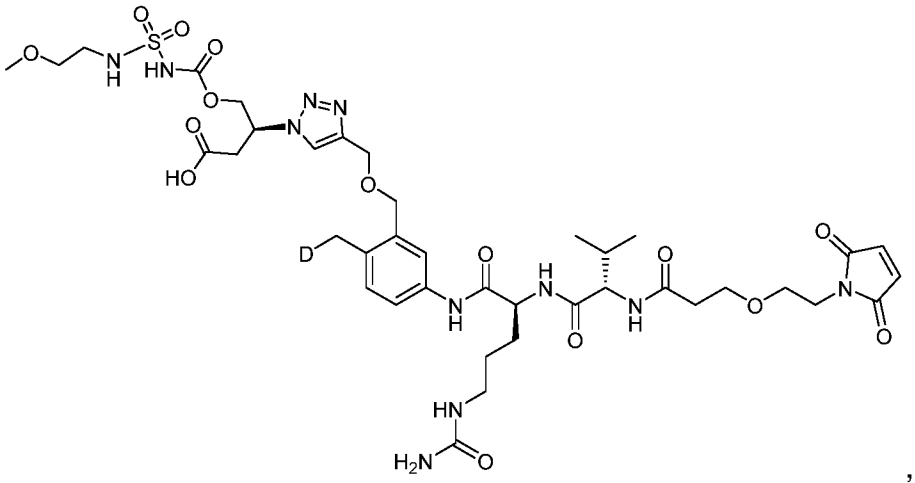

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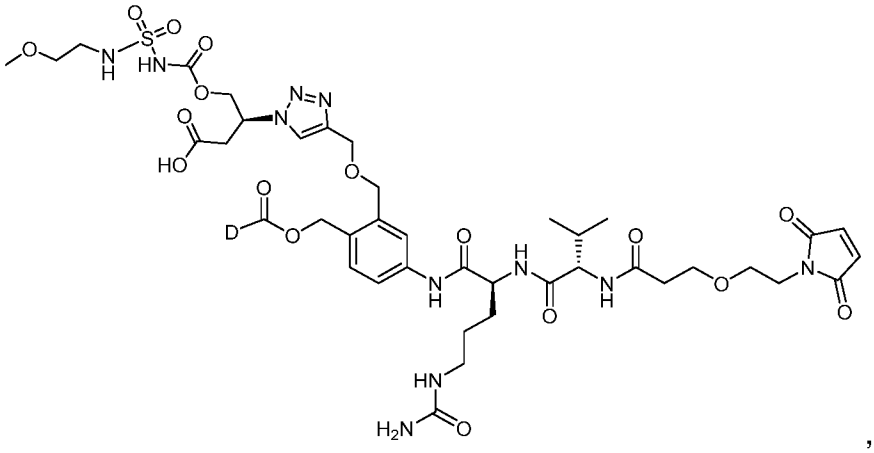

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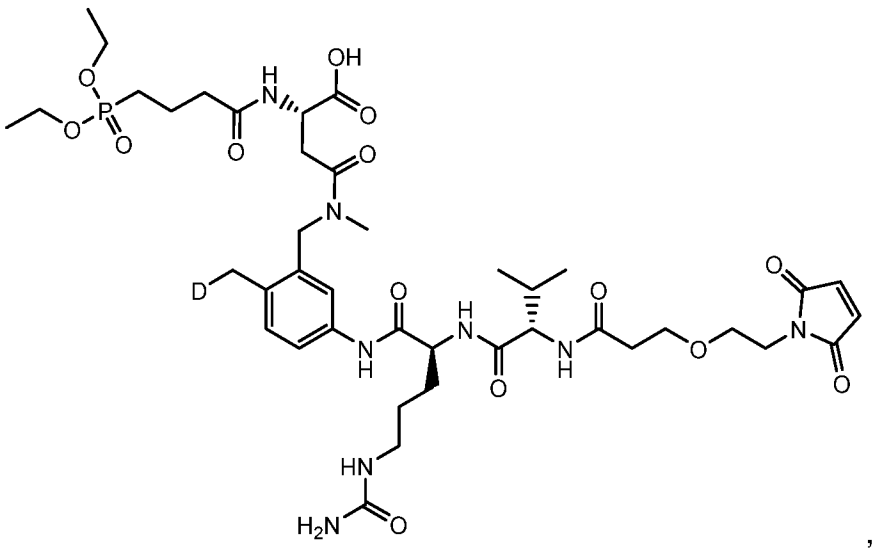

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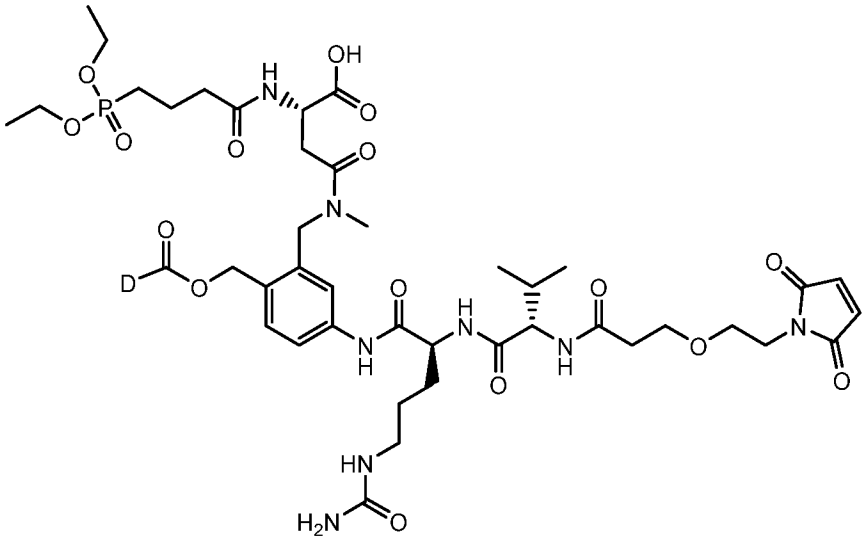

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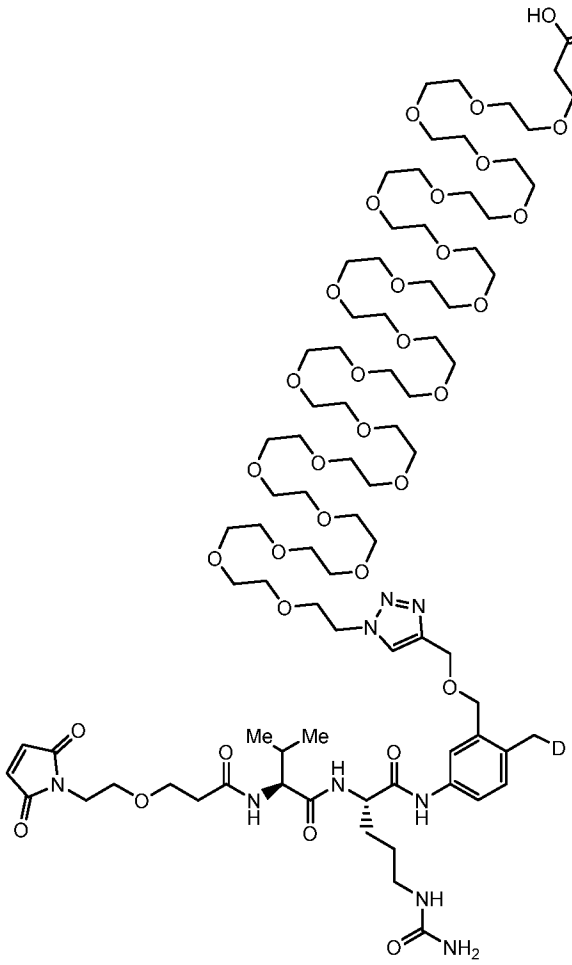

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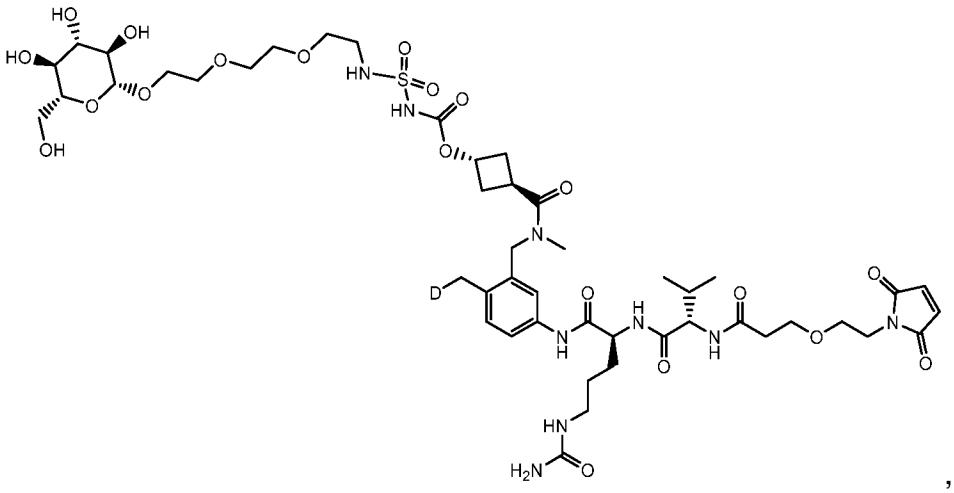

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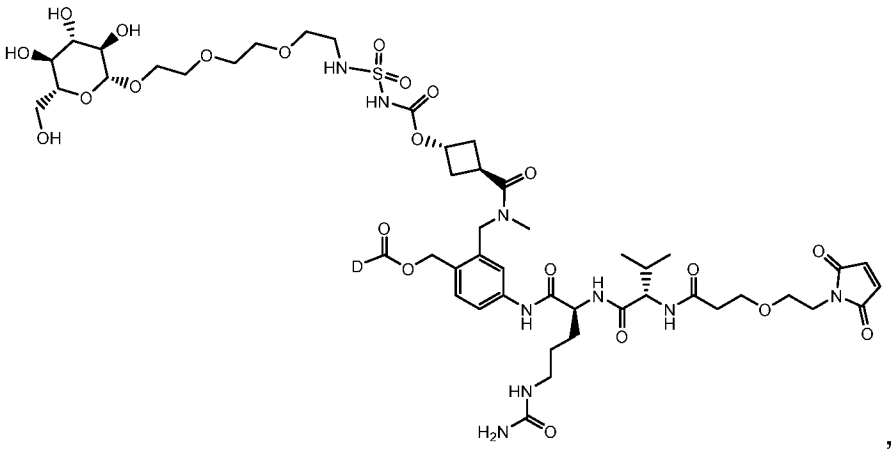

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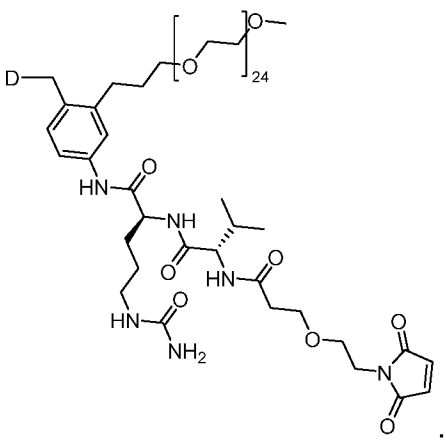

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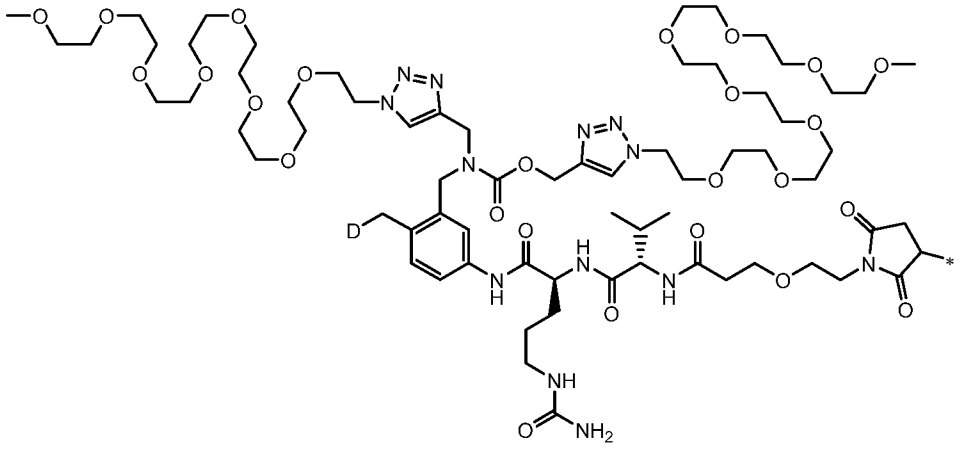
[83] In some embodiments, the antibody-drug conjugate comprises the linker-drug group, -(L-D), which comprises a formula selected from:



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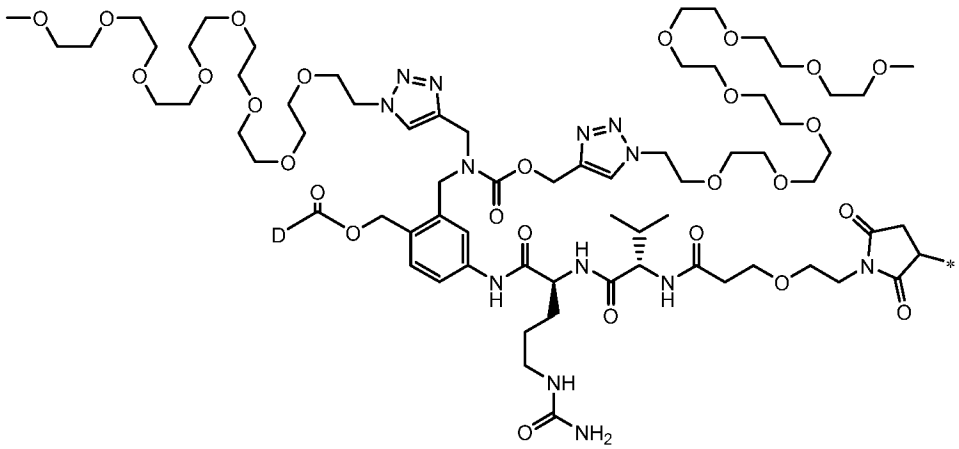

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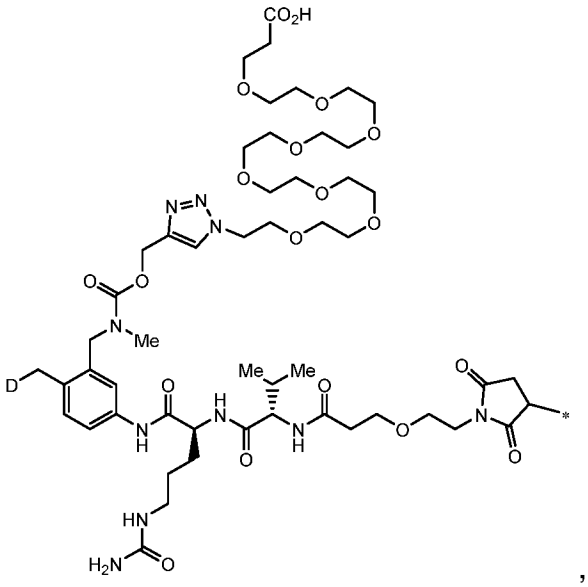

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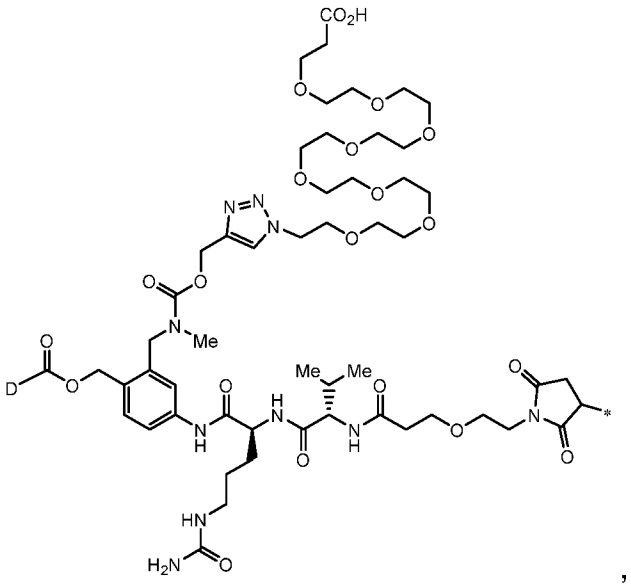

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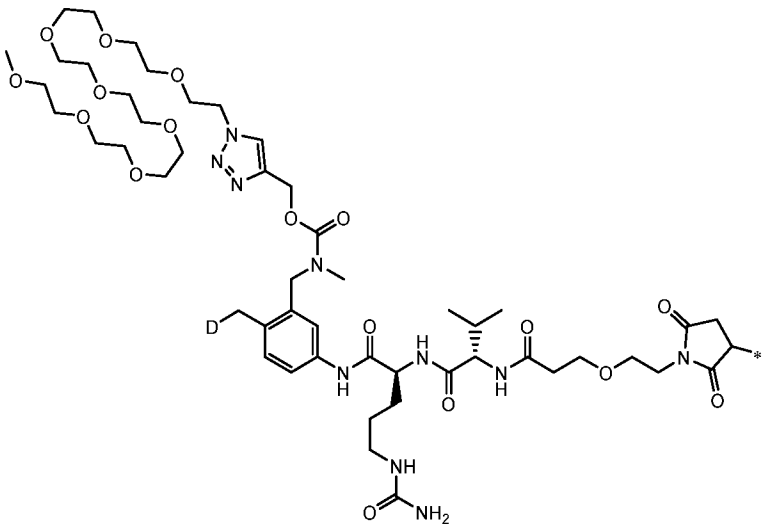

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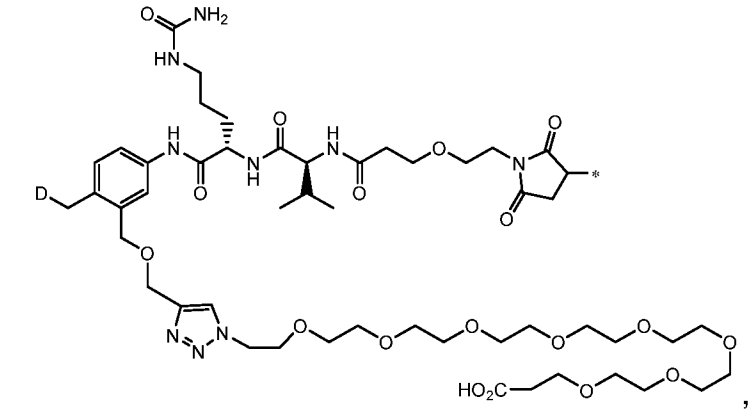

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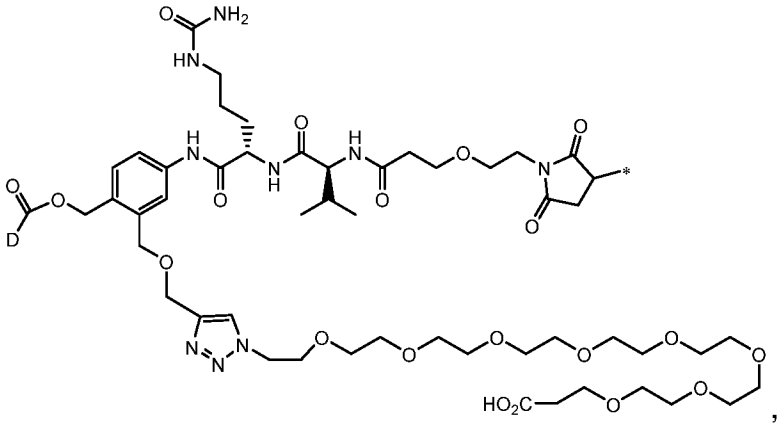

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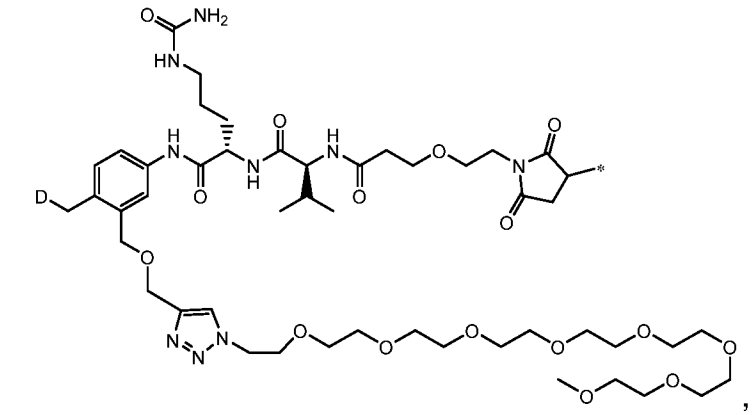

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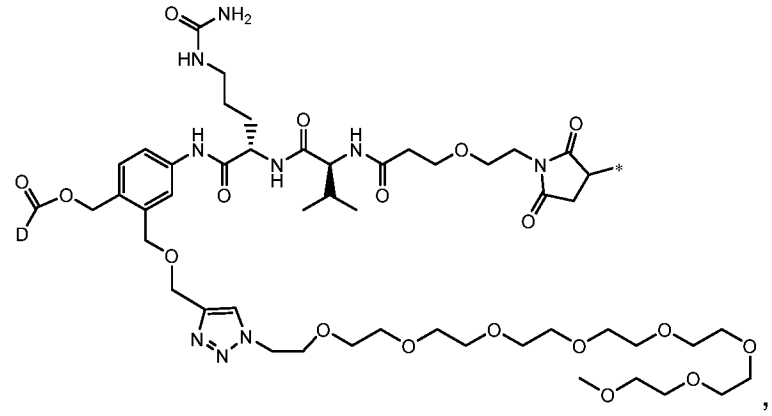

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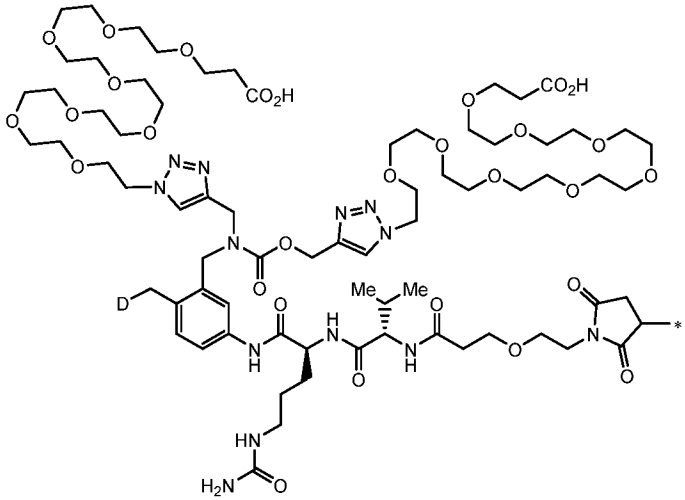

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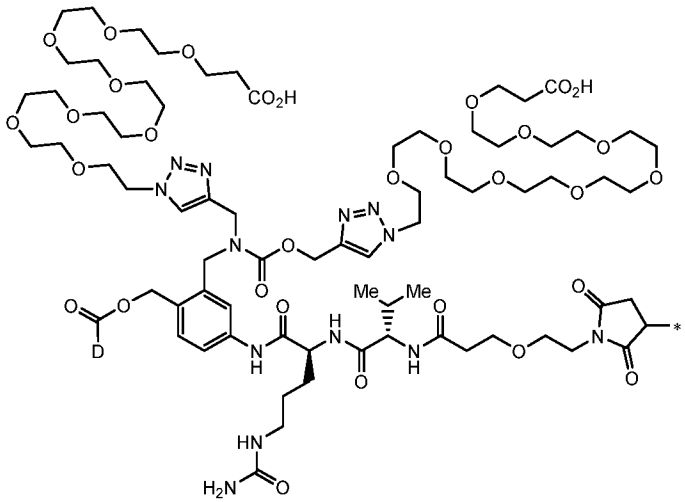

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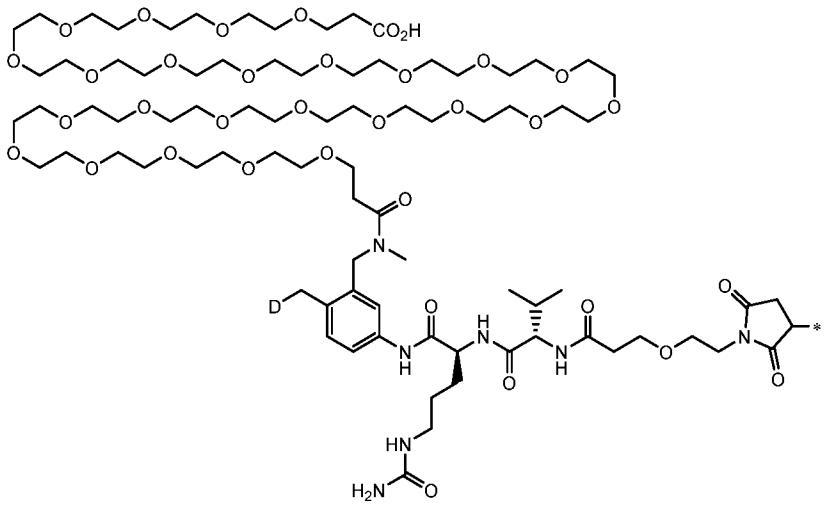

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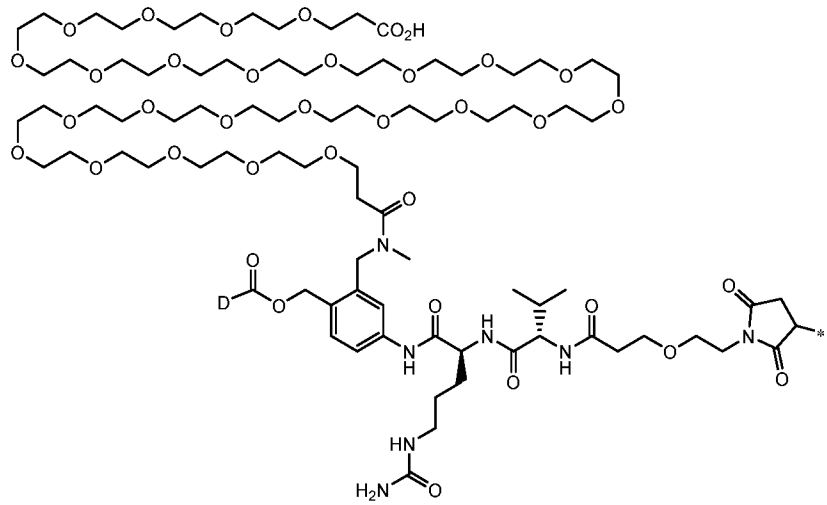

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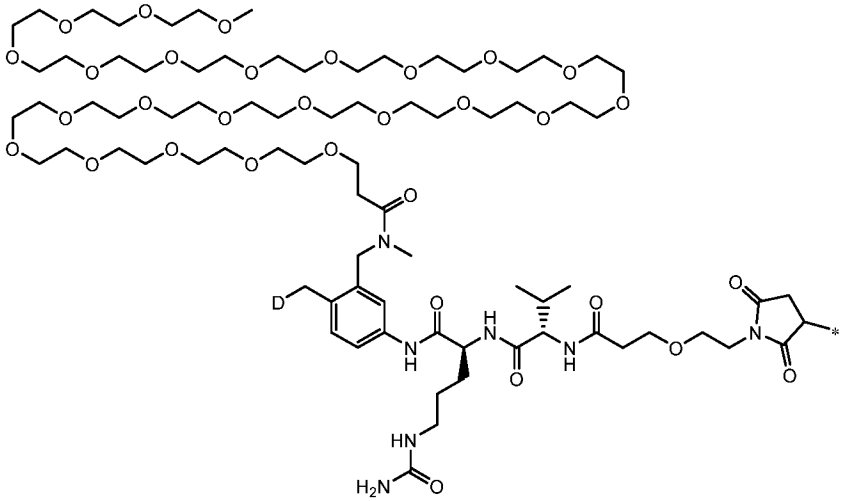

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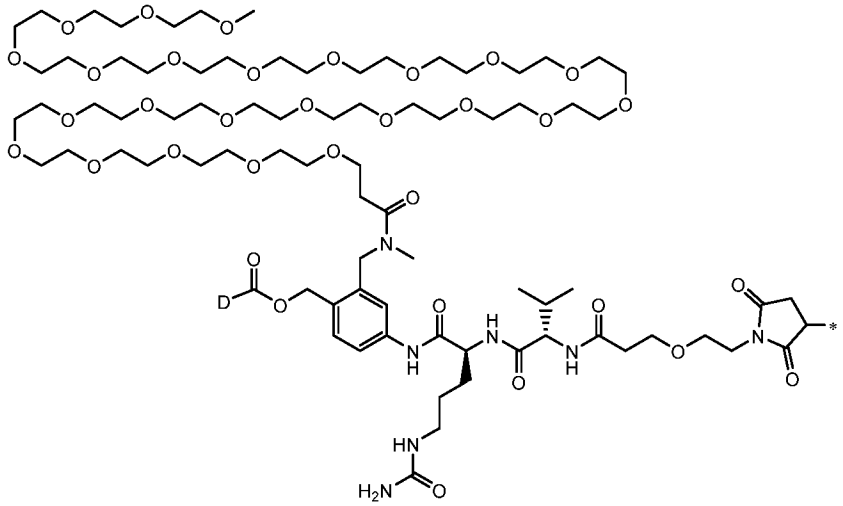

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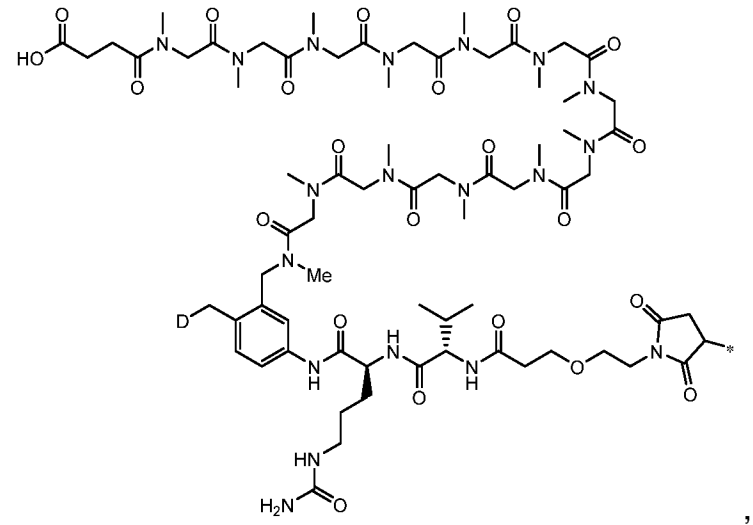

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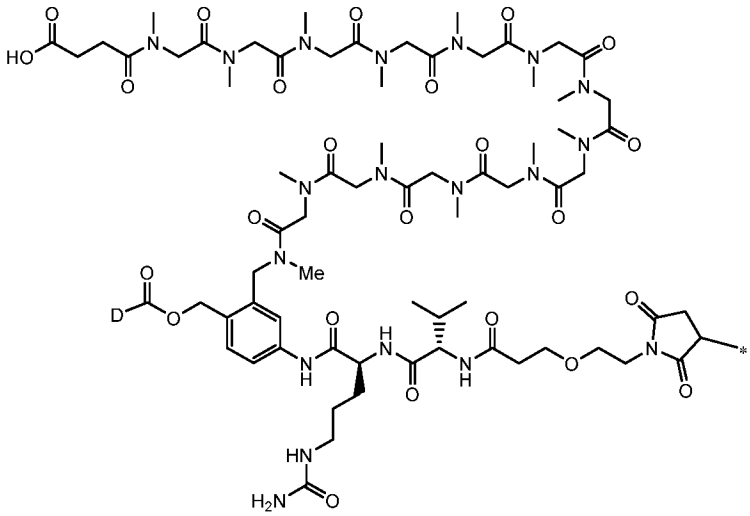

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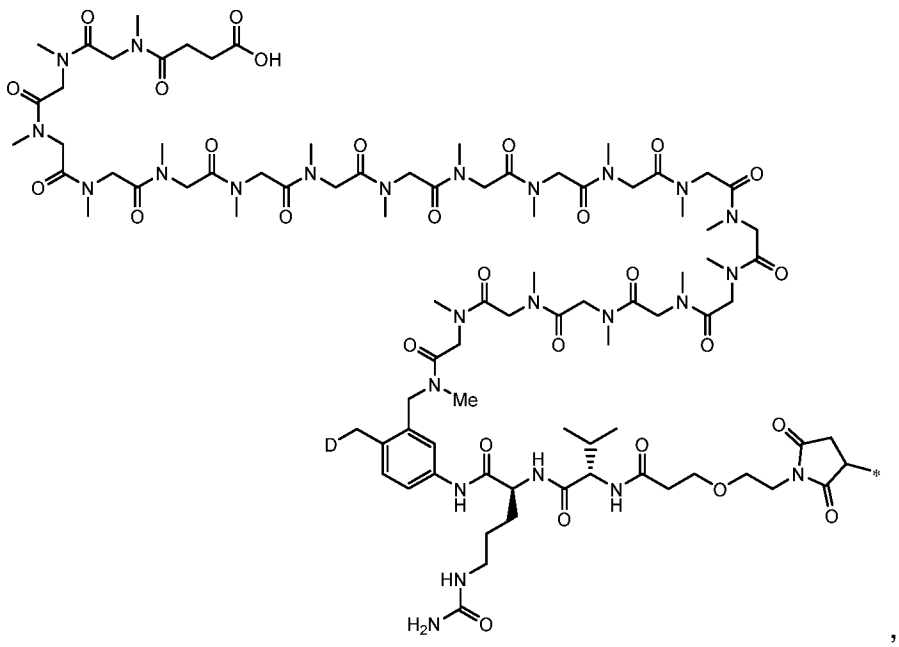

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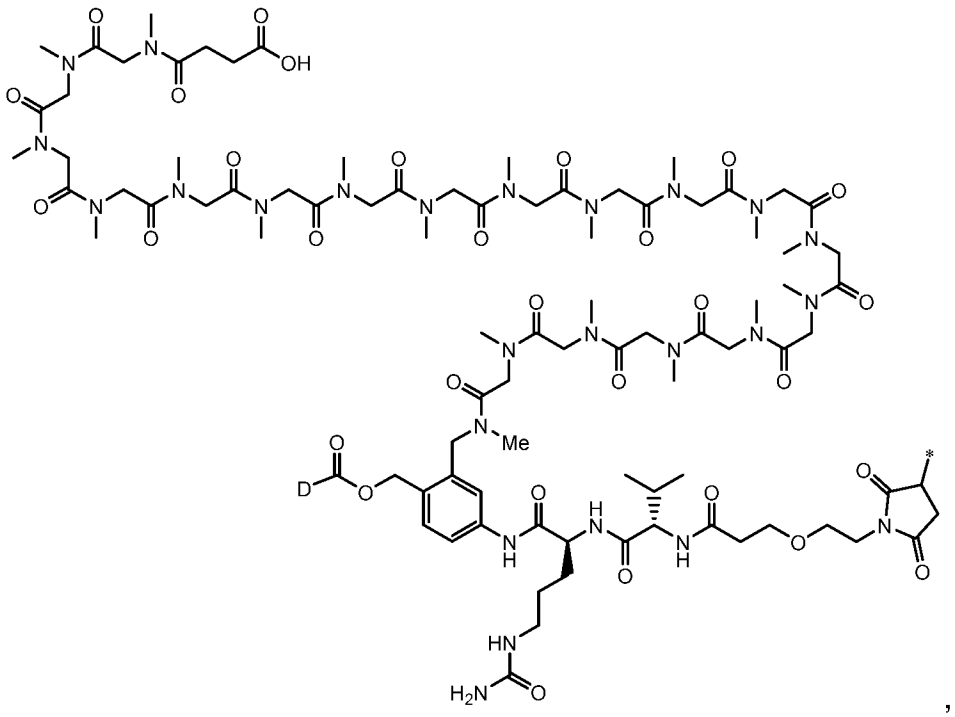

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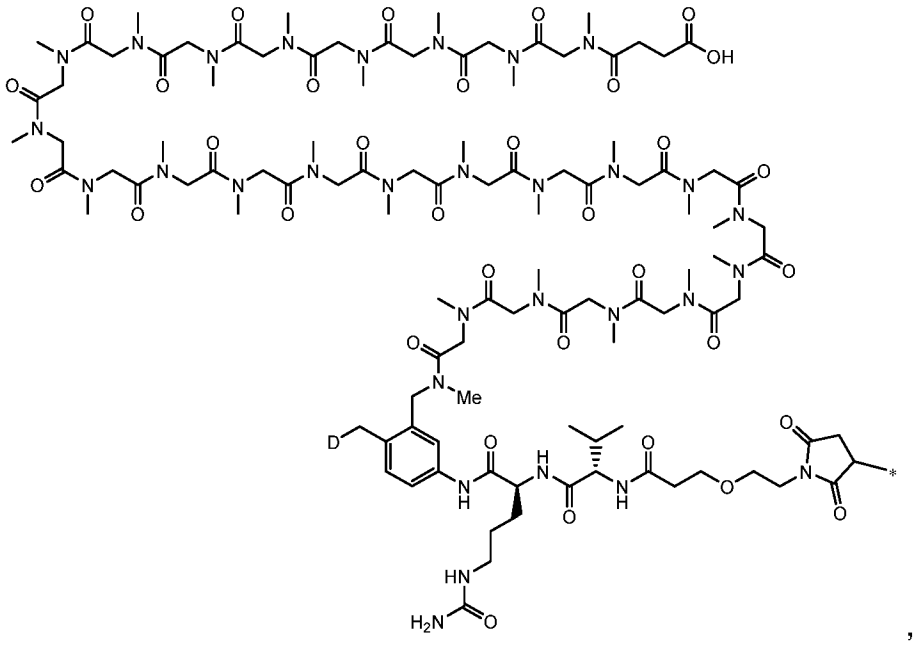

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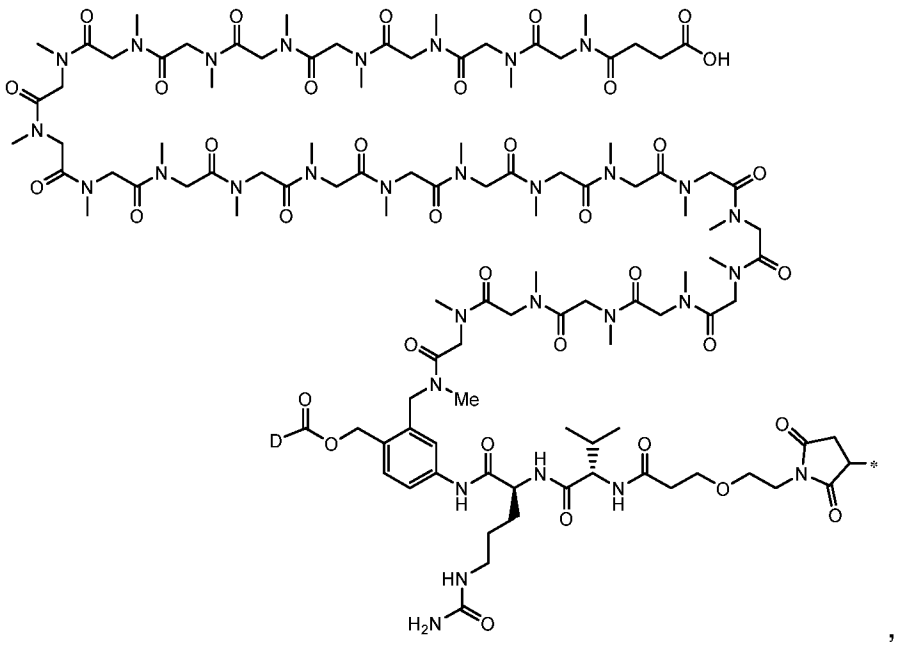

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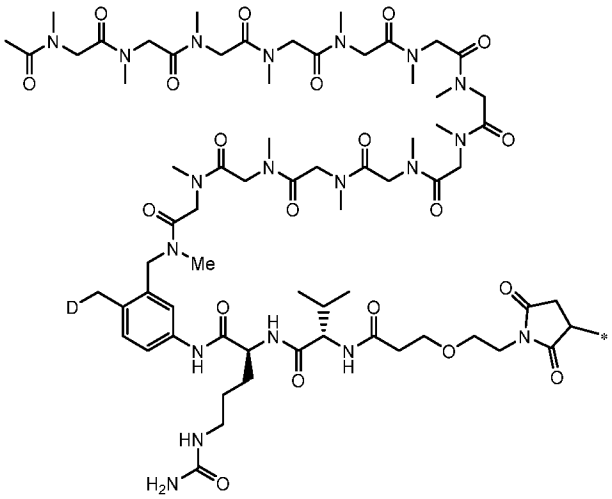

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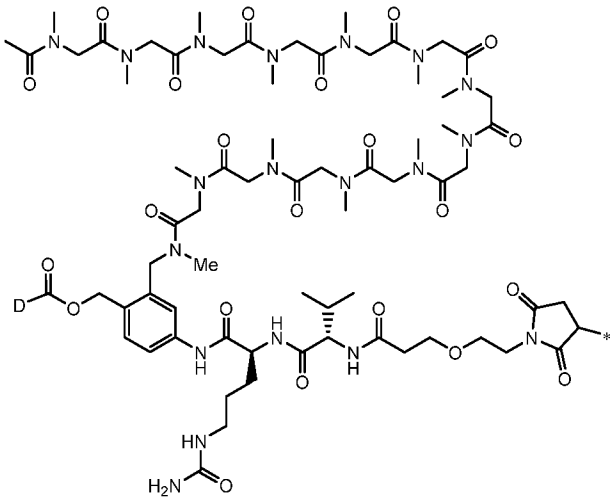

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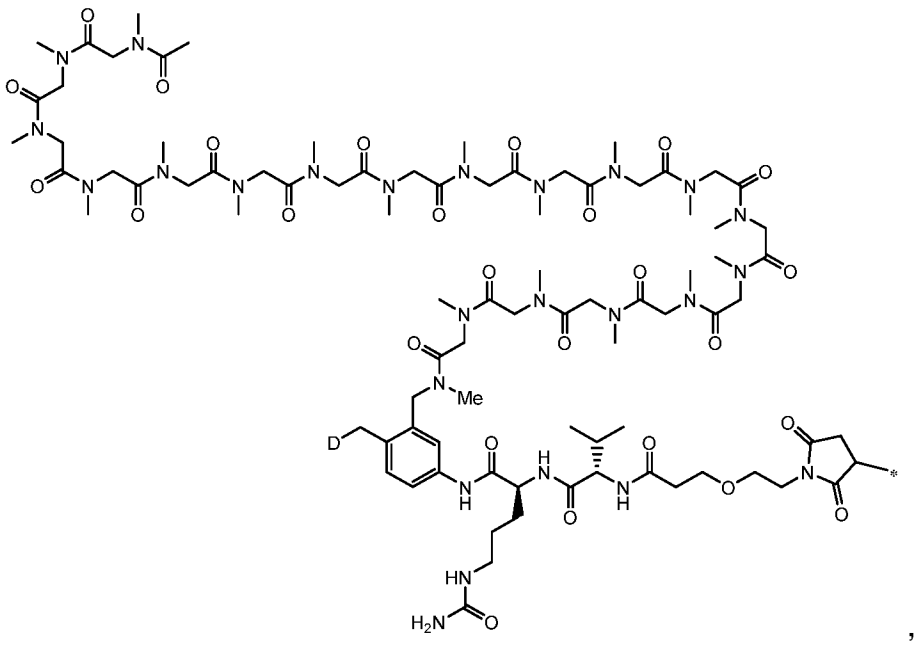

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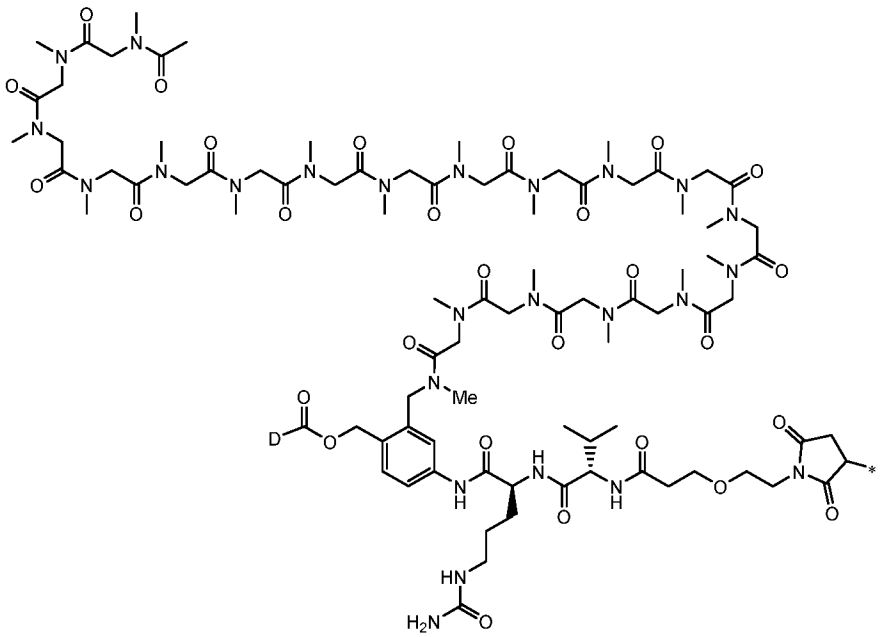

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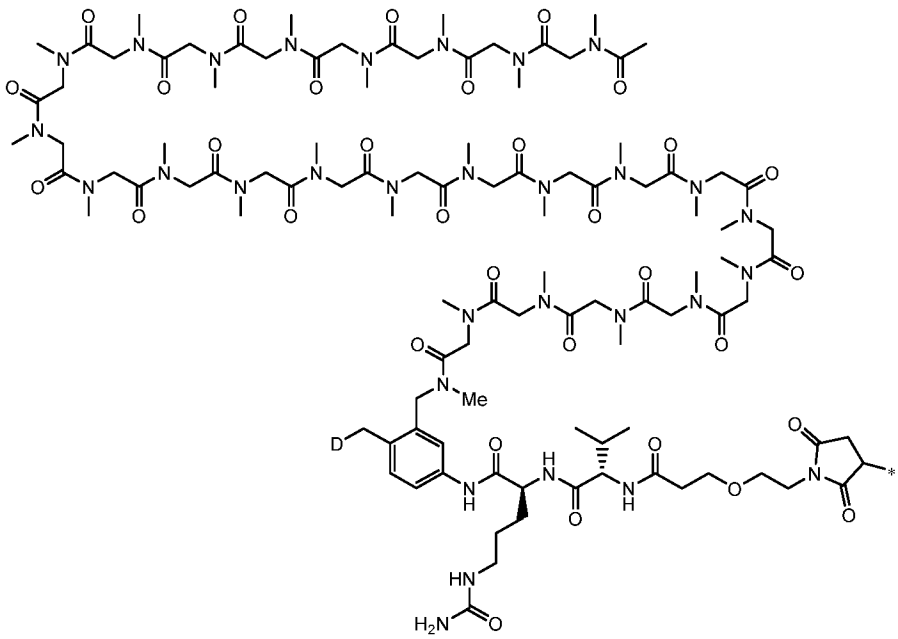

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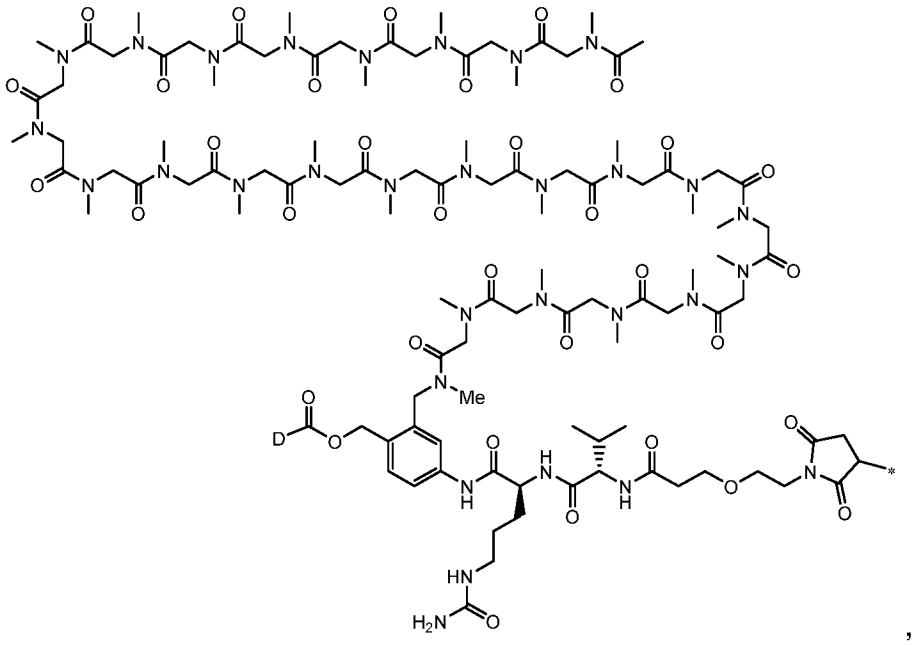

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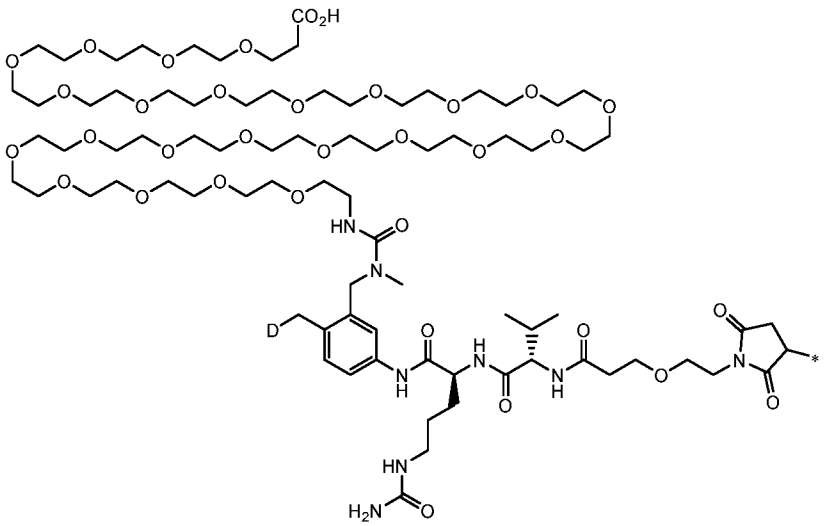

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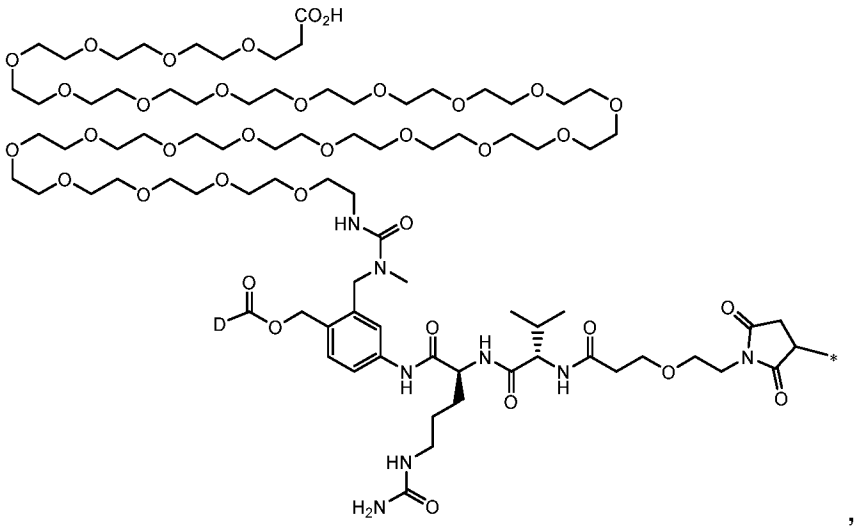

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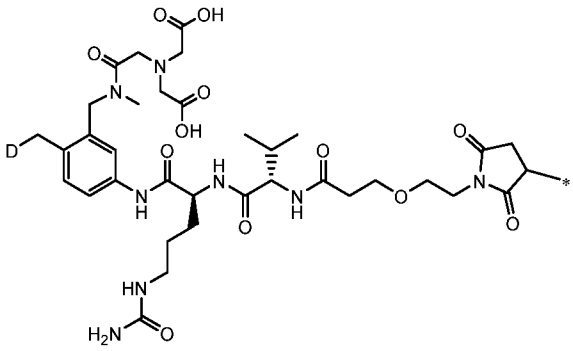

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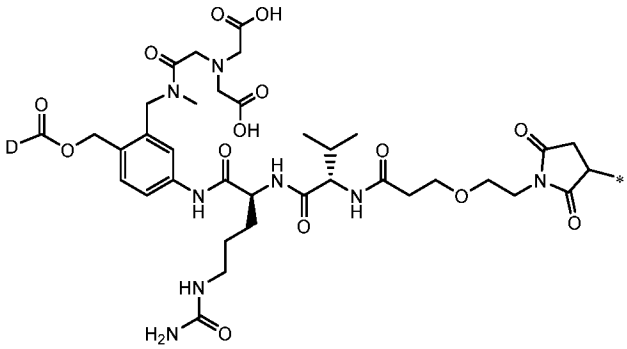

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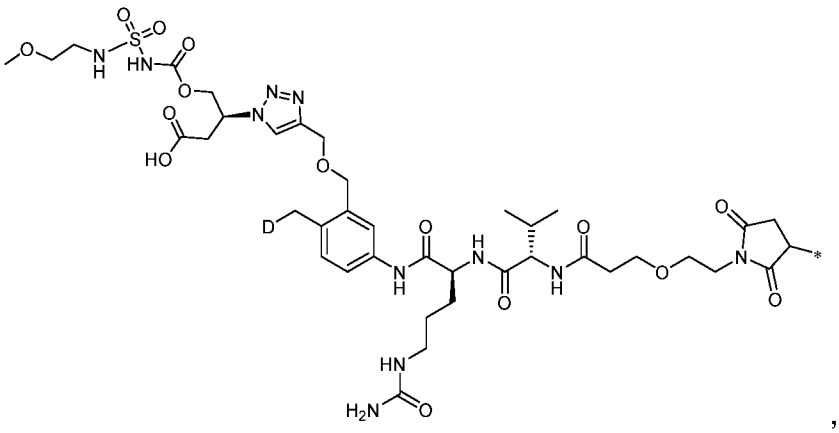

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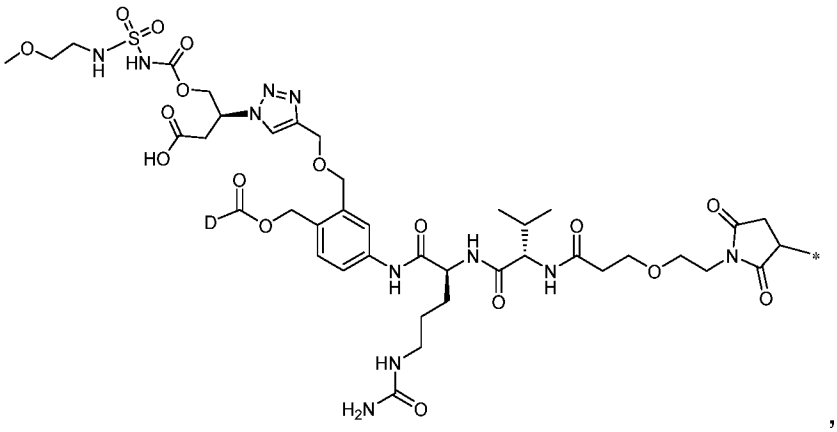

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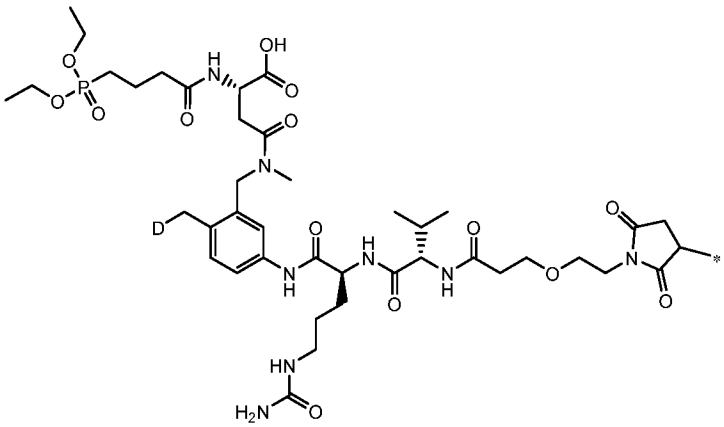

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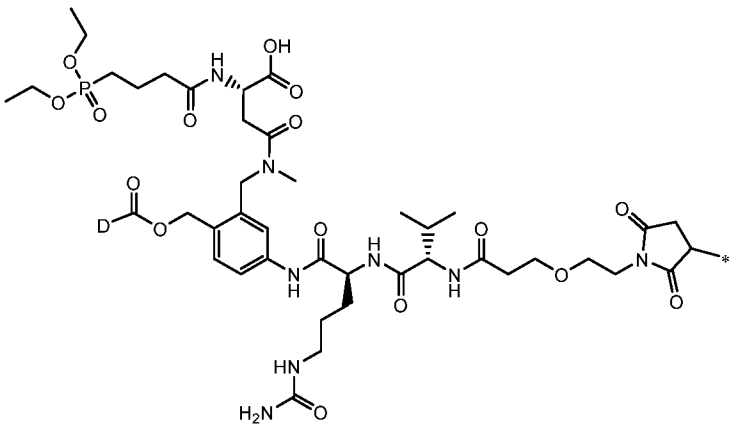

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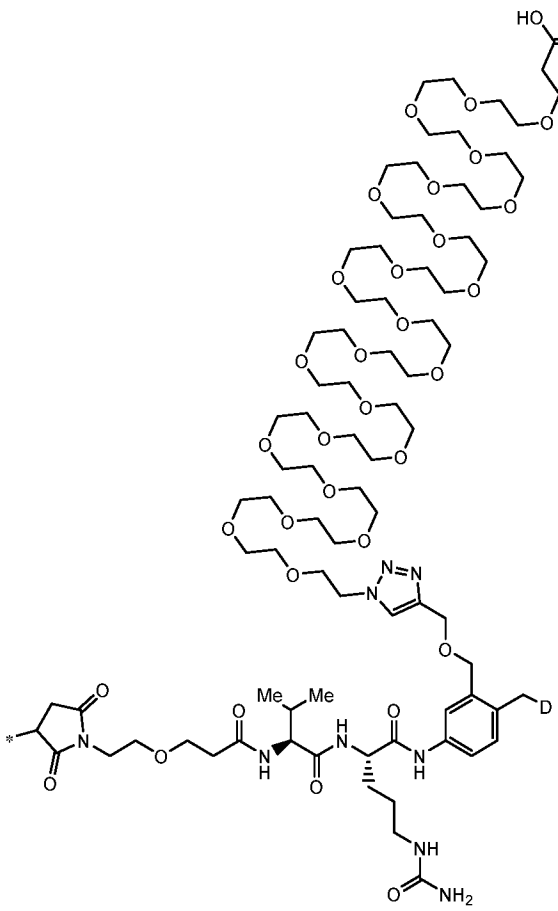

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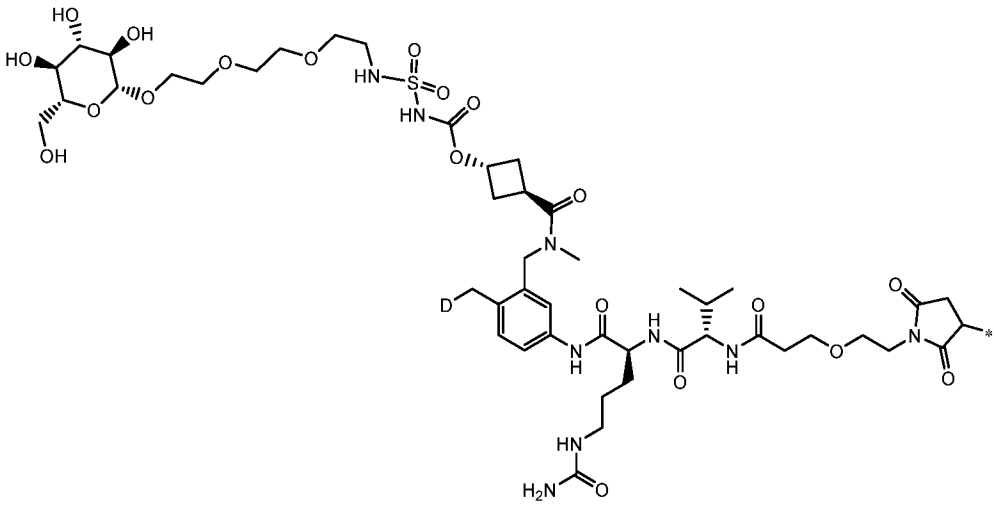

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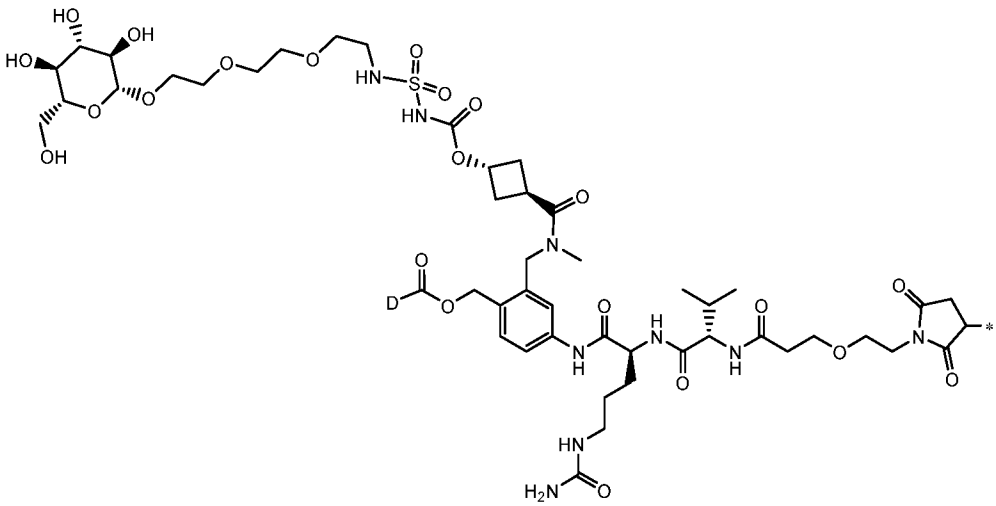
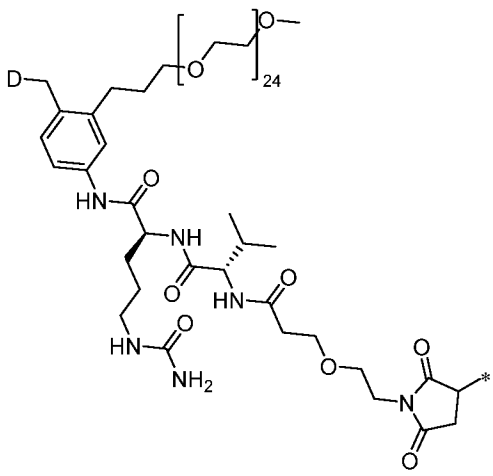


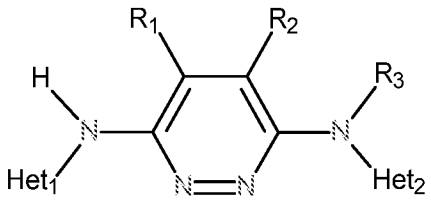
, and



,

and wherein —* is a bond to the antibody.

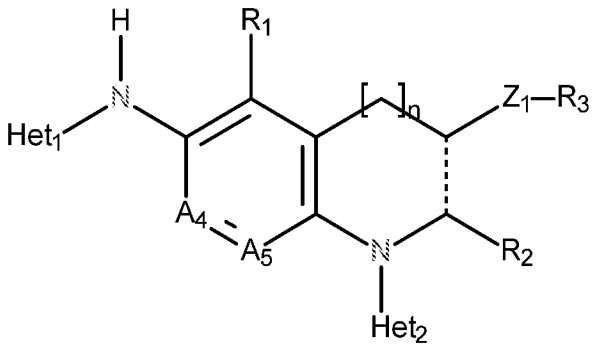
[84] In some embodiments, the Bcl-xL inhibitor (D) comprises a compound of Formula (I):



(I)

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein the variables are described above for Formula (I). In some embodiments, R1 is linear or branched C1-6alkyl and R2 is H.

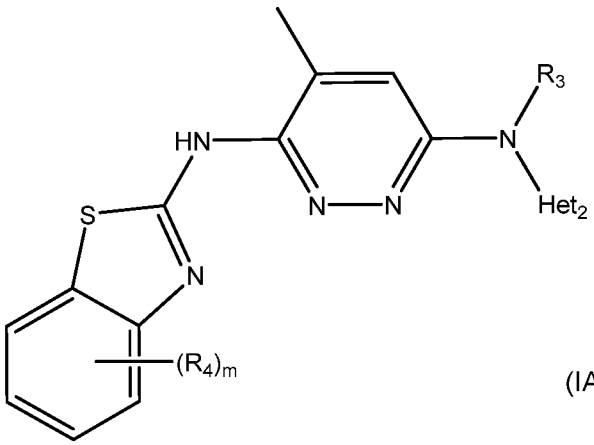
[85] In some embodiments, the Bcl-xL inhibitor (D) comprises a compound of Formula (II):



(II),

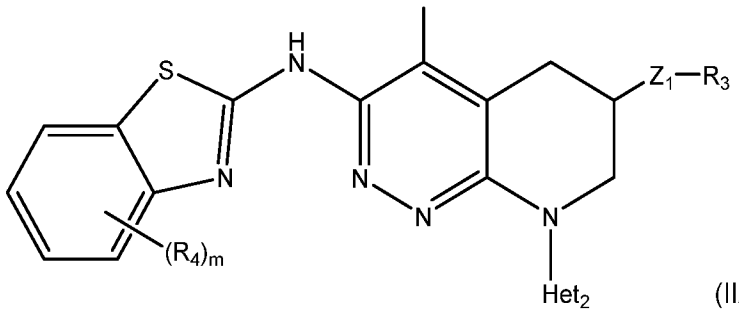
or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein the variables are described above for Formula (II). A1 and A5 both represent a nitrogen atom, R1 is linear or branched C1-6alkyl; R2 is H; n is 1; and ------ represents a single bond.

[86] In some embodiments, the Bcl-xL inhibitor (D) comprises a compound of Formula (IA) or (IIA):



(IA)

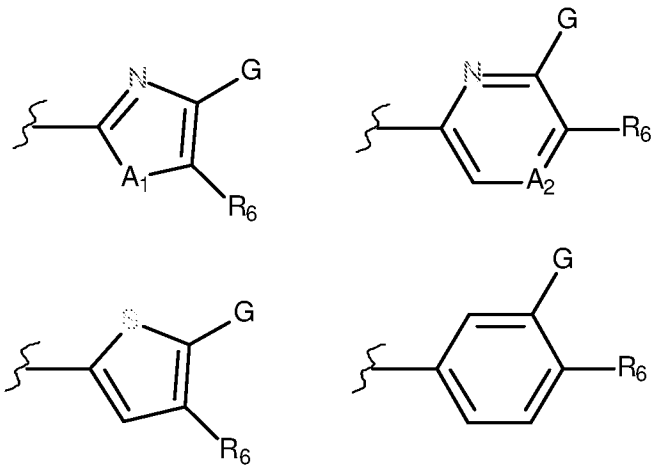
, or



(IIA)

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- Z_1 represents a bond or –O-,
- R_3 represents a group selected from: hydrogen; C_3-C_6cycloalkyl; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; and -X_1-O-R_c,
- R_a and R_b independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; and C_1-C_6alkylene-SO_2O^-,
- R_c represents a hydrogen or a linear or branched C_1-C_6alkyl group,
- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

-C(O)OH, -C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$, -NR_{G1}C(=NCN)$NR_{G1}R_{G2}$, -NR_{G1}S(O)$_2NR_{G1}R_{G2}$, -S(O)$_2R_{G3}$, -S(O)$_2NR_{G1}R_{G2}$, -NR_{G1}S(O)$_2R_{G2}$, -NR_{G1}C(=NR_{G2})$NR_{G1}R_{G2}$, -C(=S)$NR_{G1}R_{G2}$, -C(=NR_{G1})$NR_{G1}R_{G2}$, C_1-C_6alkyl

optionally substituted by a hydroxyl group, halogen, -NO_2, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, and C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms;

- R_{G3} is C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; or

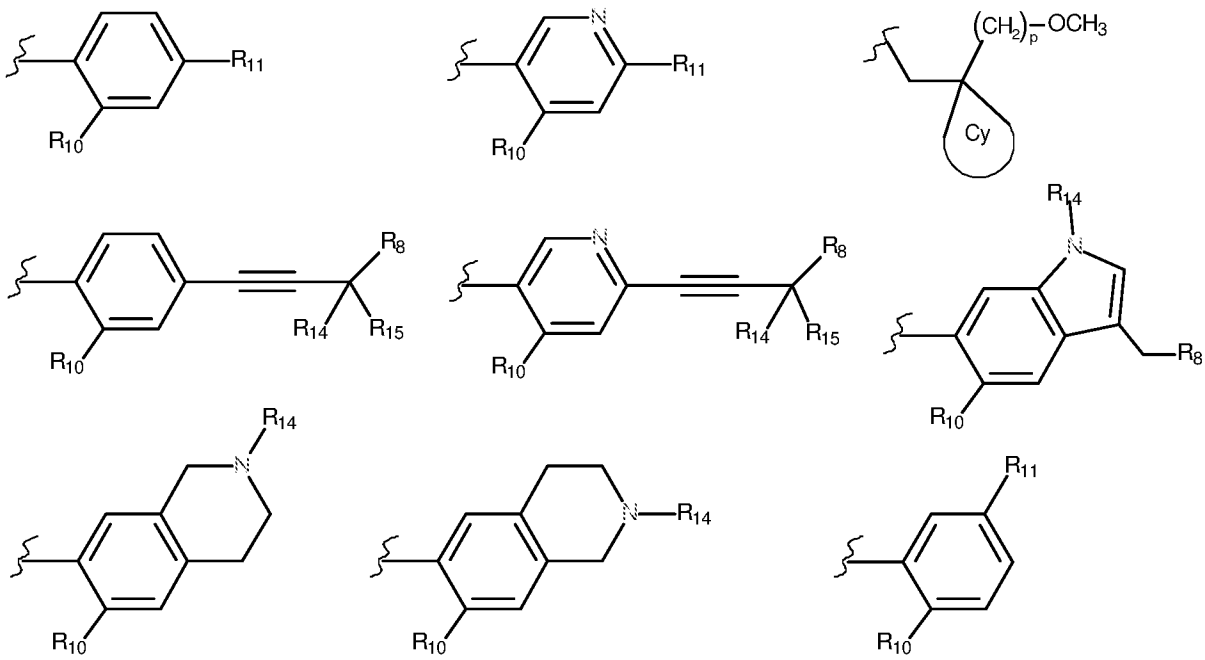
R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl;

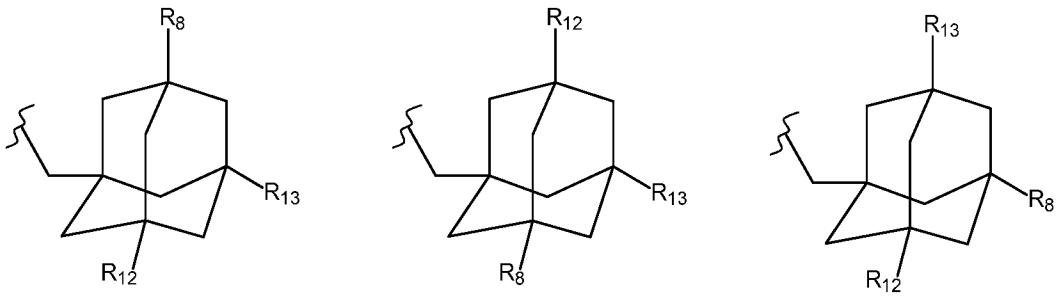
- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; halogen or –CN,
- R_6 represents a group selected from:

  -X_2-O-R_7; and

  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

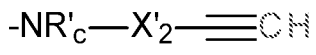
- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:





wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -$NR'_aR'_b$;
  -NR'_a-CO-OR'_c; -NR'_a-CO-R'_c; -$N^+R'_aR'_bR'_c$; -O-R'_c; -NH-X'_2-$N^+R'_aR'_bR'_c$; -O-X'_2-$NR'_aR'_b$; -X'_2-$NR'_aR'_b$: -NR'_c-X'_2-N_3 and :

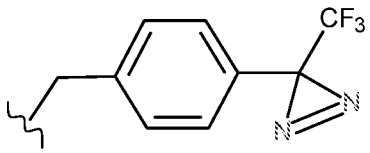


,

- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two

hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

the group:



or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- m=0, 1 or 2,

- p=1, 2, 3 or 4,

- B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl.

[87] In some embodiments, for Formula (IA) or (IIA), G is selected from the group consisting of: -C(O)OH, $-C(O)OR_{G3}$, $-C(O)NR_{G1}R_{G2}$, $-C(O)R_{G2}$, $-NR_{G1}C(O)R_{G2}$, $-NR_{G1}C(O)NR_{G1}R_{G2}$,
$-OC(O)NR_{G1}R_{G2}$, $-NR_{G1}C(O)OR_{G3}$, $-C(=NOR_{G1})NR_{G1}R_{G2}$,
$-NR_{G1}C(=NCN)NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2NR_{G1}R_{G2}$, $-S(O)_2R_{G3}$, $-S(O)_2NR_{G1}R_{G2}$,

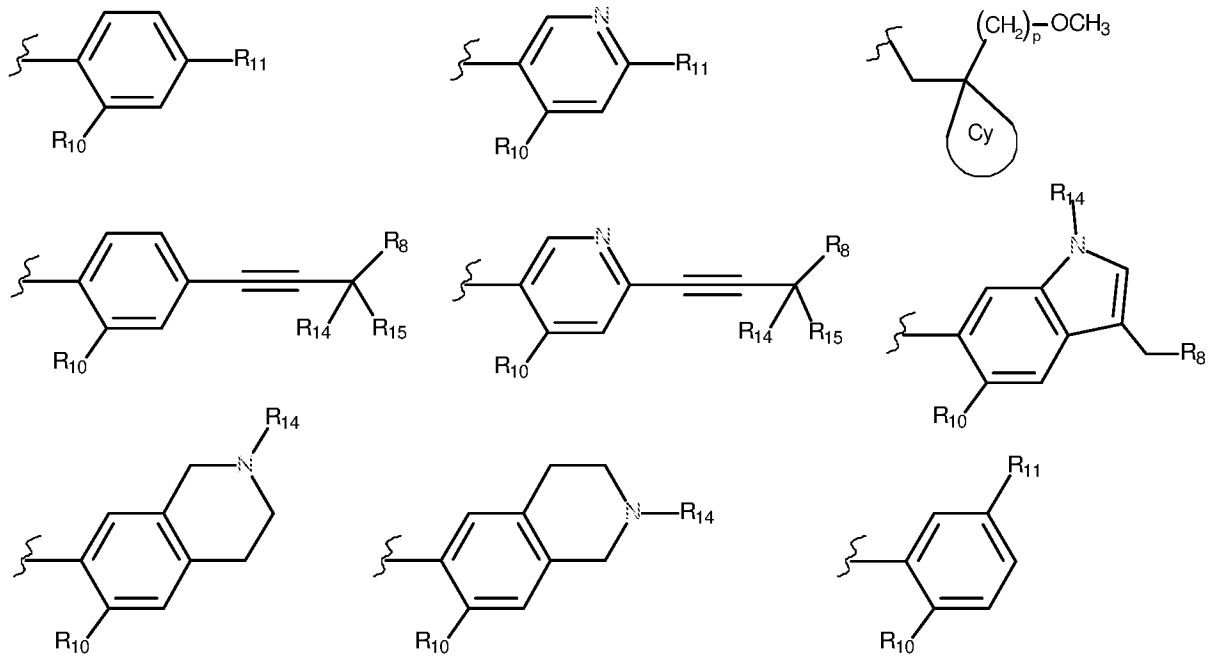
$-NR_{G1}S(O)_2R_{G2}$, $-NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, $-C(=S)NR_{G1}R_{G2}$, $-C(=NR_{G1})NR_{G1}R_{G2}$, halogen, $-NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, and C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms;

- R_{G3} is C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; or

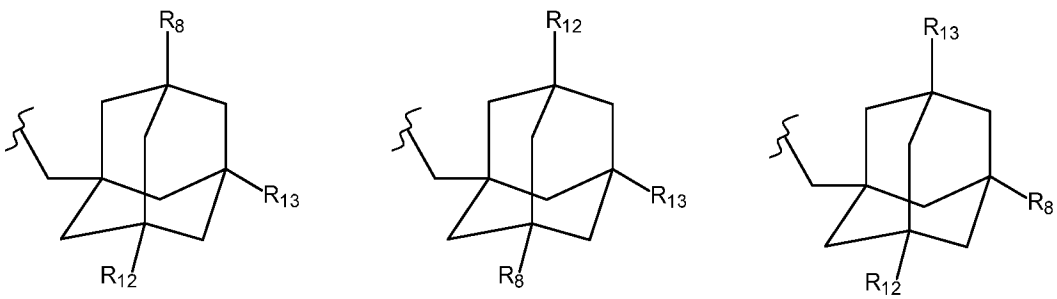
- R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl.

[88] In some embodiments, for Formula (I), (II), (IA) or (IIA), R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:

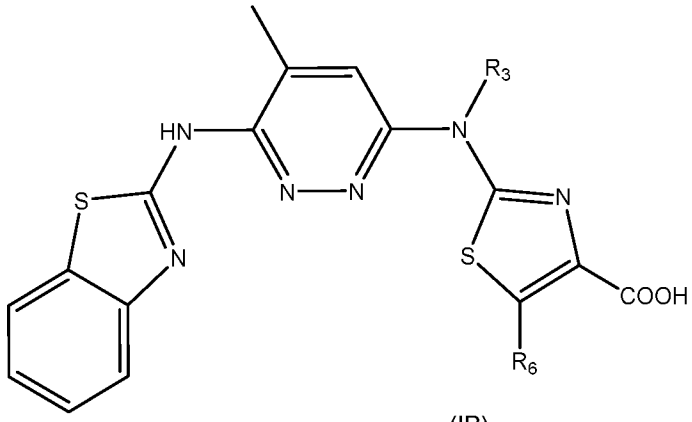


wherein Cy represents a C_3-C_8cycloalkyl.

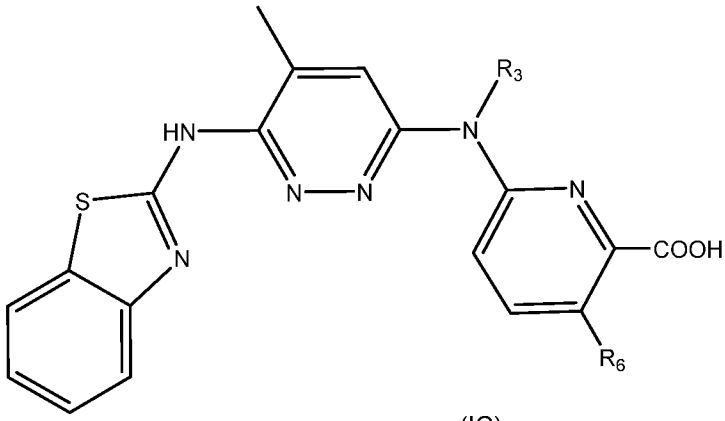
[89] In some embodiments, for Formula (I), (II), (IA) or (IIA), R_7 represents a group selected from:



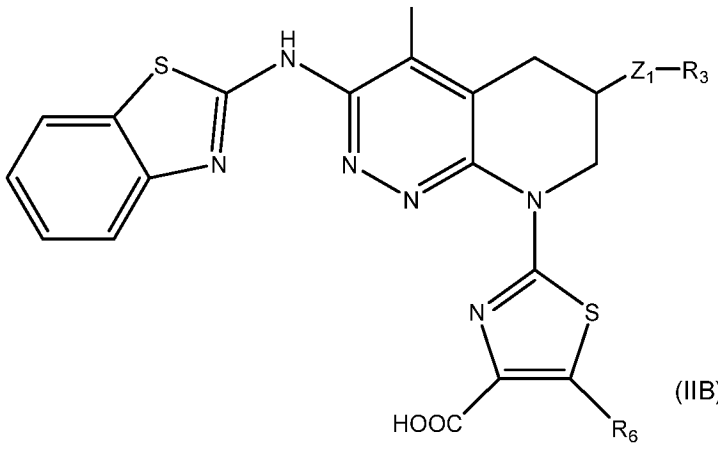
[90] In some embodiments, the Bcl-xL inhibitor (D) comprises a compound of Formula (IB), (IC), (IIB) or (IIC):



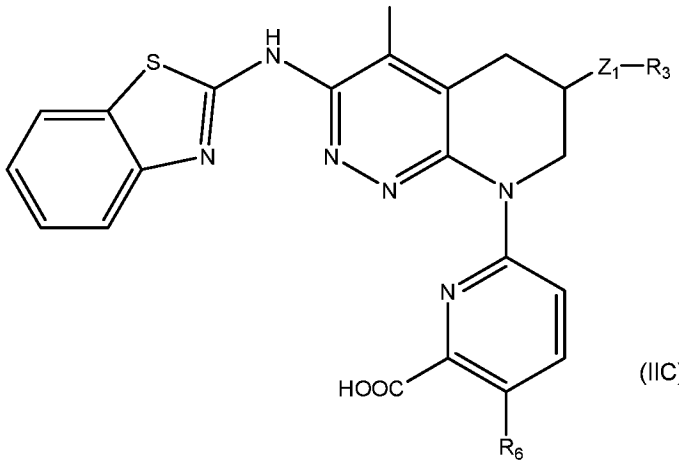
(IB) ,



(IC) ,



(IIB) , or

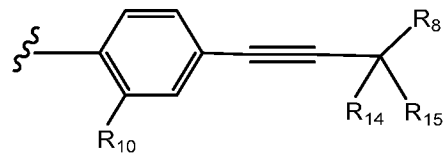


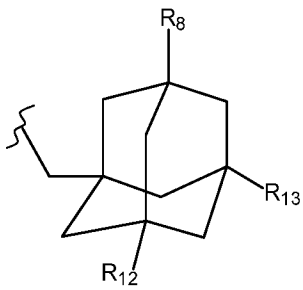
(IIC)

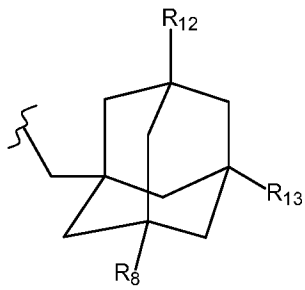
,

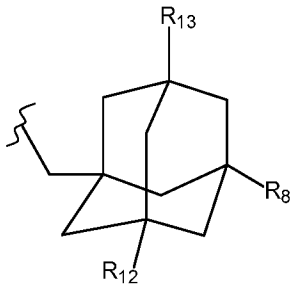
or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- for formula (IB) or (IC), R_3 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; and -X_1-O-R_c;
  for formula (IIB) or (IIC), Z_1 represents a bond, and R_3 represents hydrogen; or Z_1 represents –O-, and R_3 represents –X_1-NR_aR_b,
- R_a and R_b independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; and C_1-C_6alkylene-SO_2O^-,
- R_c represents a hydrogen or a linear or branched C_1-C_6alkyl group
- R_6 represents –X_2-O-R_7 or an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,
- R_7 represents a group selected from:









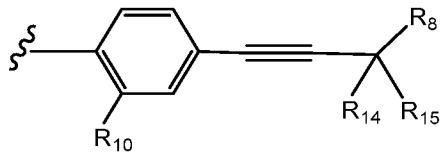
,

- R_8 represents a group selected from: -$NR'_aR'_b$; -O-X'_2-$NR'_aR'_b$; and -X'_2-$NR'_aR'_b$,
- R_{10} represents fluorine,

- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-$NR'_dR'_e$;

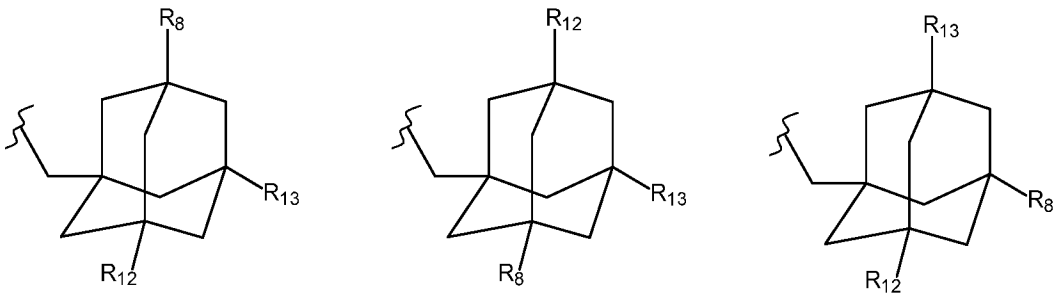
  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,
- R'_d, R'_e independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,
- B_3 represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, and oxo.

In some embodiments, R_7 represents the following group:



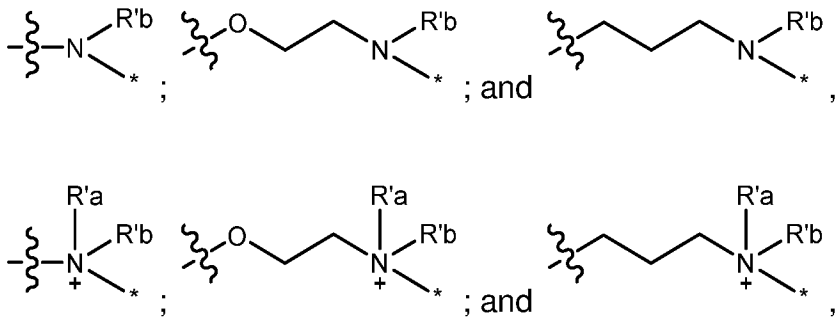
.

In some embodiments, R_7 represents a group selected from:



.

[91] In some embodiments, for Formula (I), (IA), (IB), (IC), (II), (IIA), (IIB) or (IIC), R_8 represents a group selected from:

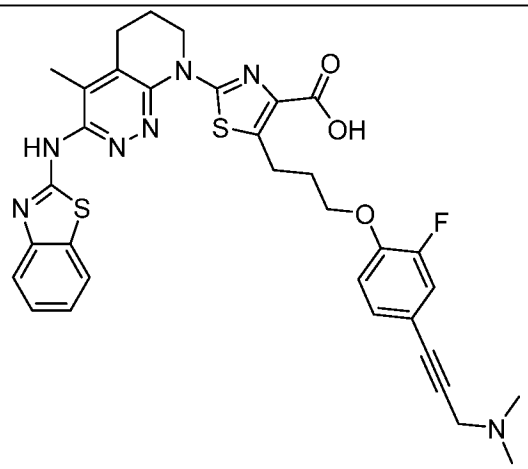
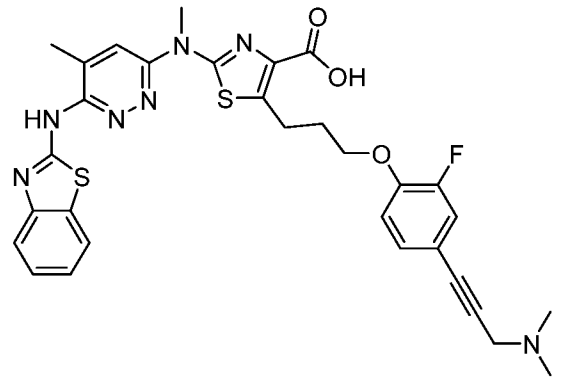
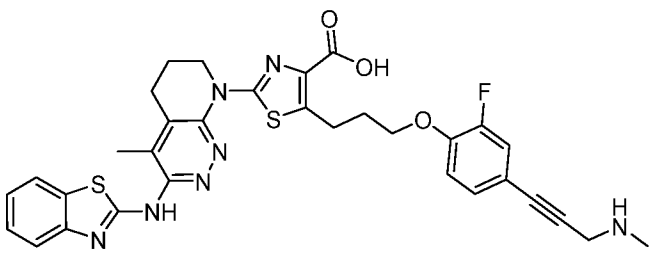
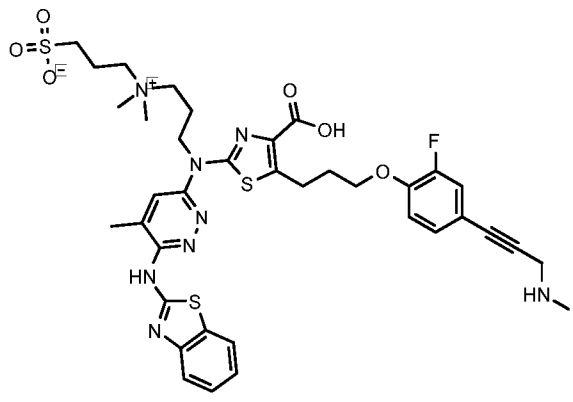


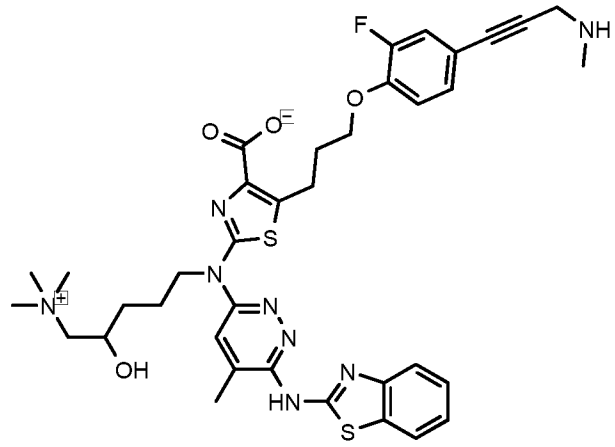
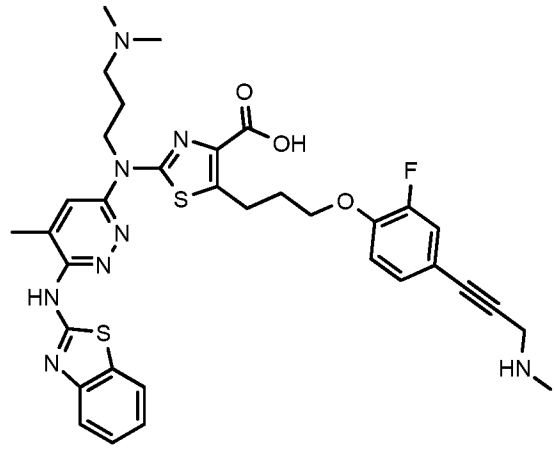
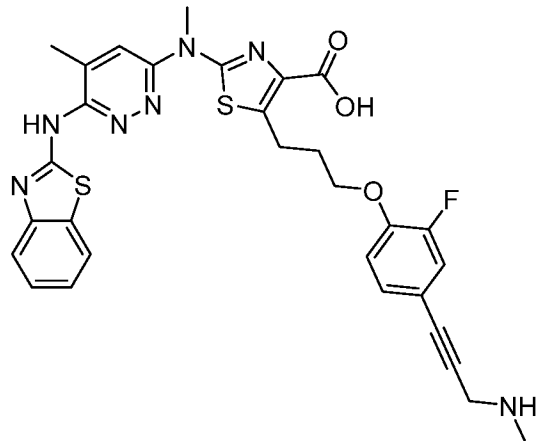
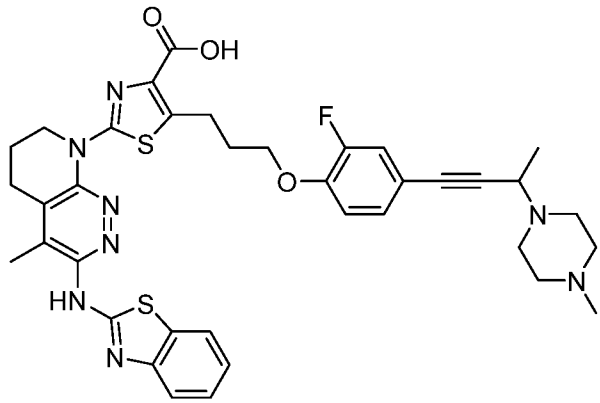
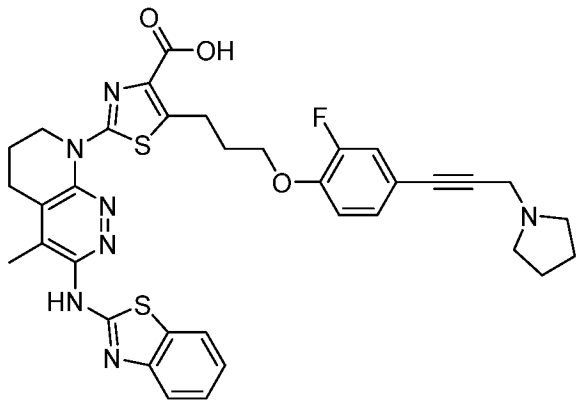
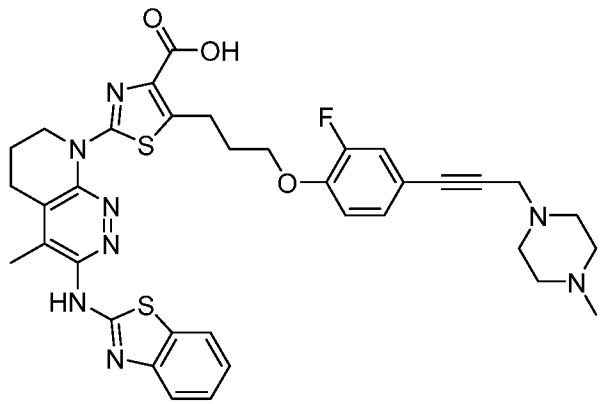
wherein —— * represents a bond to the linker.

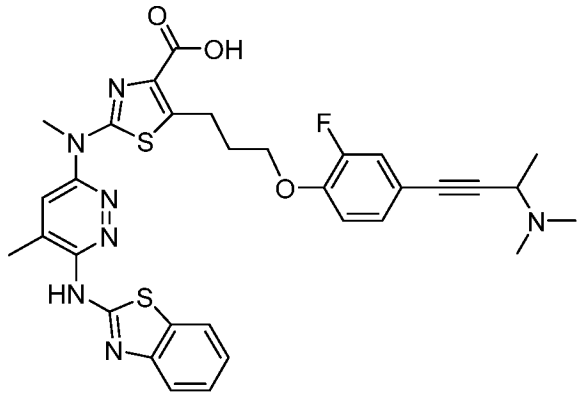
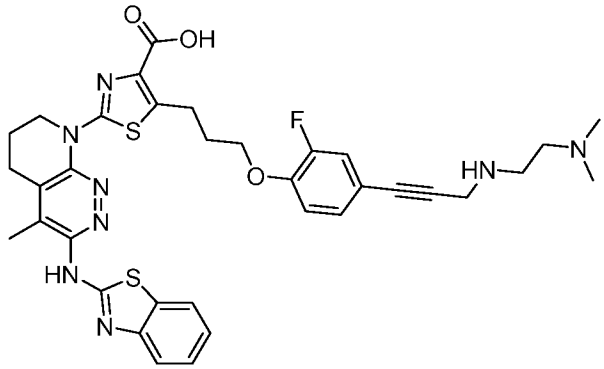
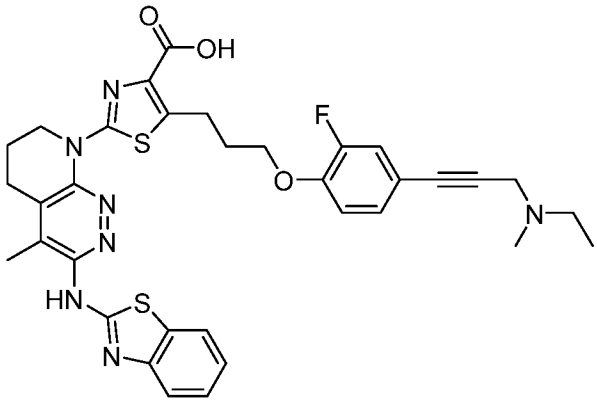
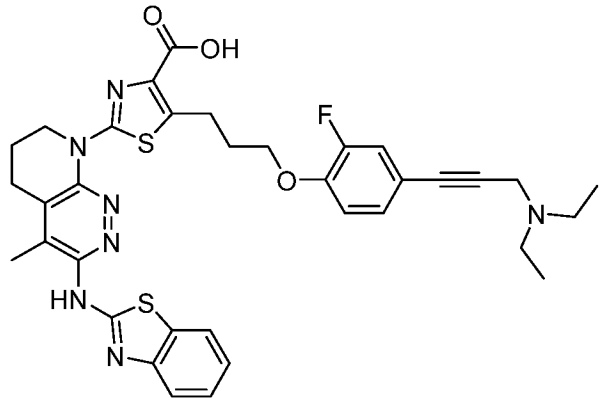
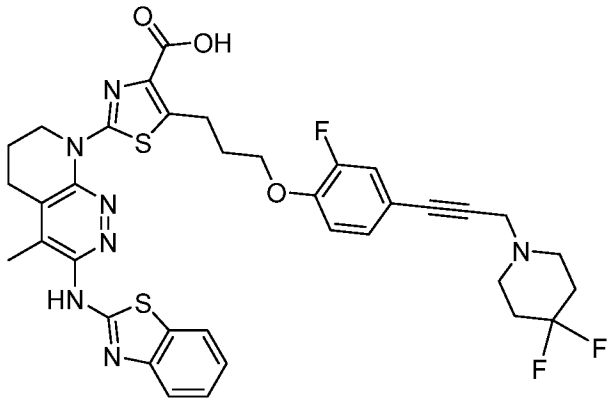
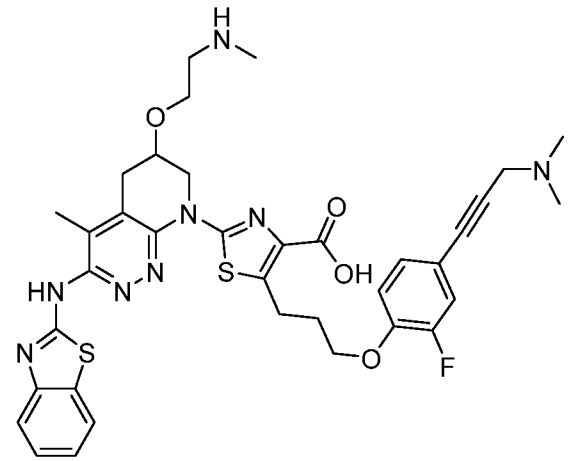
[92] In some embodiments, B3 represents a C3-C8heterocycloalkyl group selected from a pyrrolidinyl group, a piperidinyl group, a piperazinyl group, a morpholinyl group, an azepanyl group, and a 2,8-diazaspiro[4,5]decanyl group.

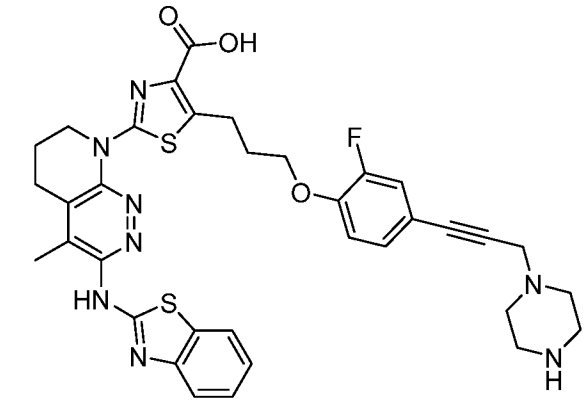
[93] In some embodiments, D represents a Bcl-xL inhibitor attached to the linker L by a covalent bond, wherein the Bcl-xL inhibitor is selected from a compound in Table A1:

Table A1

 P1	 P2
 P3	 P4

 P5	 P6
 P7	 P8
 P9	 P10

 P11	 P12
 P13	 P14
 P15	 P16



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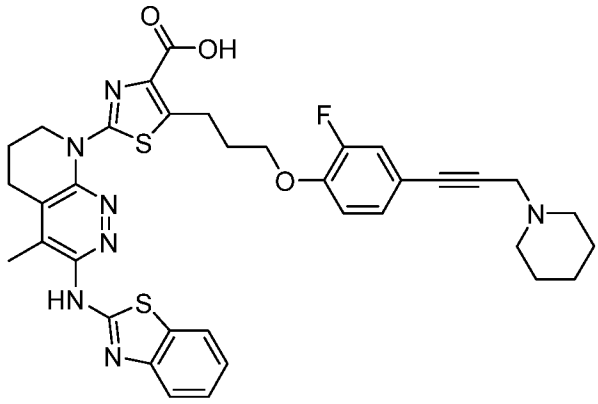

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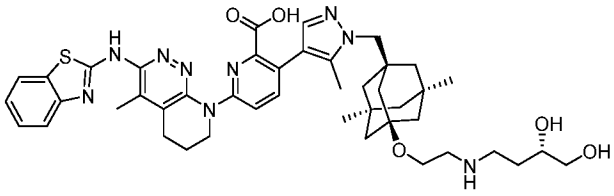

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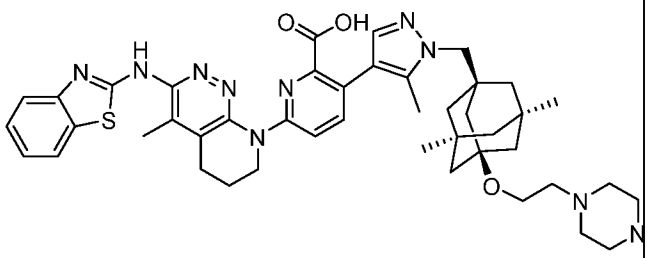

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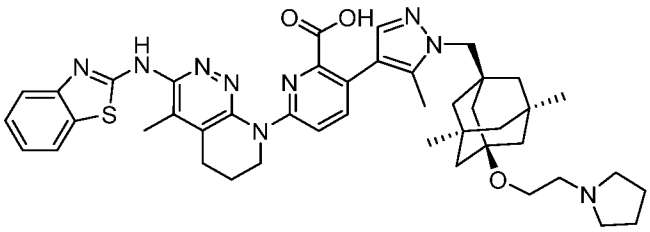

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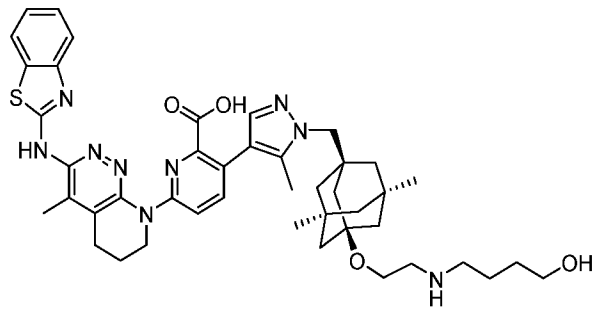

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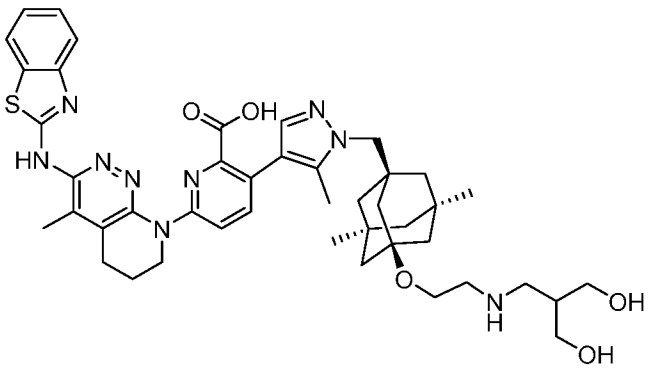

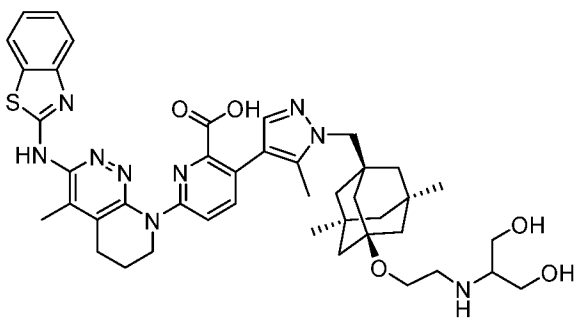

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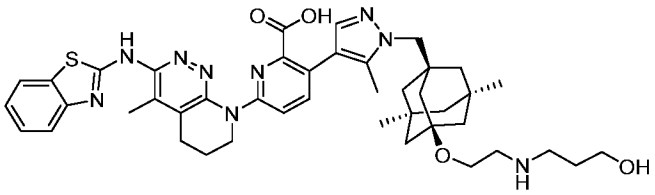
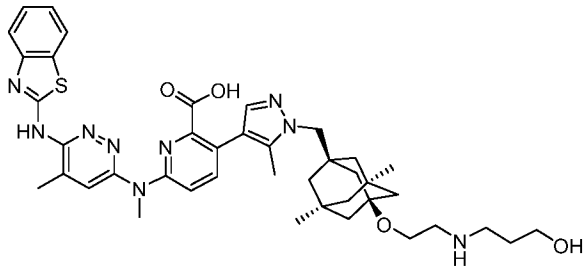
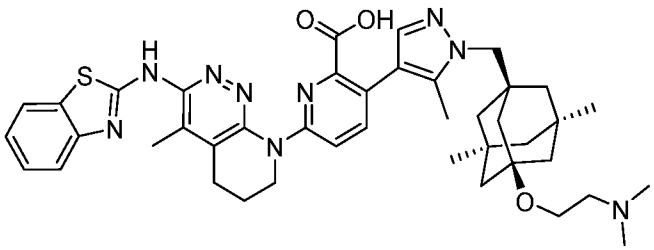
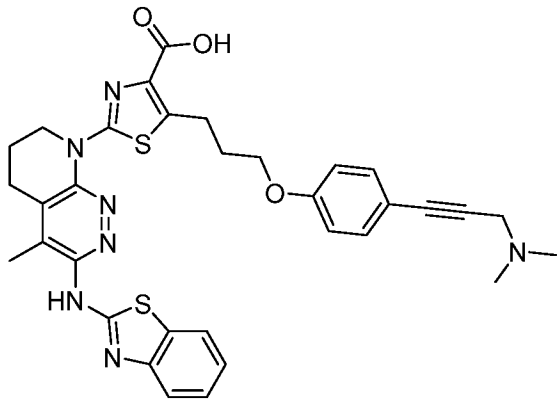
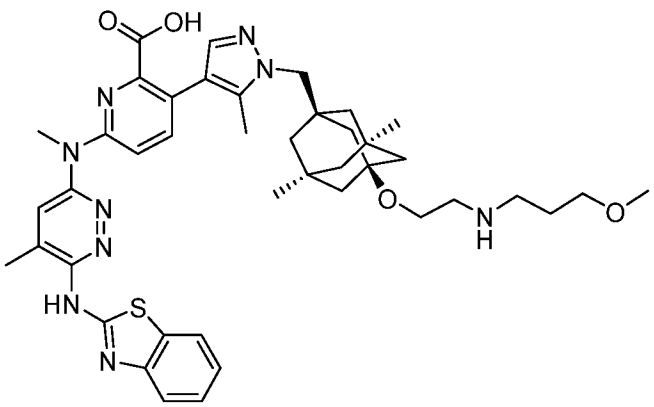
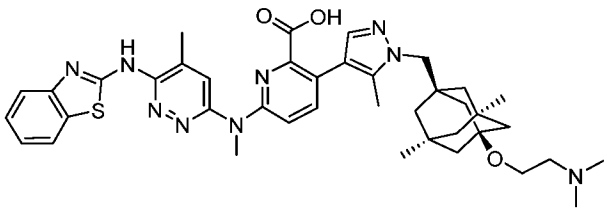
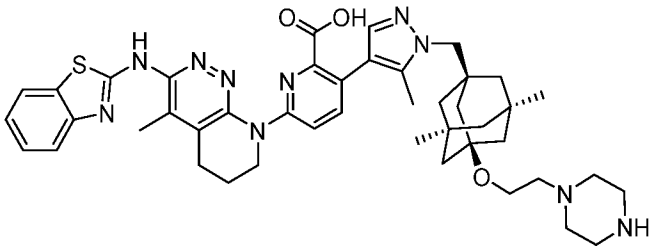
 P25	 P26
 P27	 P28
 P29	 P30



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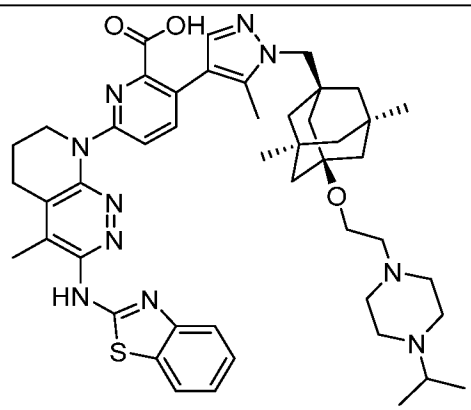

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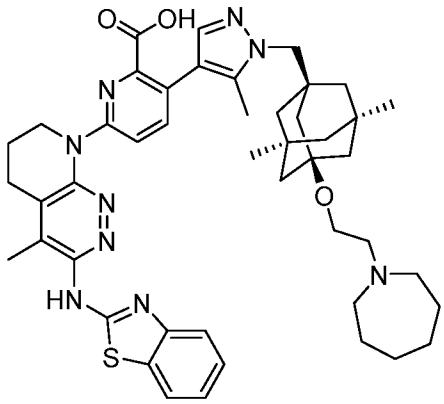

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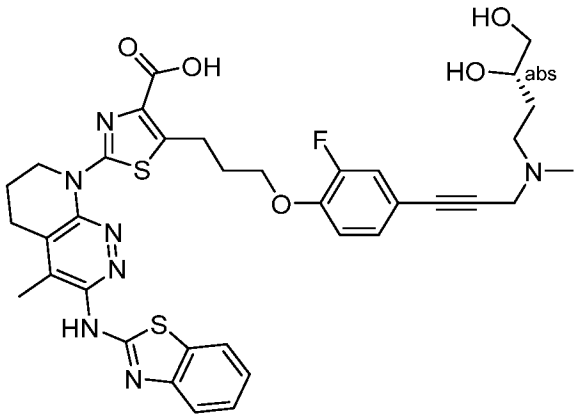

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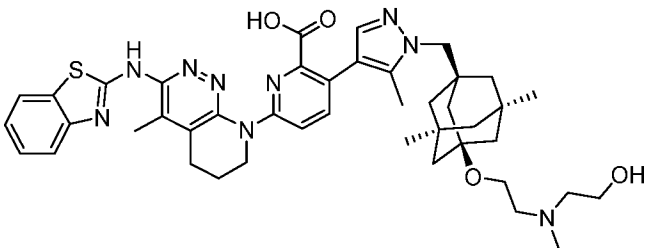

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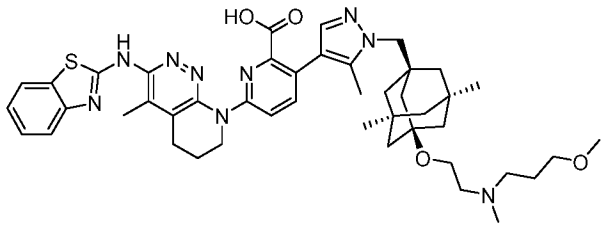

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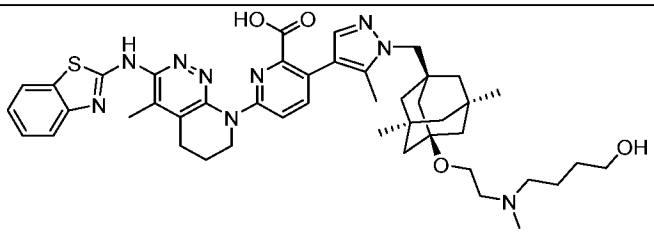

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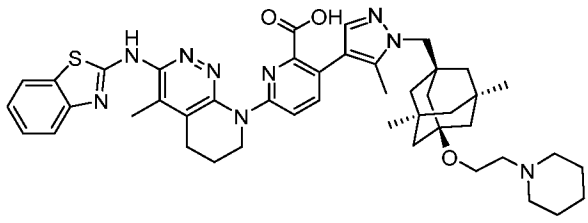

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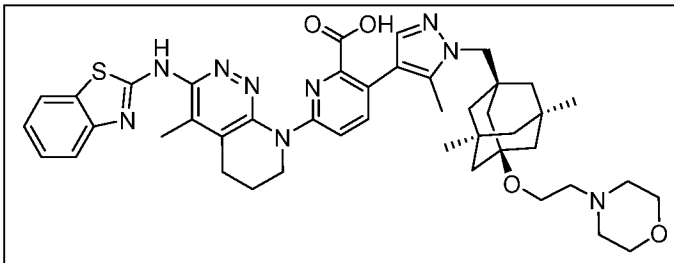

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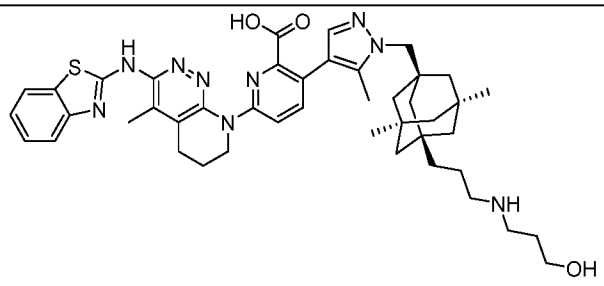

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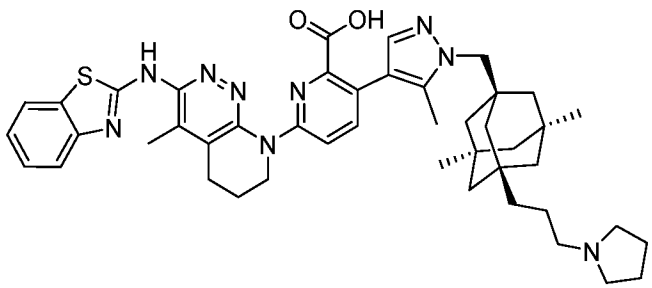

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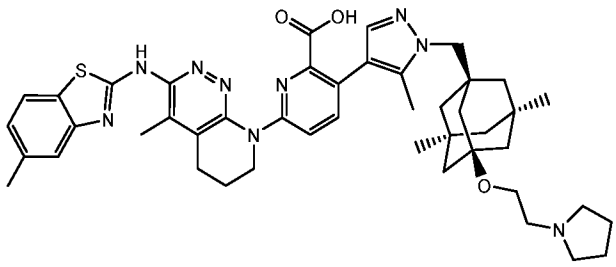

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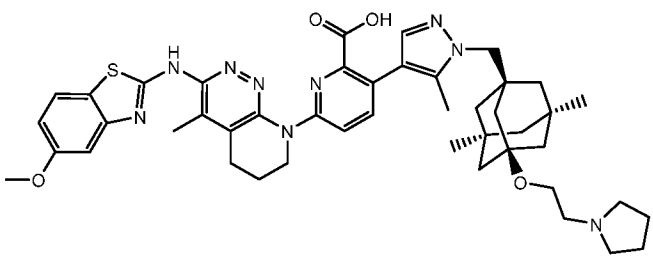

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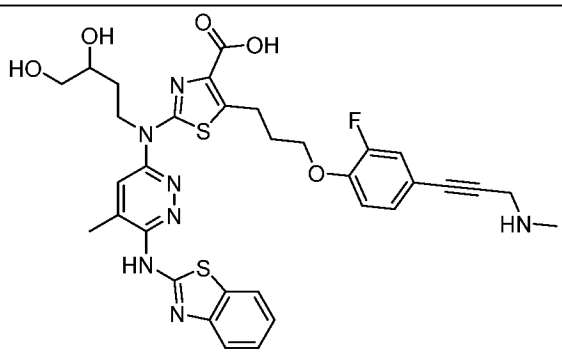

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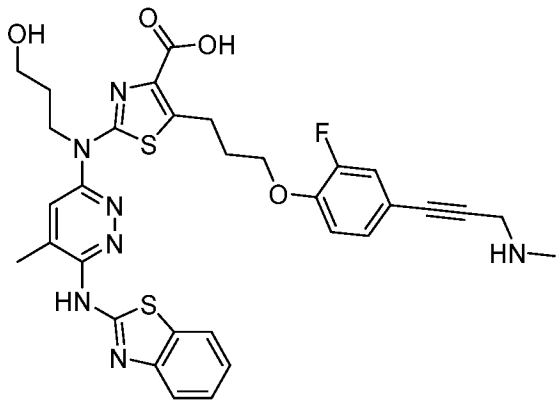

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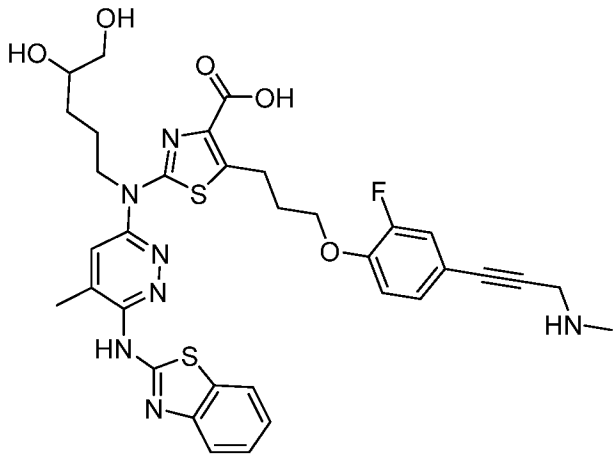

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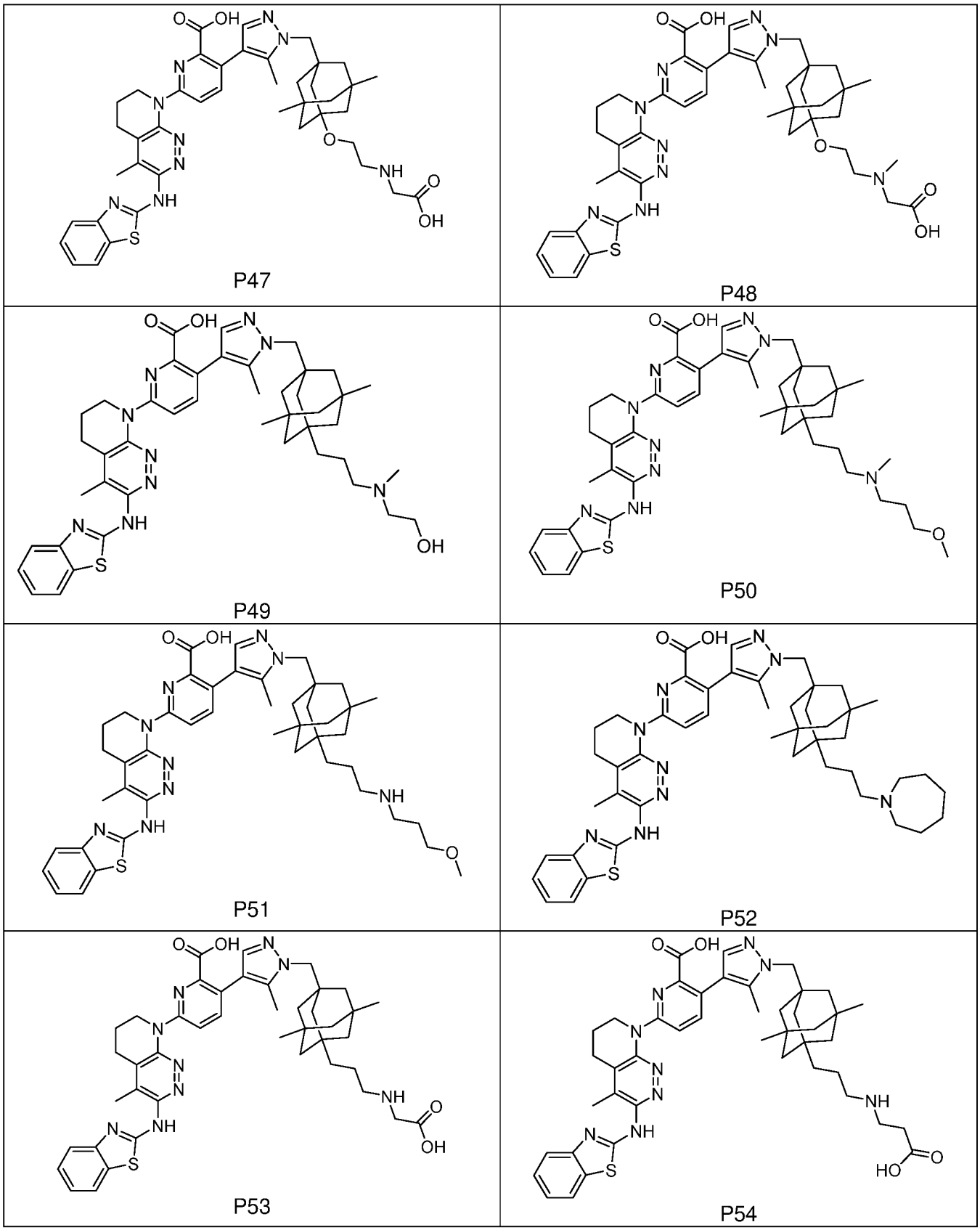
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P48
P49
P50
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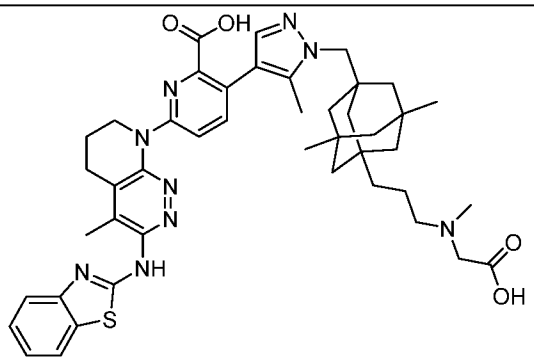
P53
P54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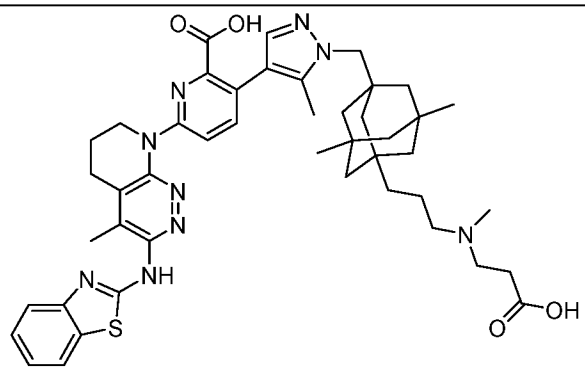
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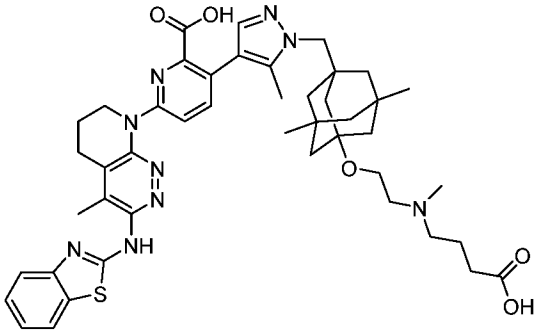
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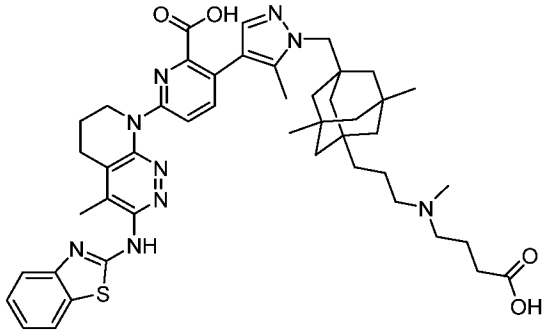
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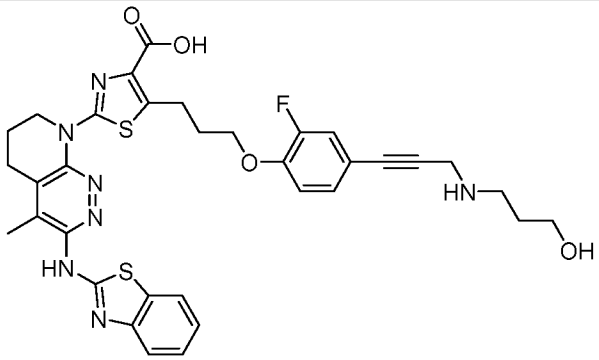
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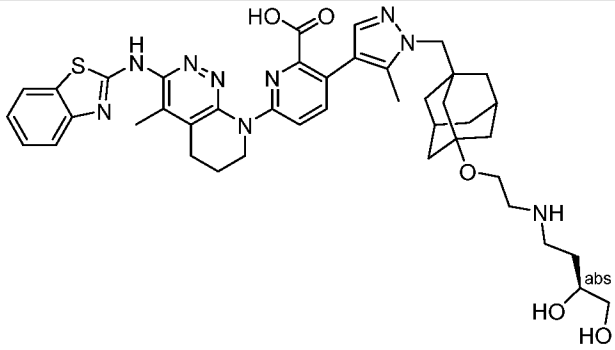
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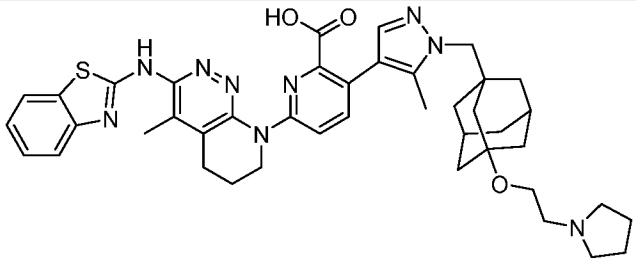
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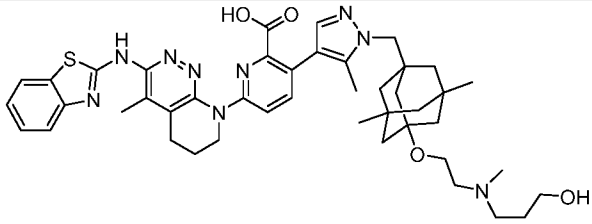
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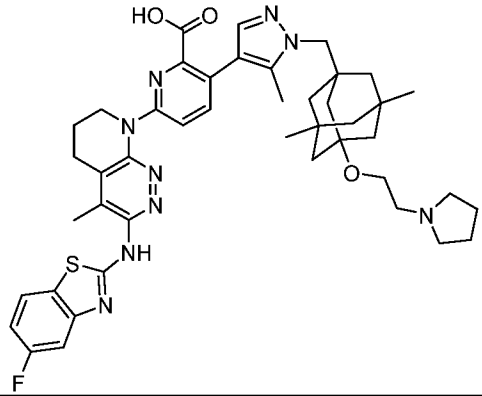

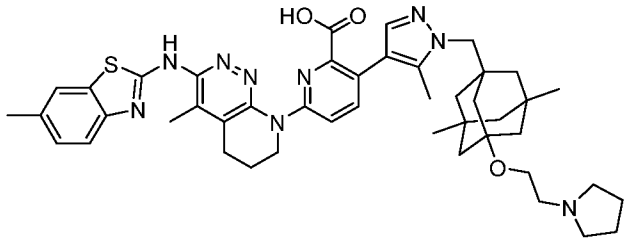
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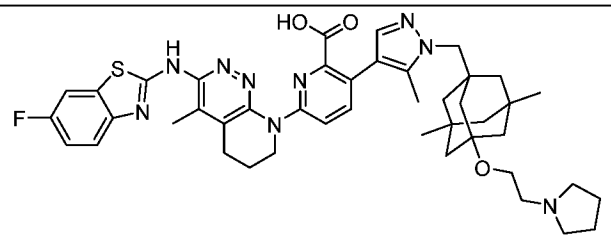
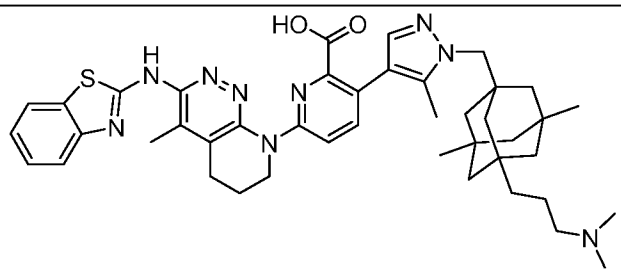
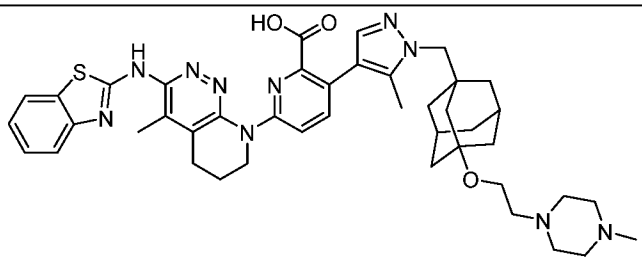
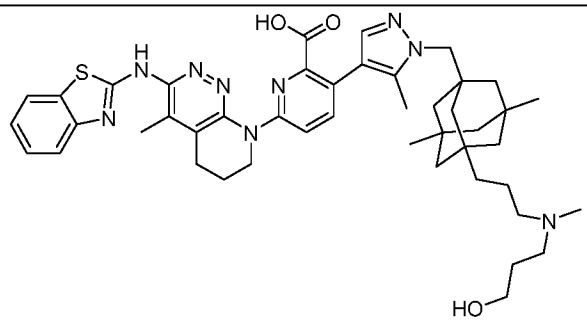
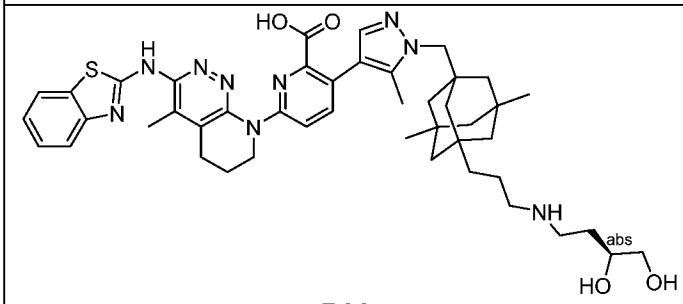
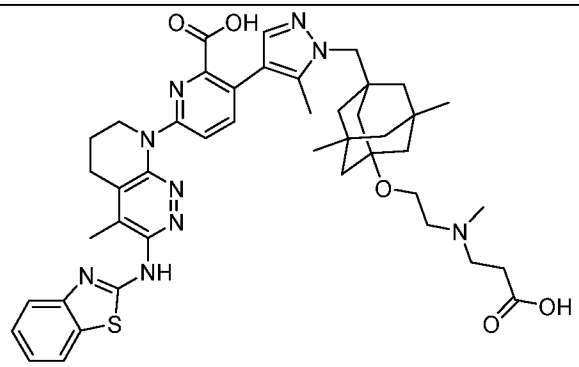
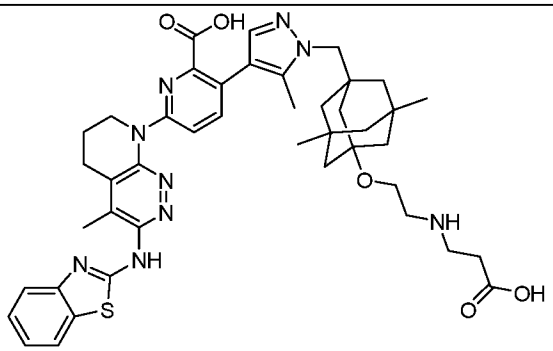
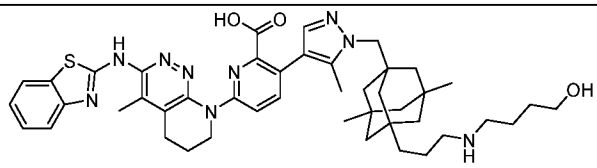
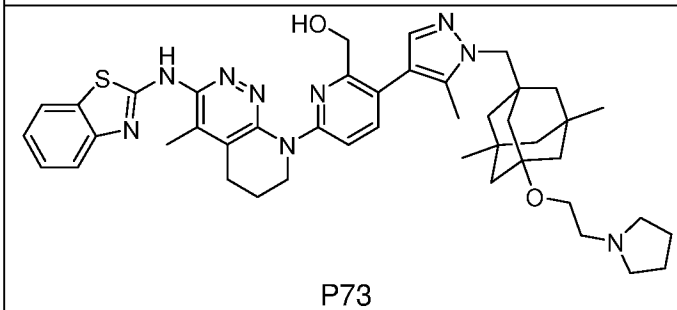
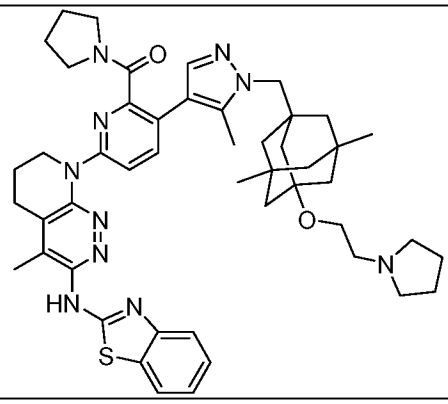
 P65	 P66
 P67	 P68
 P69	 P70
 P71	 P72
 P73	

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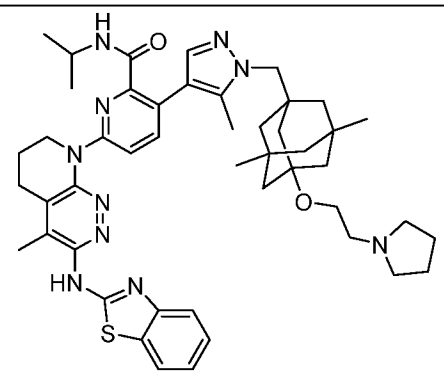
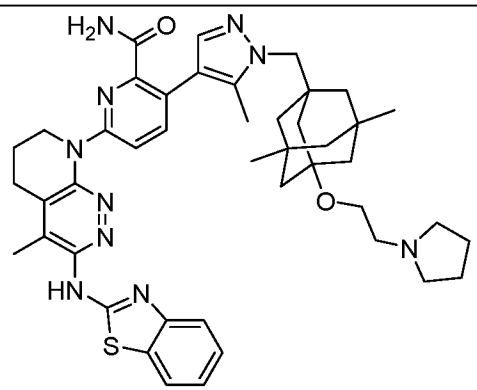
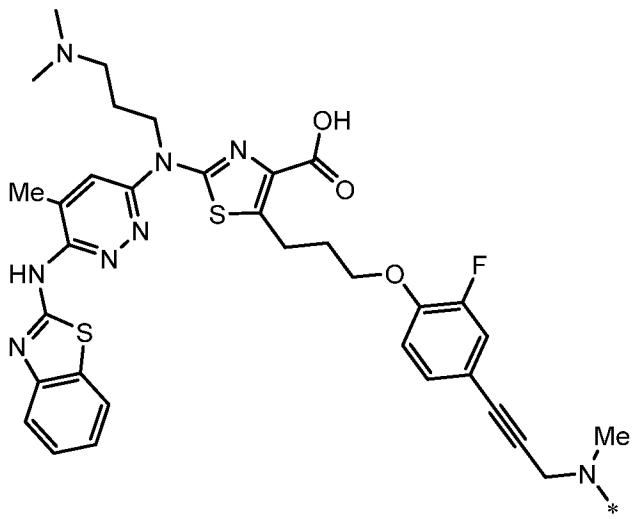
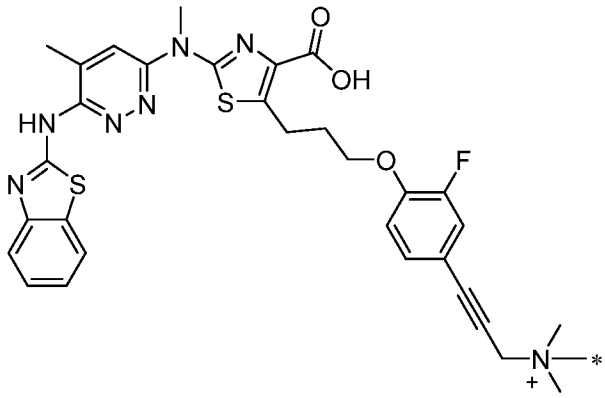
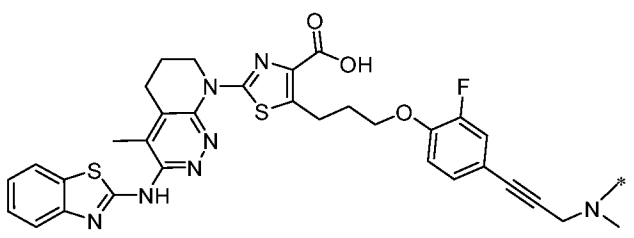
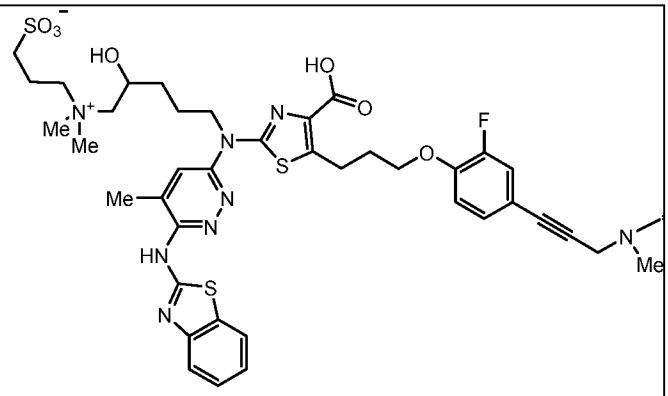
 P75	 P76

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing.

[94] In some embodiments, D comprises a formula selected from any one of the formulae in Table A2, or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing.

Table A2

 P1a	 P2a
 P3a	



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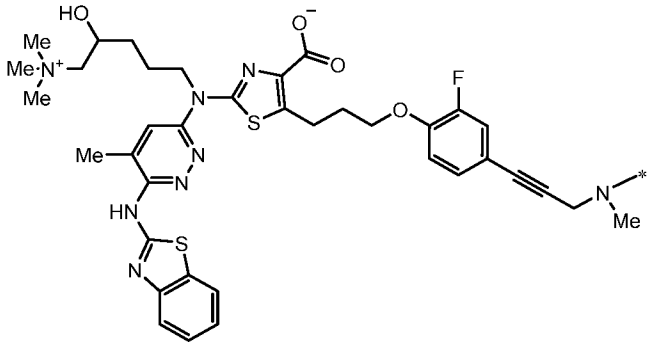

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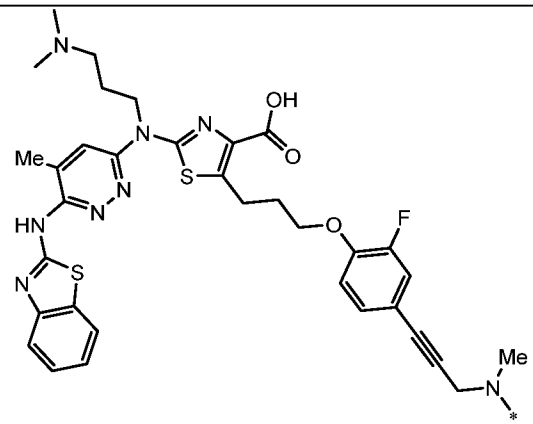

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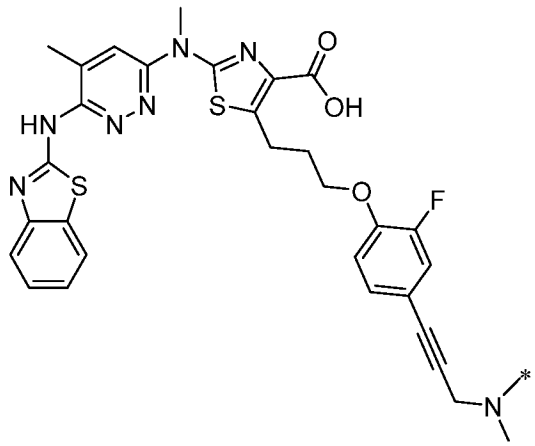

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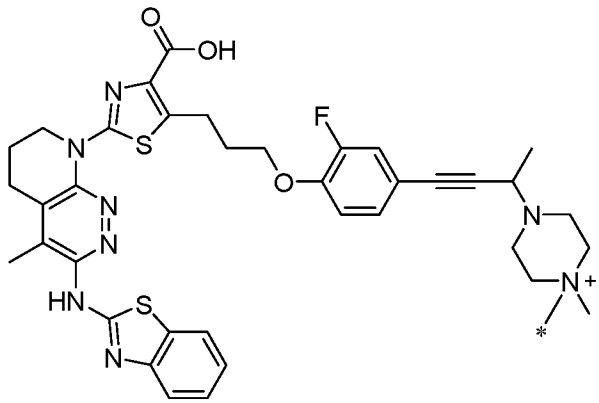

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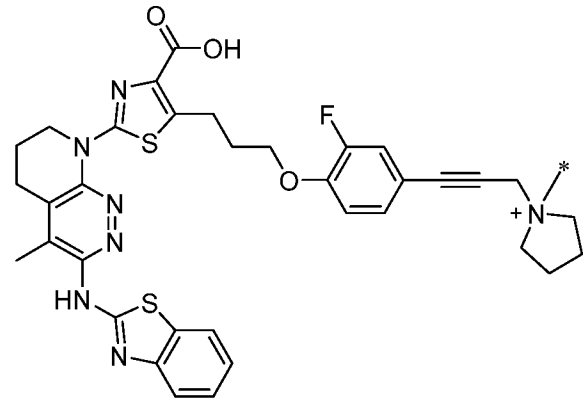
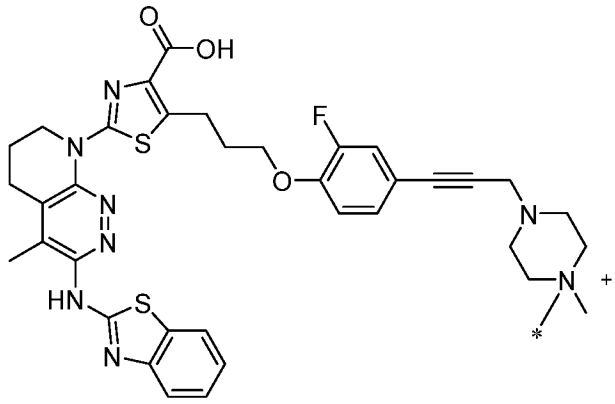
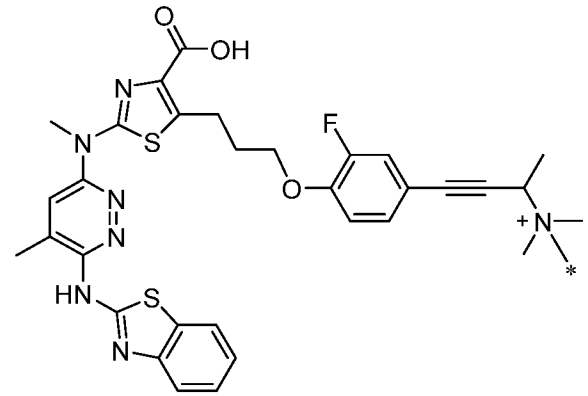
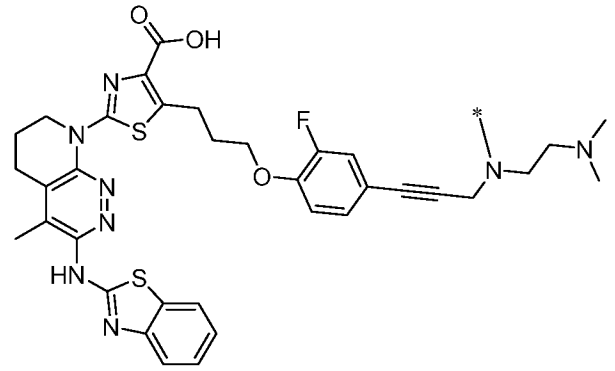
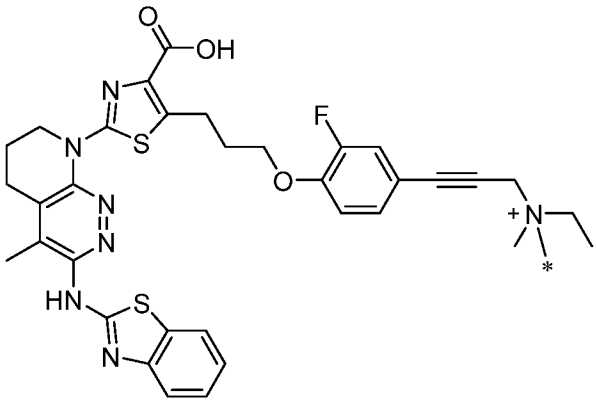
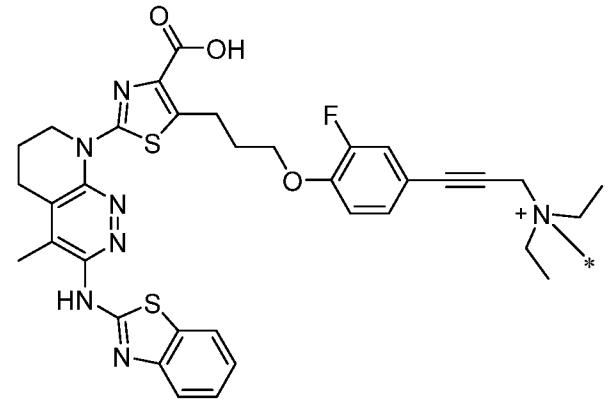
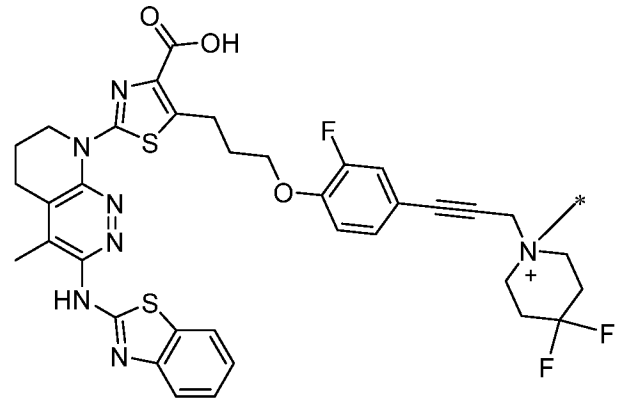
 P9a	 P10a
 P11a	 P12a
 P13a	 P14a



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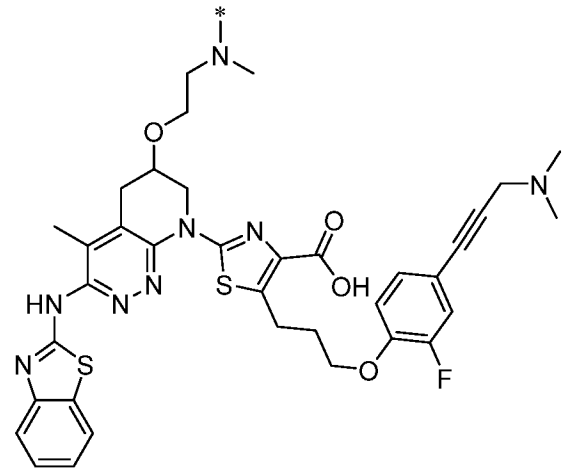

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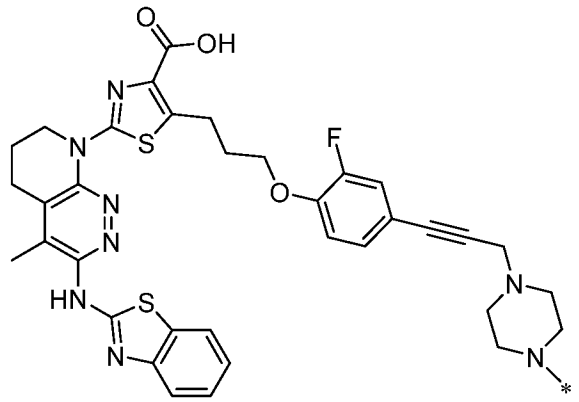

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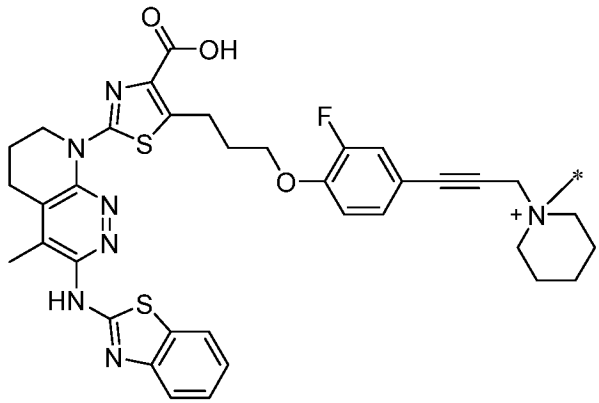

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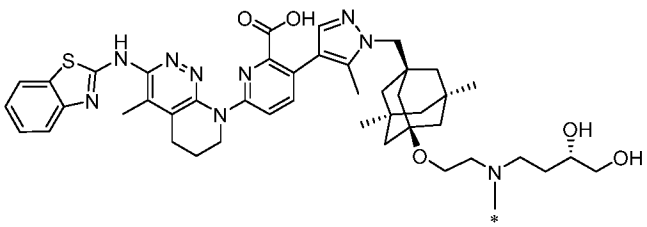

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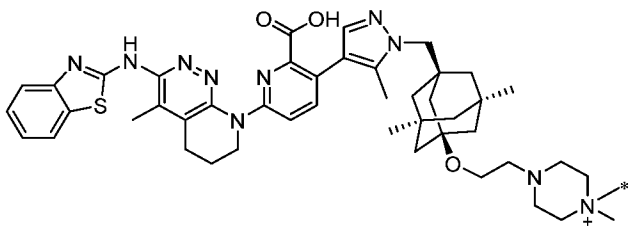

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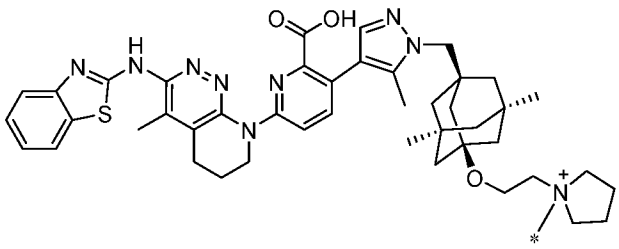
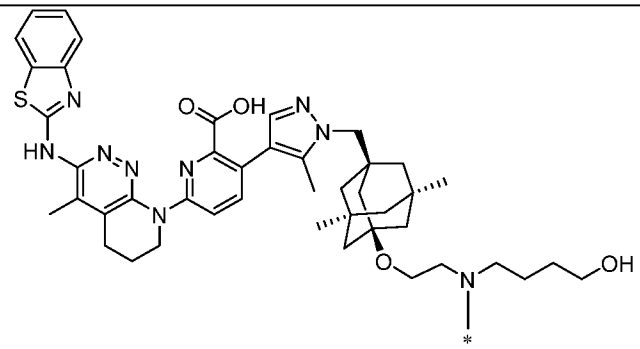
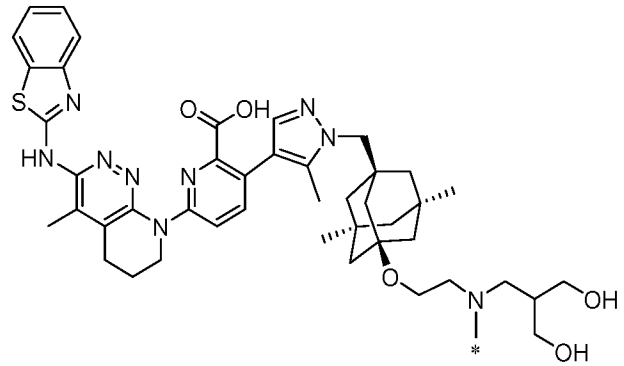
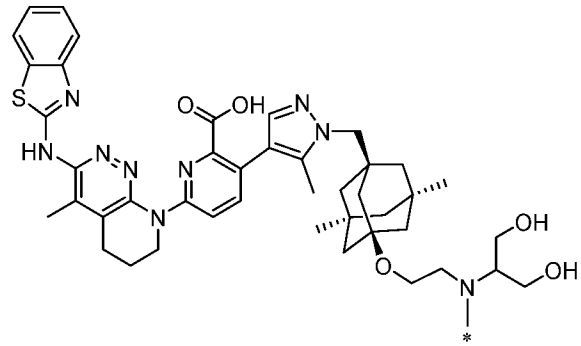
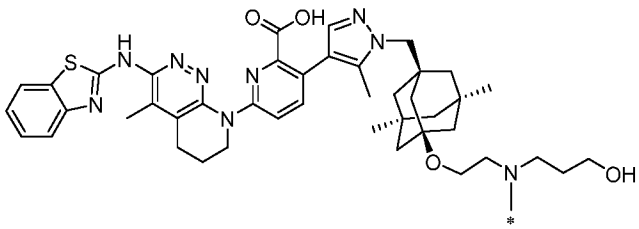
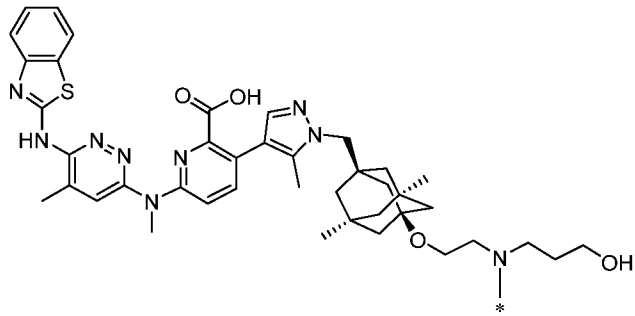
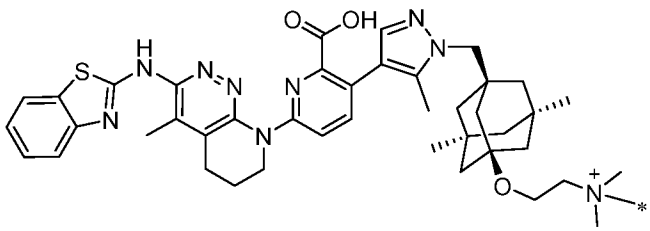
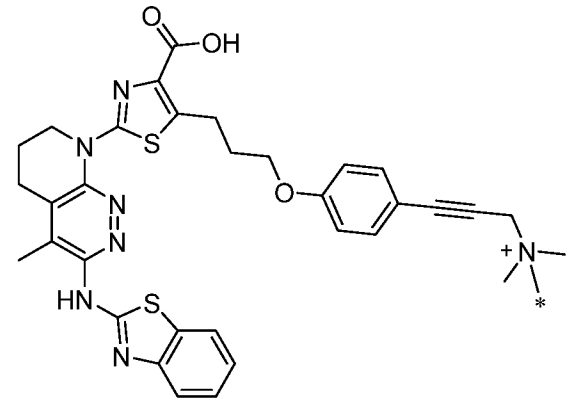
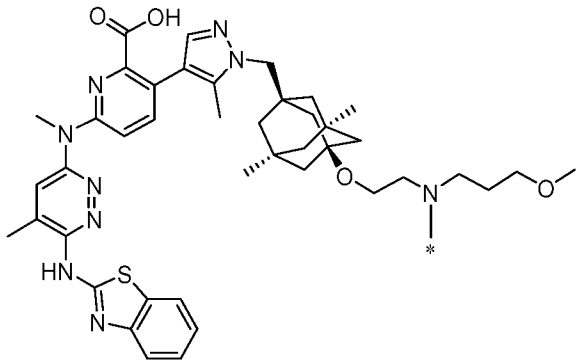
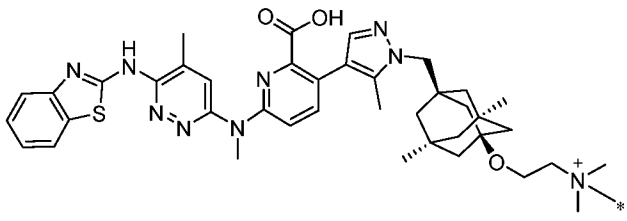
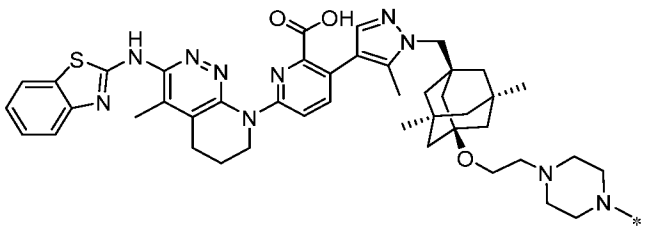
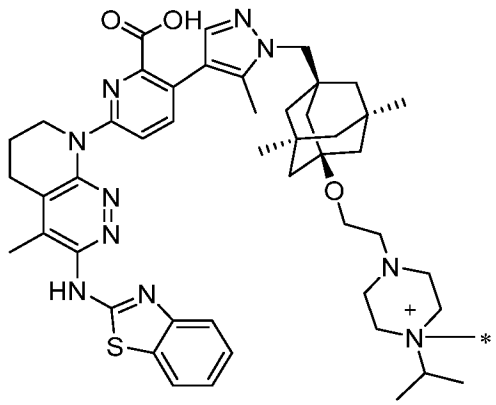
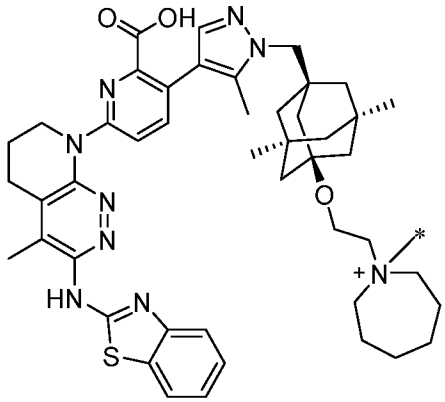


P21a	 P22a
 P23a	 P24a
 P25a	 P26a
 P27a	

	 P28a
 P29a	 P30a
 P31a	 P32a



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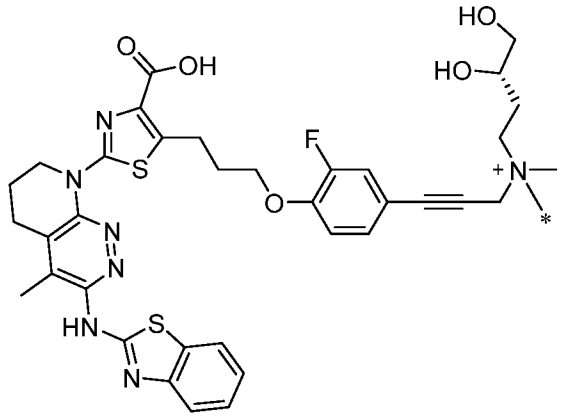

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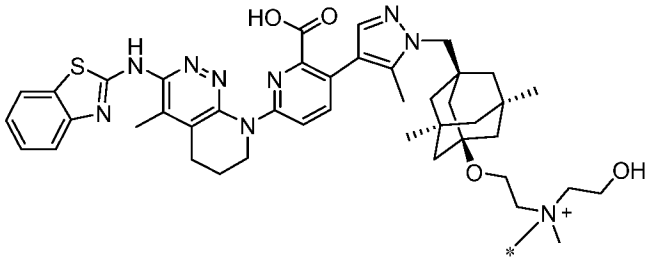

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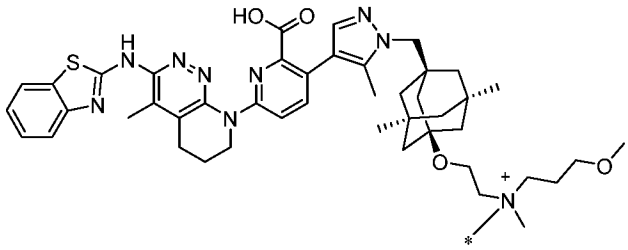
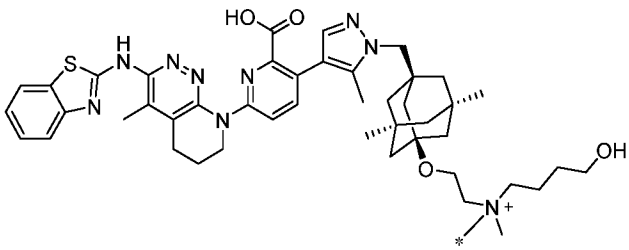


P36a



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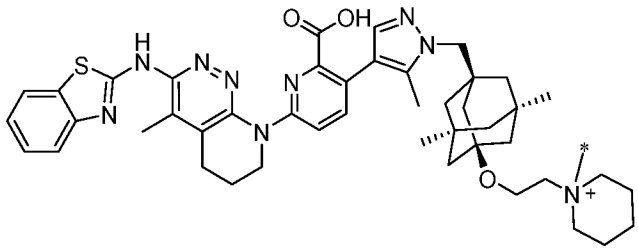

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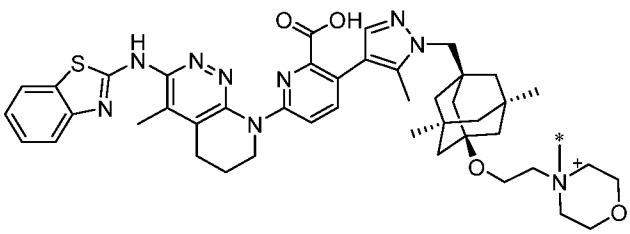

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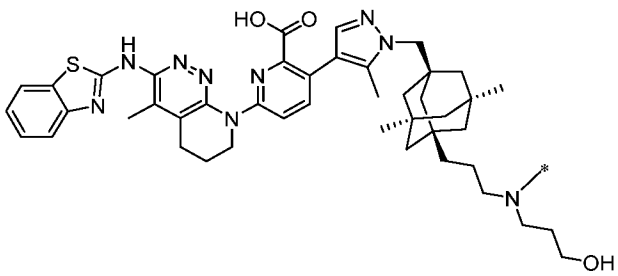

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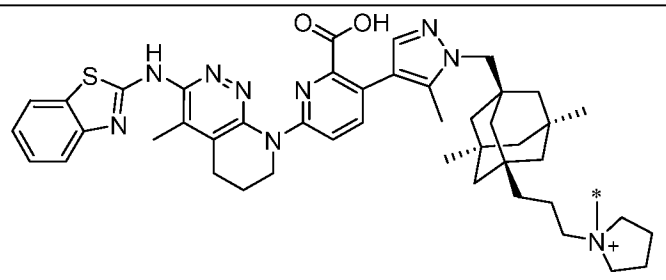

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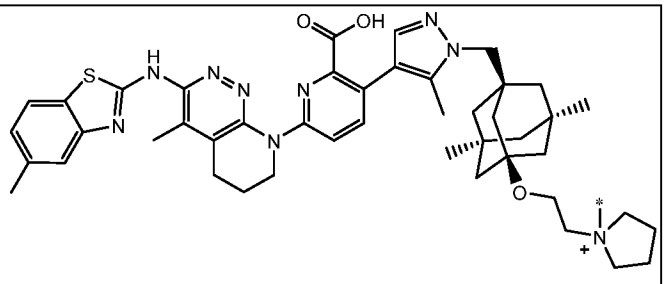
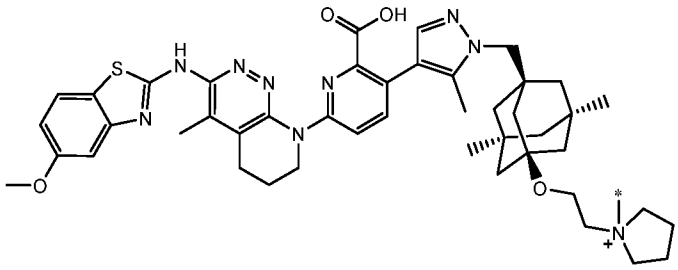


P42a



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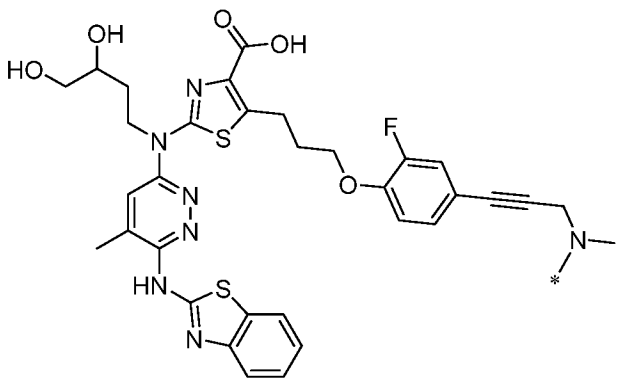

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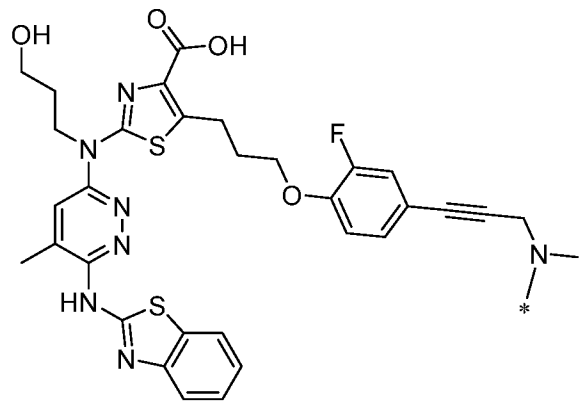

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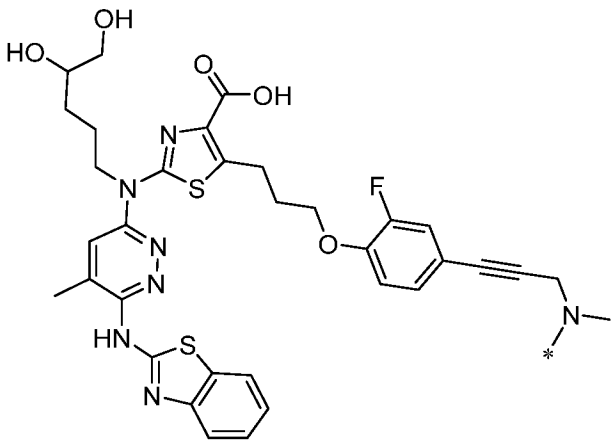

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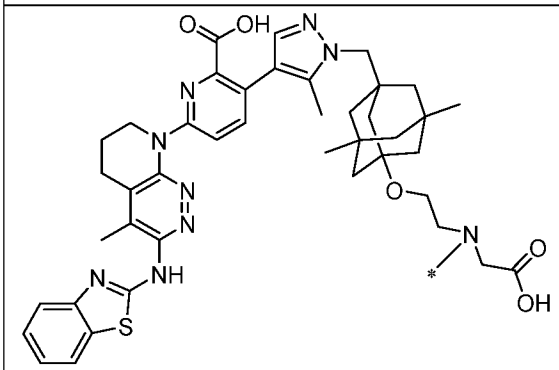

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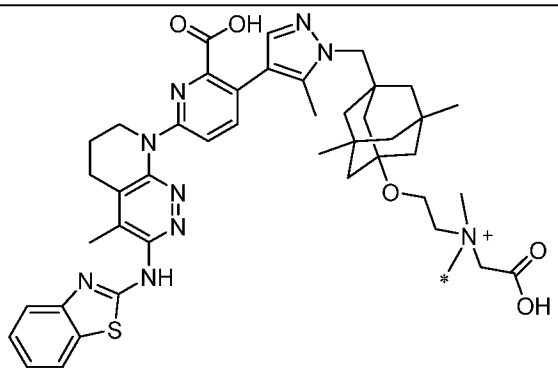

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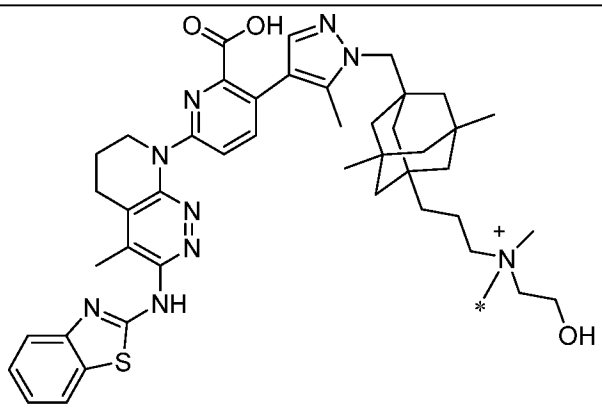
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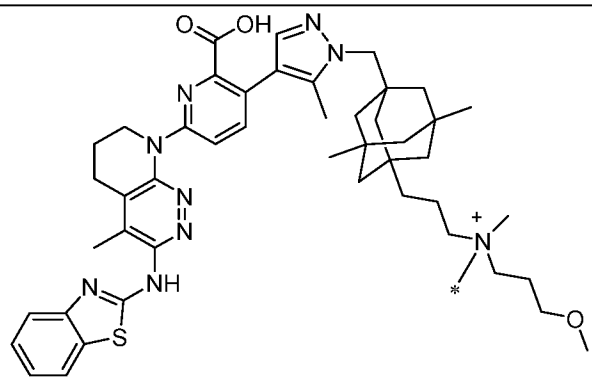
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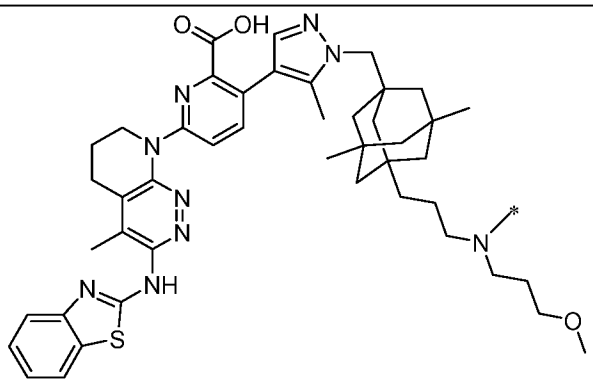
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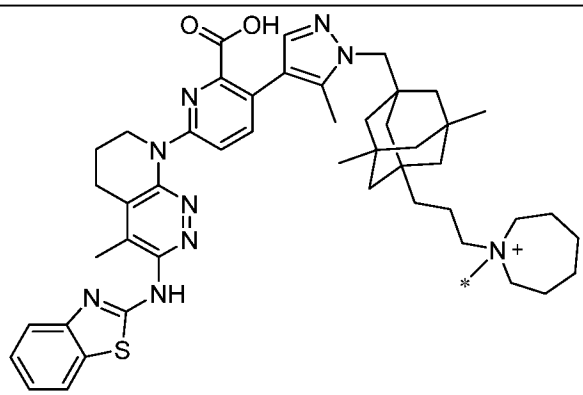
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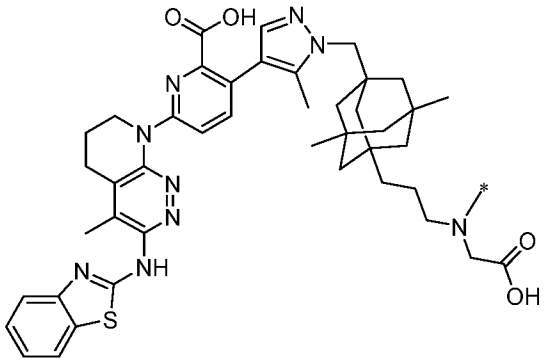
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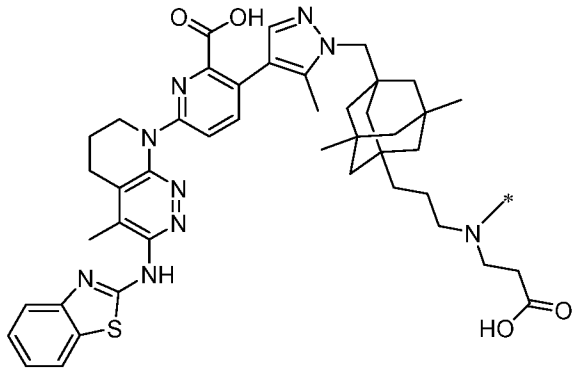
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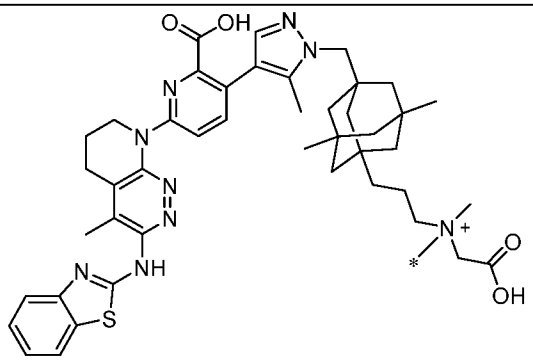
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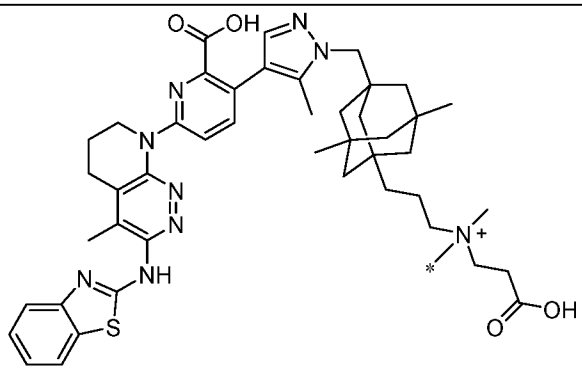
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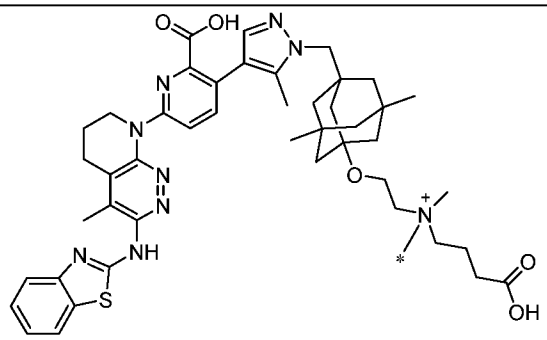

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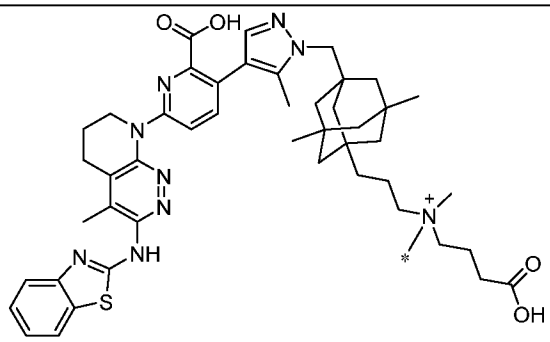

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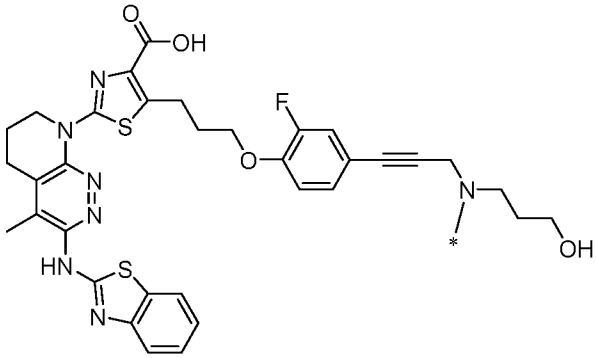

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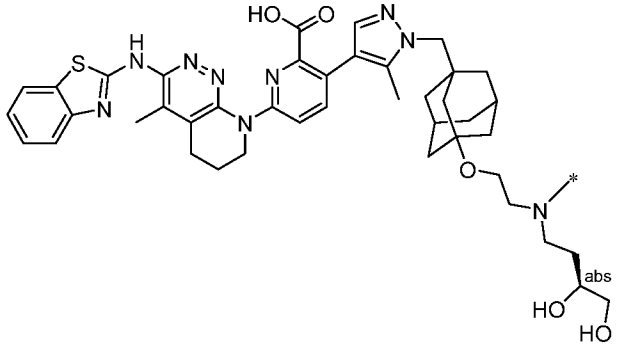

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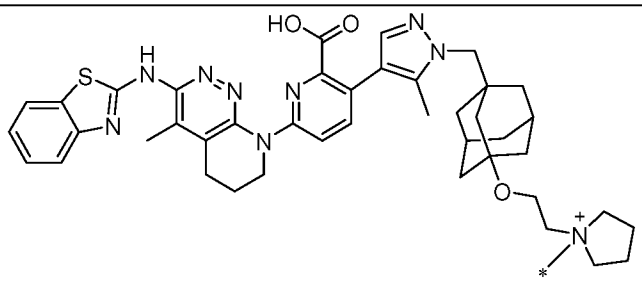

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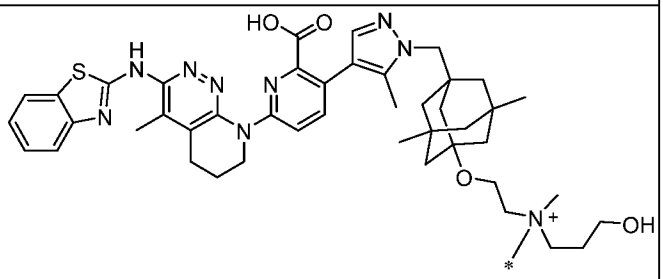

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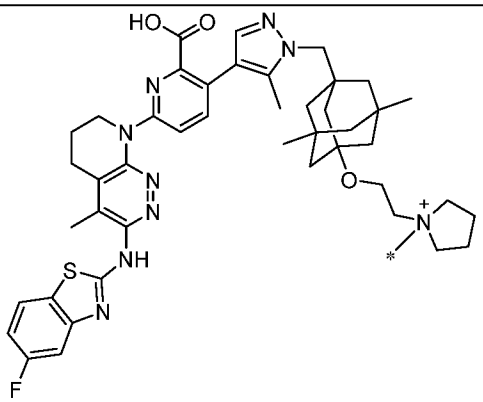

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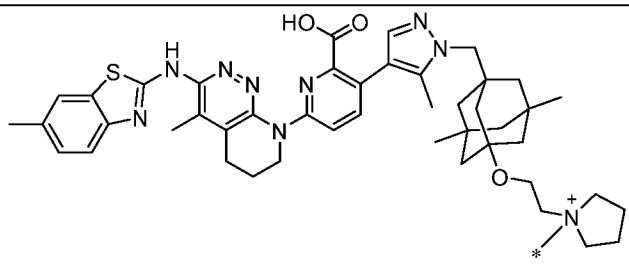

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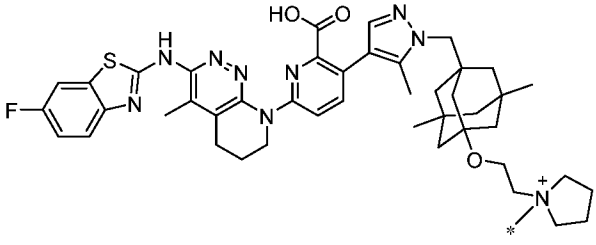

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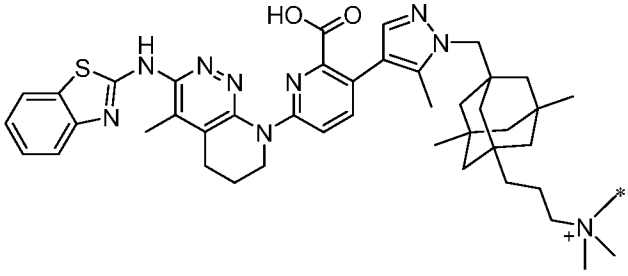

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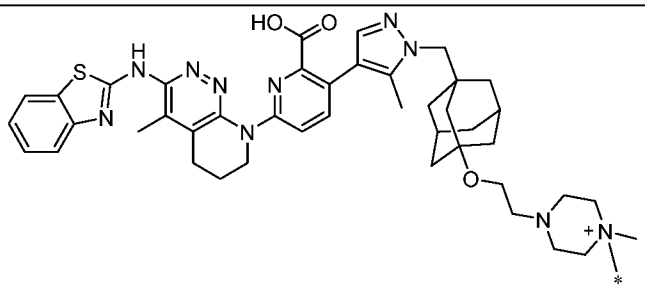
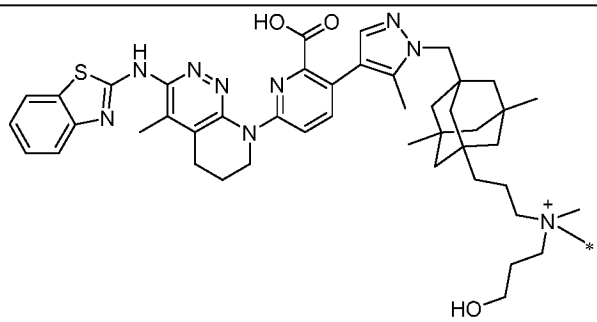


P67a



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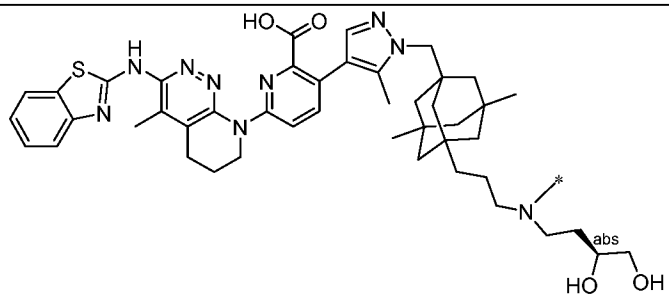

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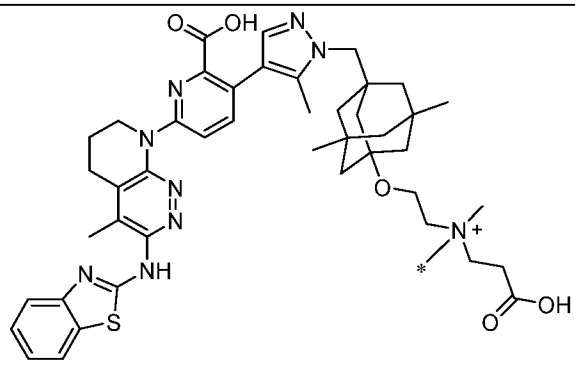

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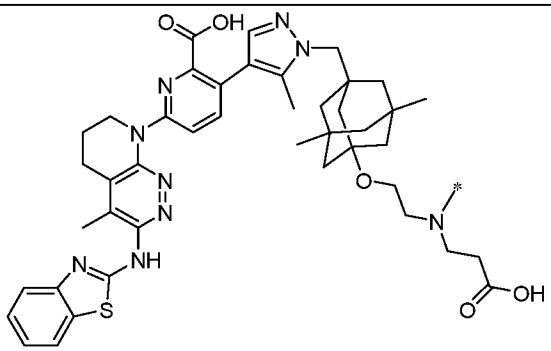

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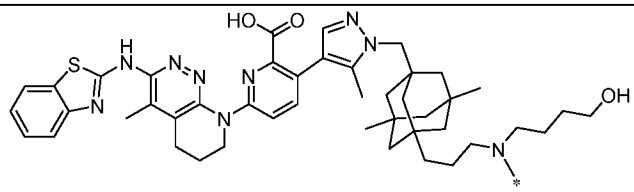

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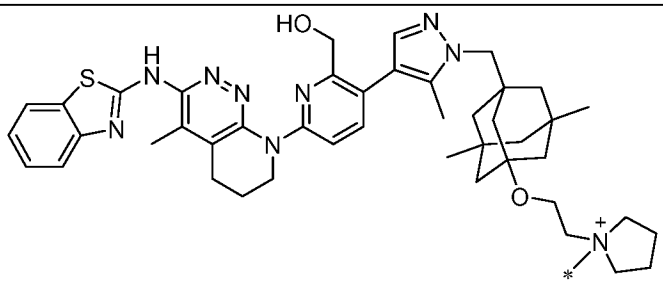
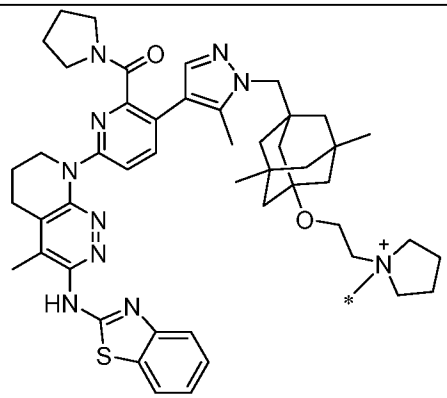
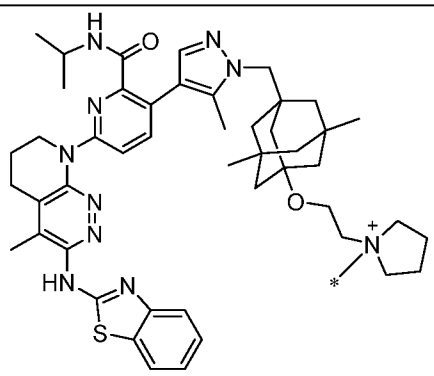
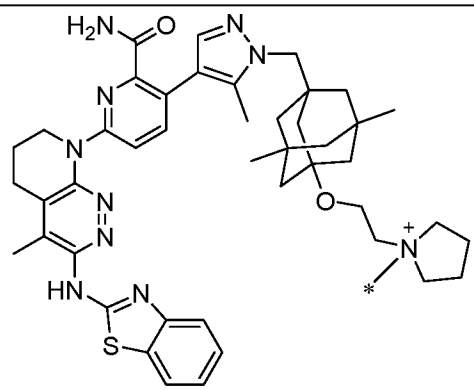


P73a



P74a

 P75a	 P76a

wherein ——* represents a bond to the linker.

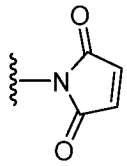
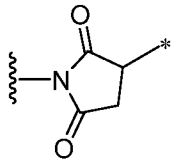
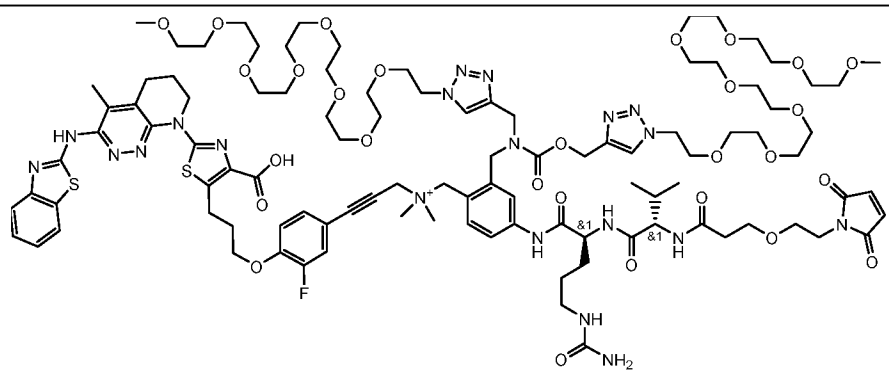
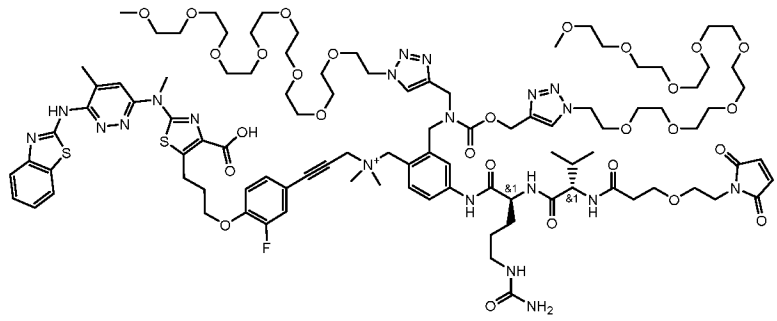
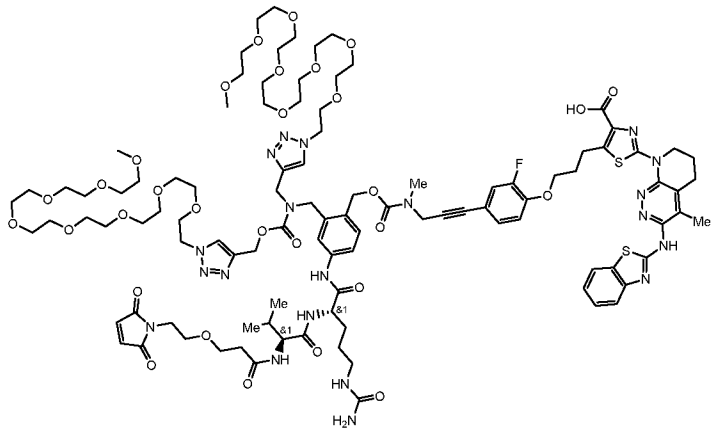
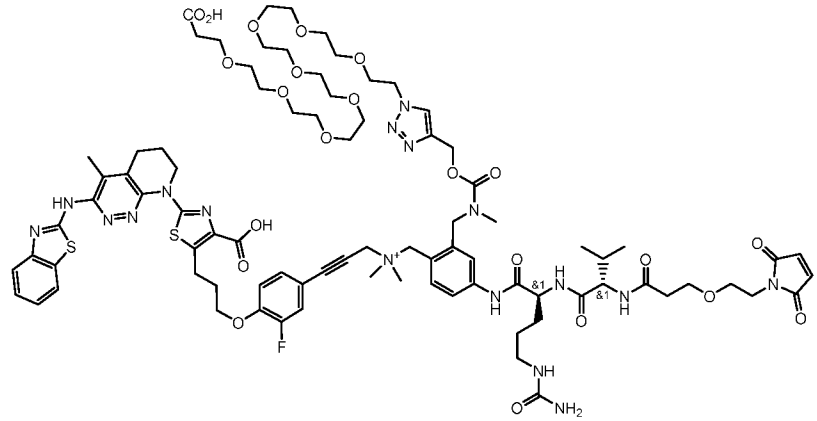
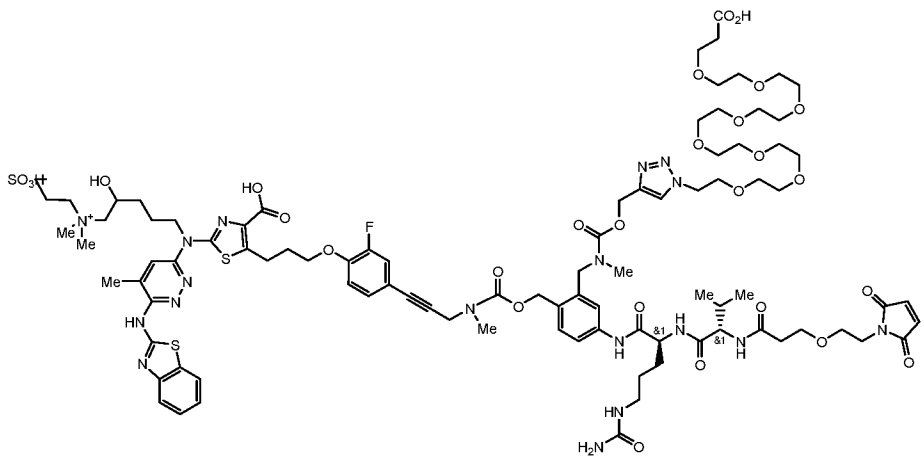
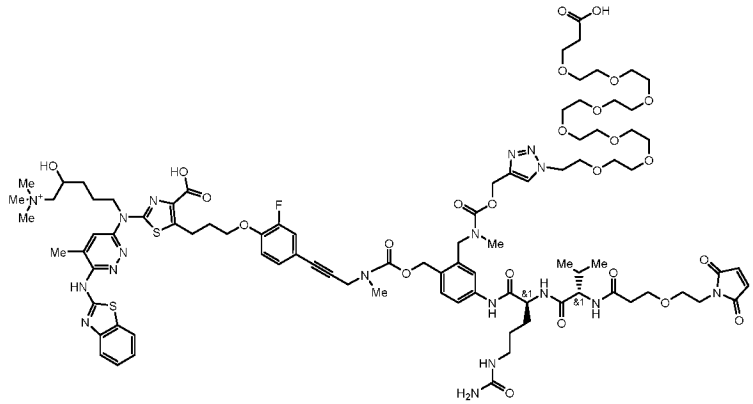
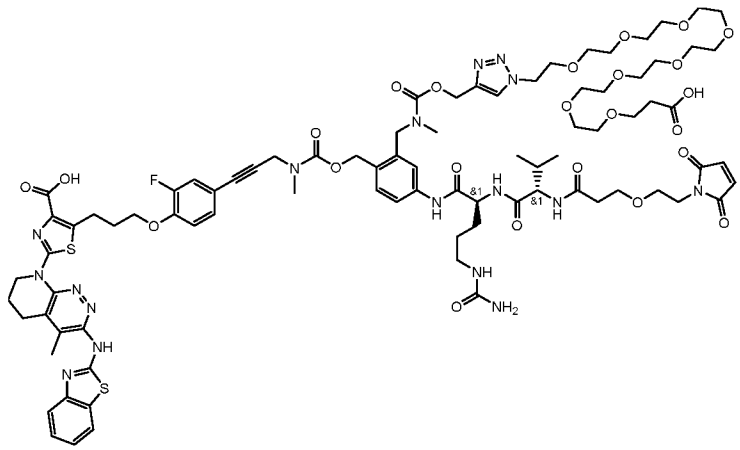
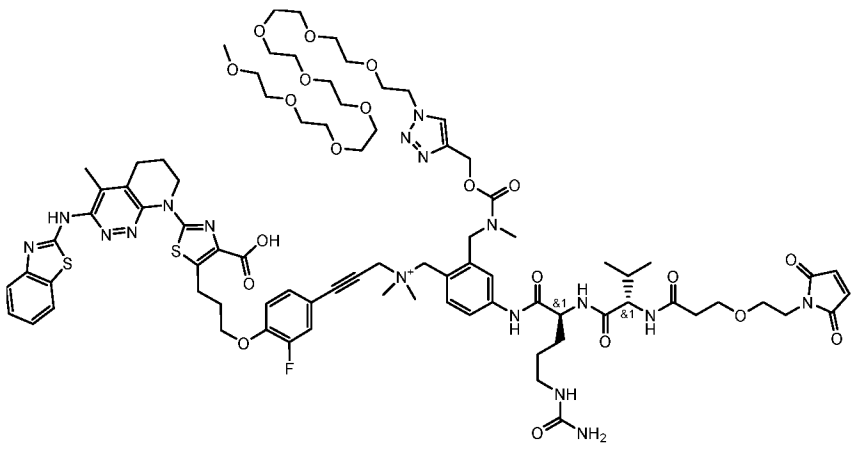
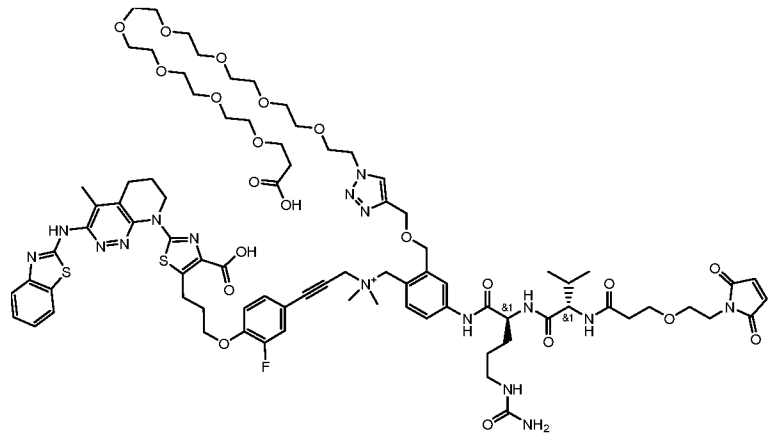
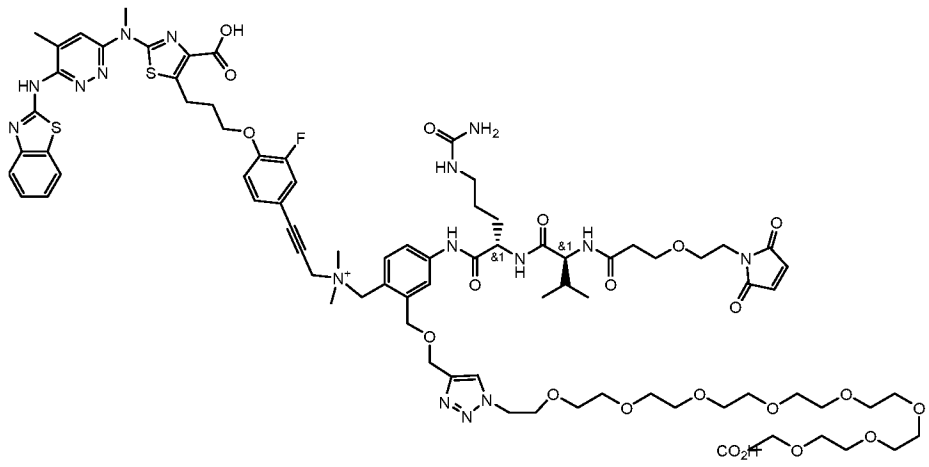
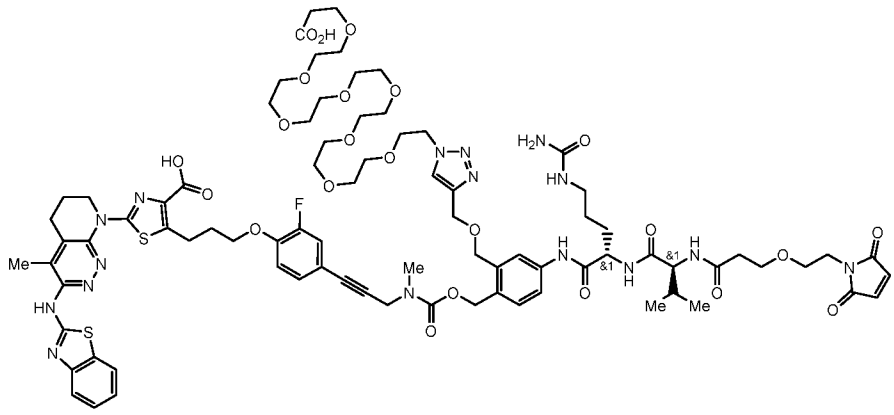
[95] In some embodiments, -(L-D) is formed from a compound selected from Table B or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt thereof. In some embodiments, the maleimide group  in the compound of Table B form a covalent bond with the antibody or antigen-binding fragment thereof (Ab) to form the ADC compound of formula (1) comprising a  moiety, wherein * indicates the connection point to Ab. For compounds in Table A1, Table A2, Table B and Table 1, depending on their electronic charge, these compounds can contain one pharmaceutically acceptable monovalent anionic counterion M_1^-. In some embodiments, the monovalent anionic counterion M_1^- can be selected from bromide, chloride, iodide, acetate, trifluoroacetate, benzoate, mesylate, tosylate, triflate, formate, or the like. In some embodiments, the monovalent anionic counterion M_1^- is trifluoroacetate or formate.

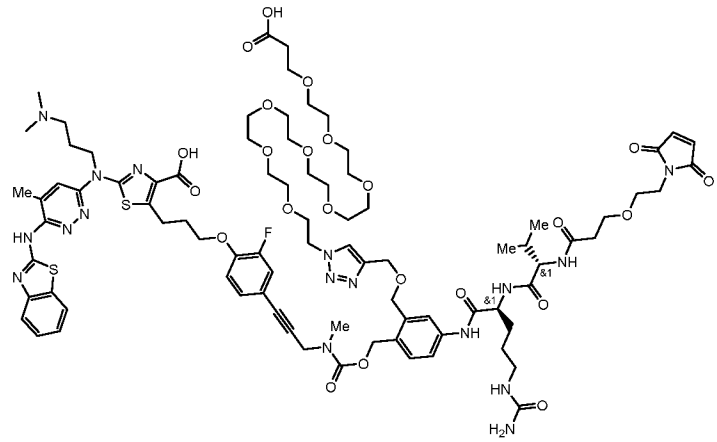
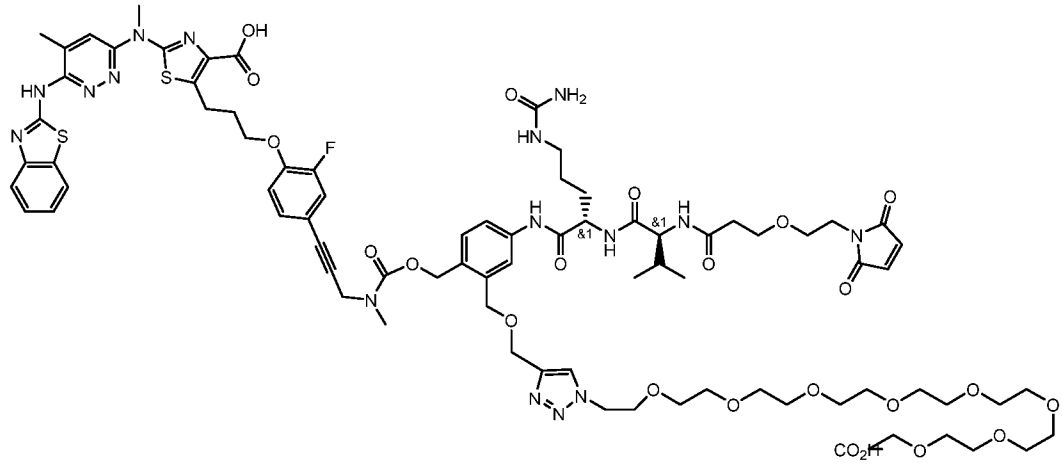
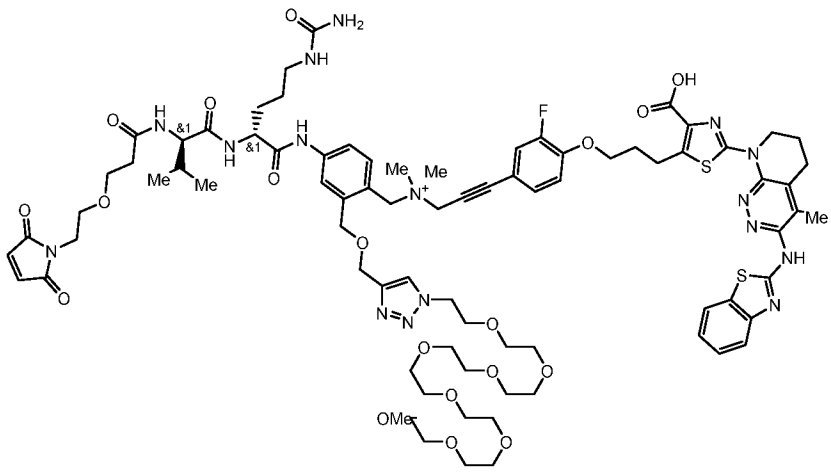
Table B. Exemplary Linker Drug Groups

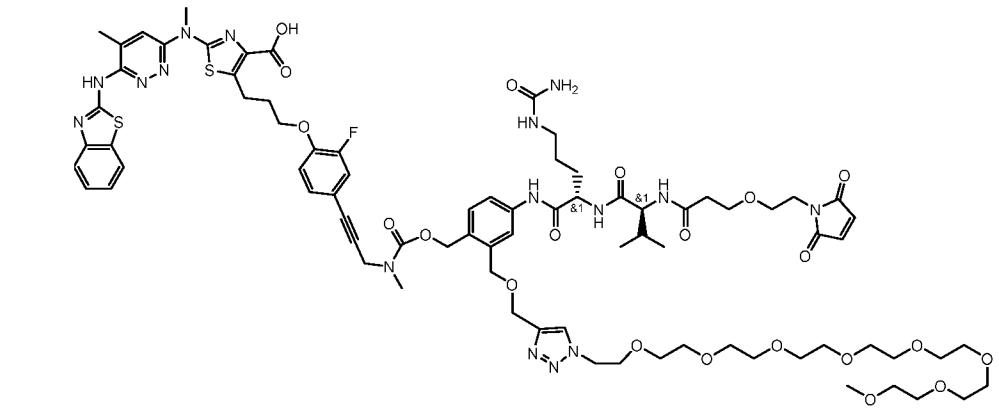
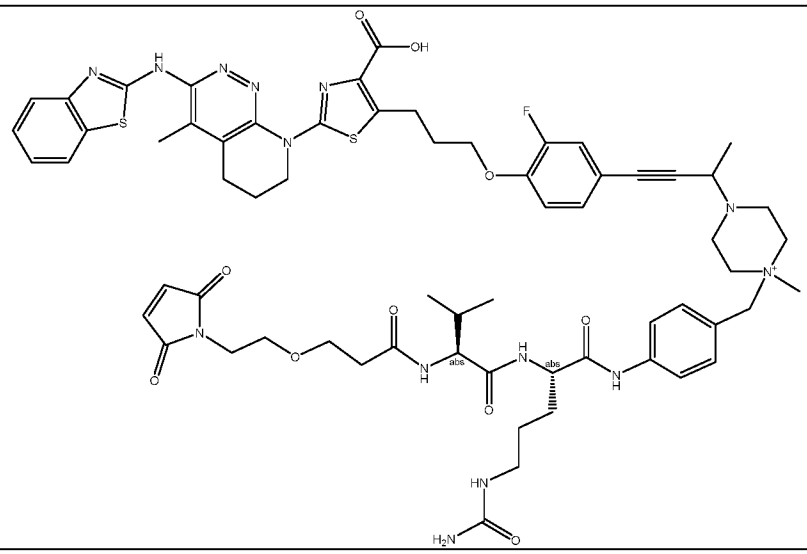
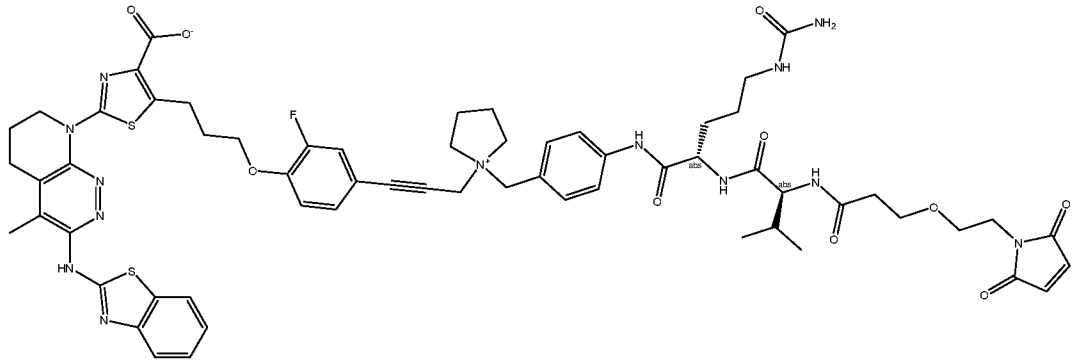
Name	Linker Payload Structure
L1A-P1	

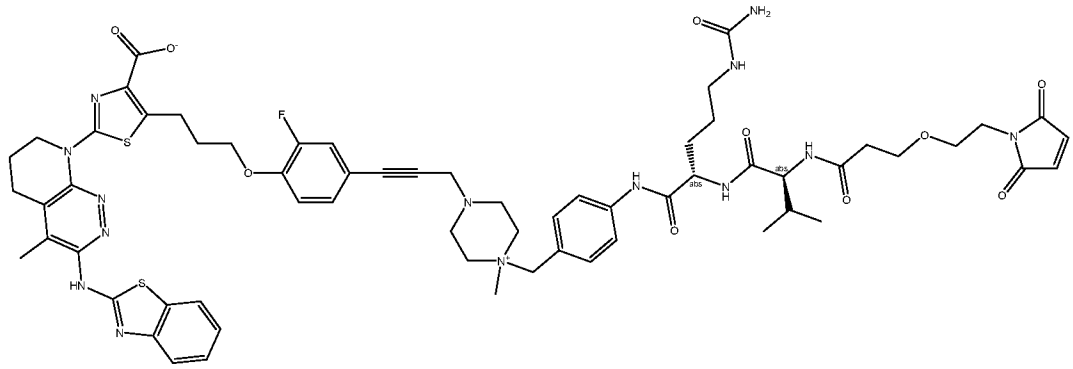
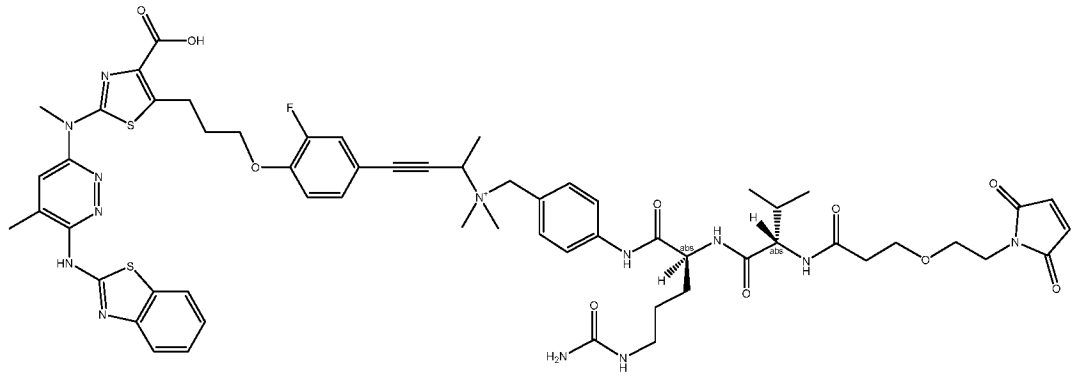
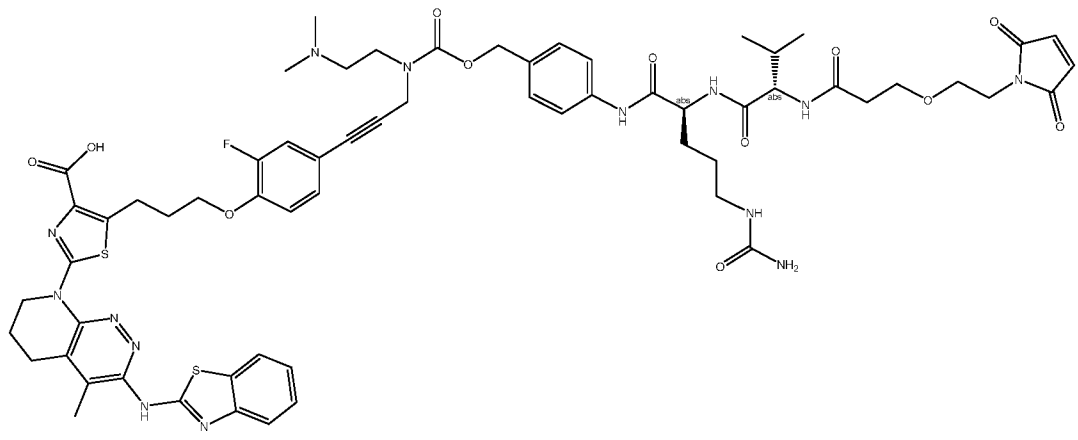
L1A-P2	
L1C-P3	
L3A-P1	
L3C-P4	

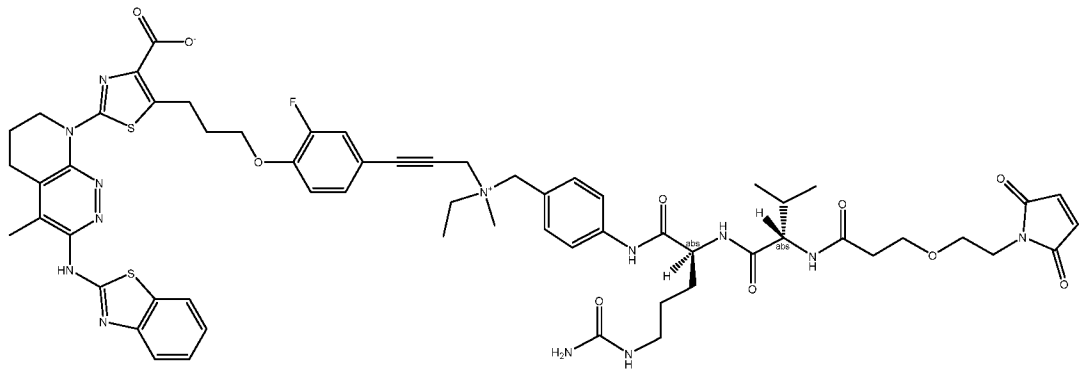
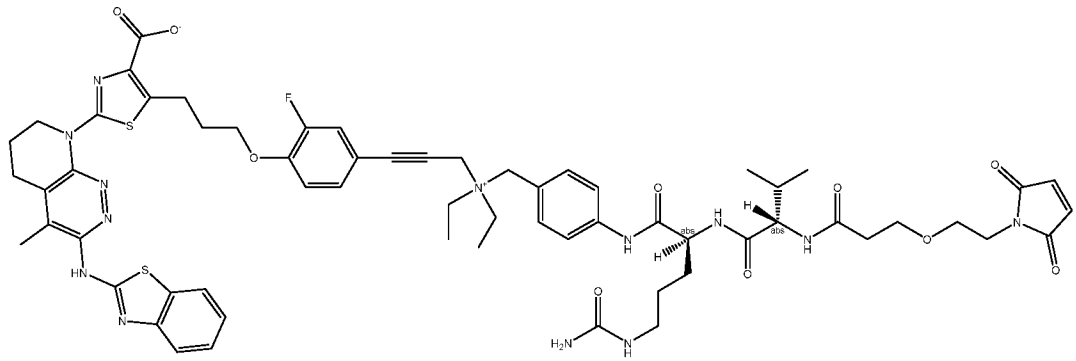
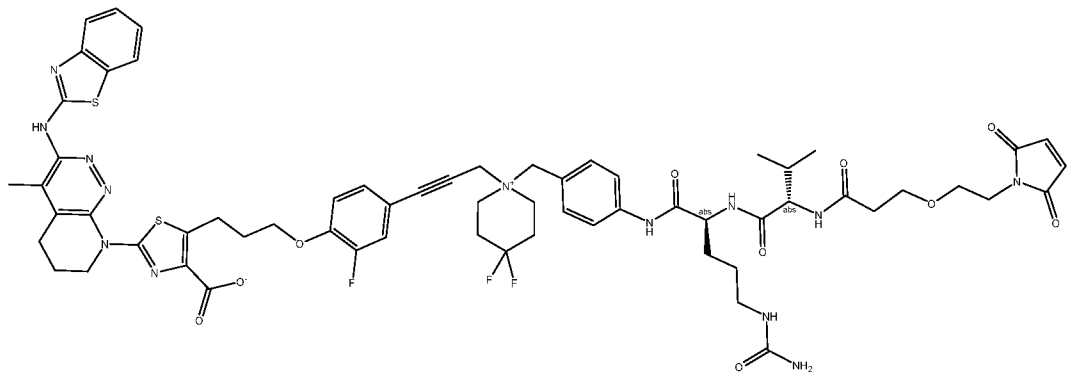
L3C-P5	
L3C-P3	
L4A-P1	

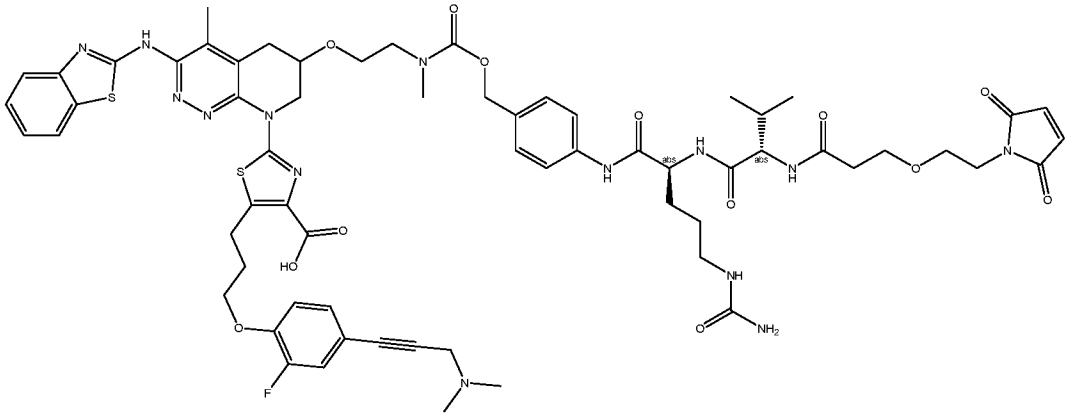
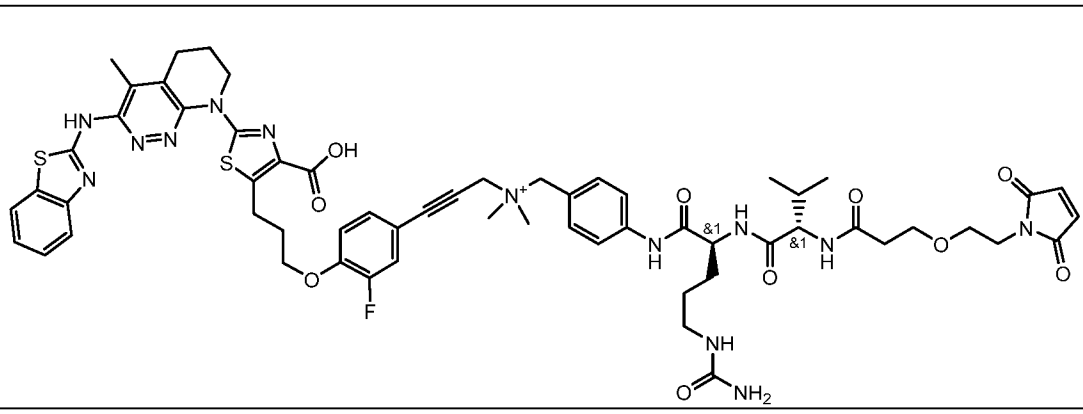
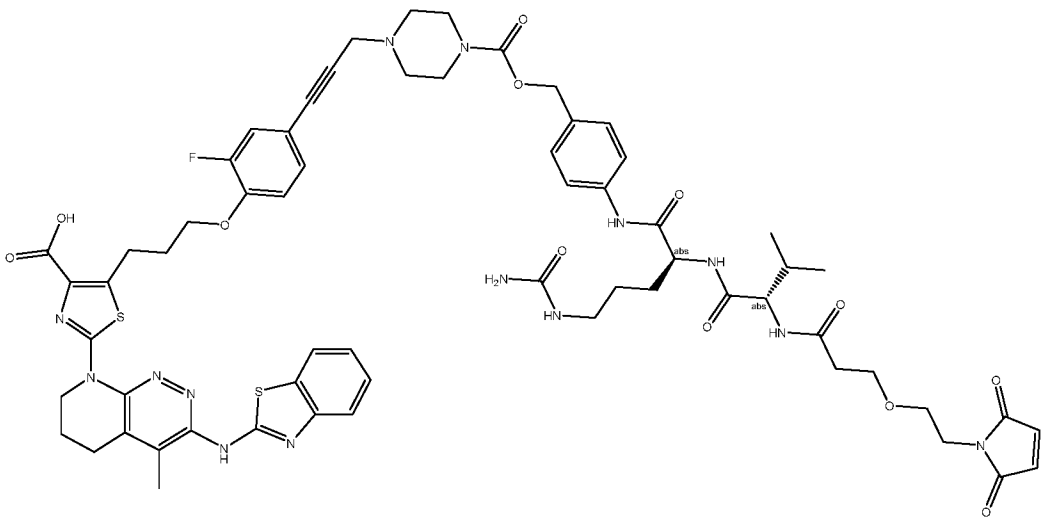
L7A-P1	
L7A-P2	
L7C-P3	

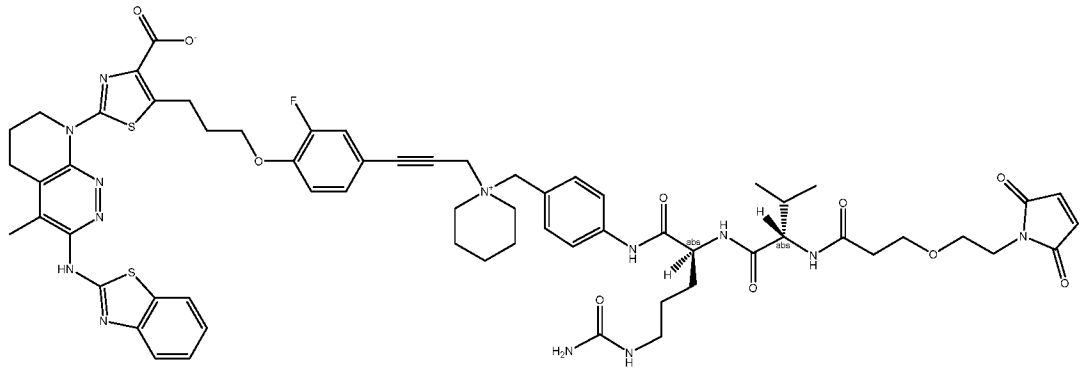
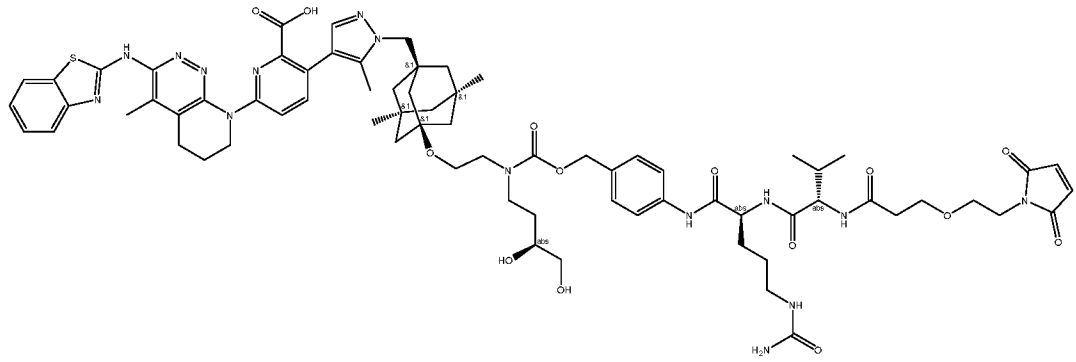
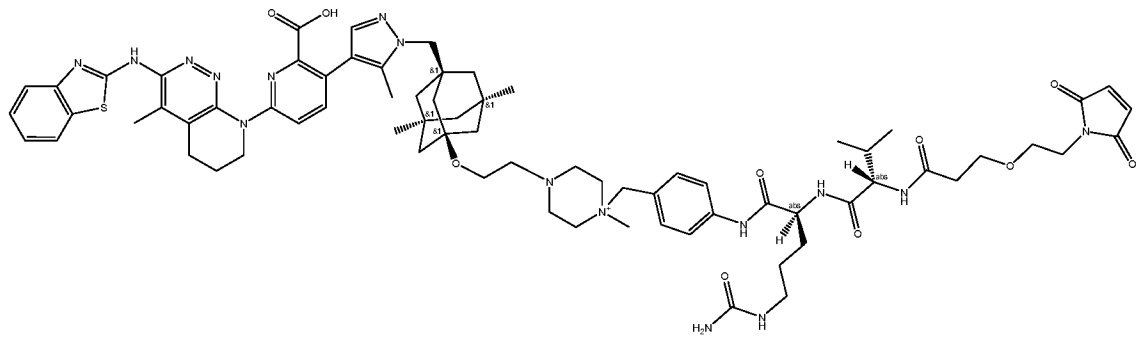
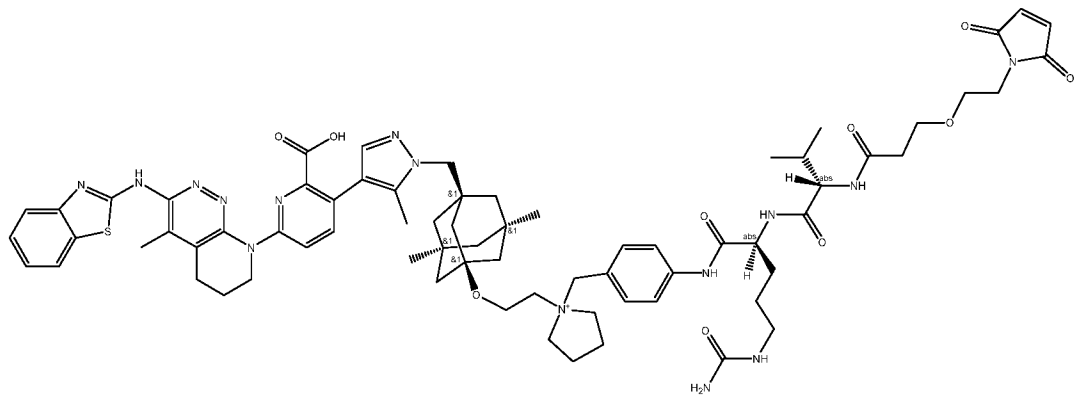
L7C-P6	
L7C-P7	
L8A-P1	

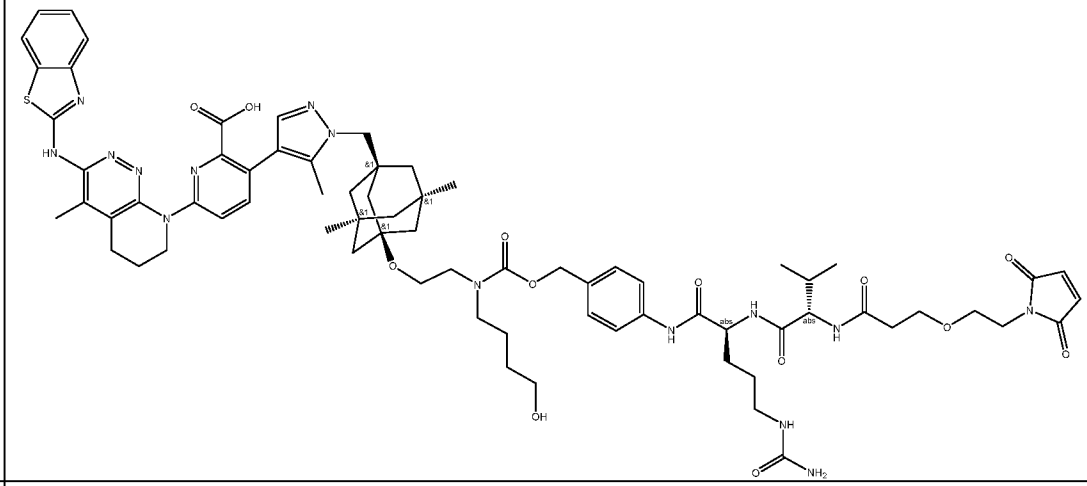
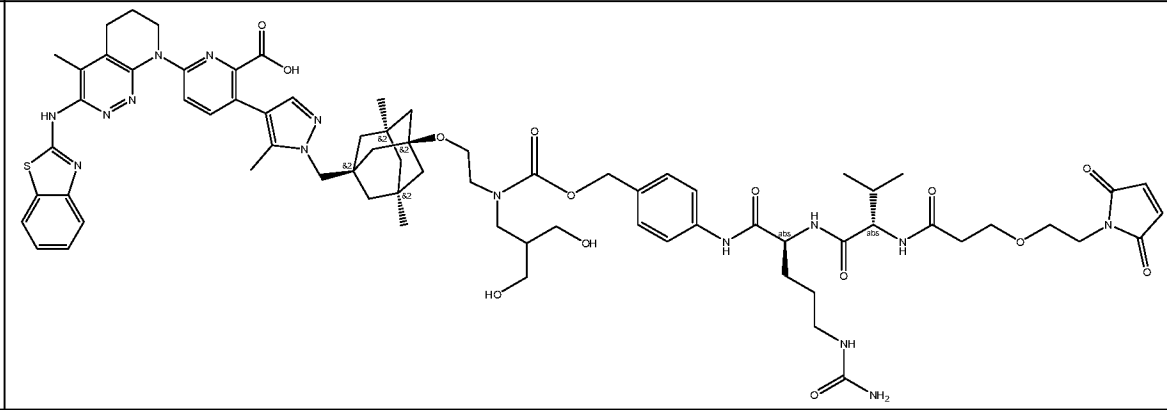
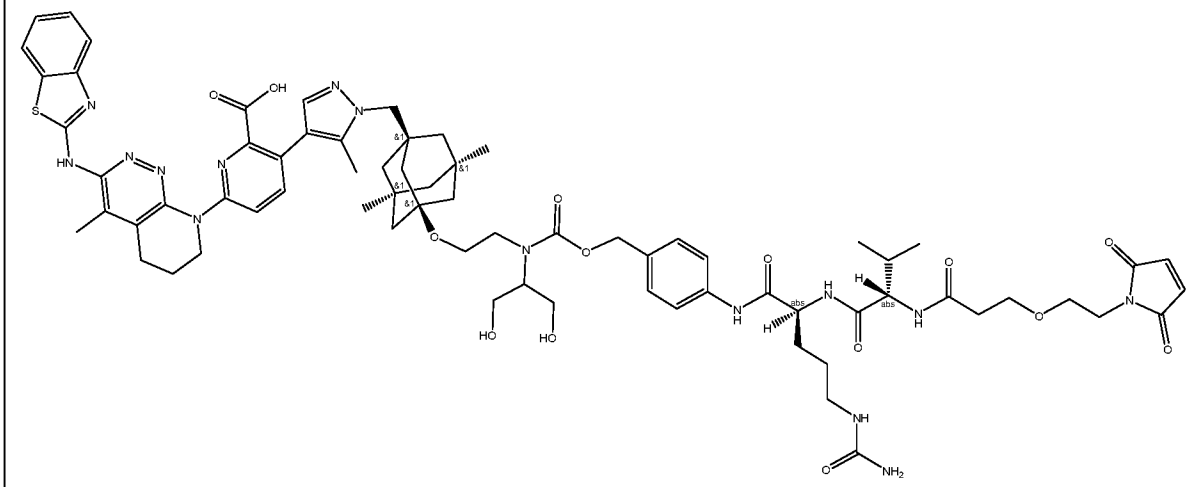
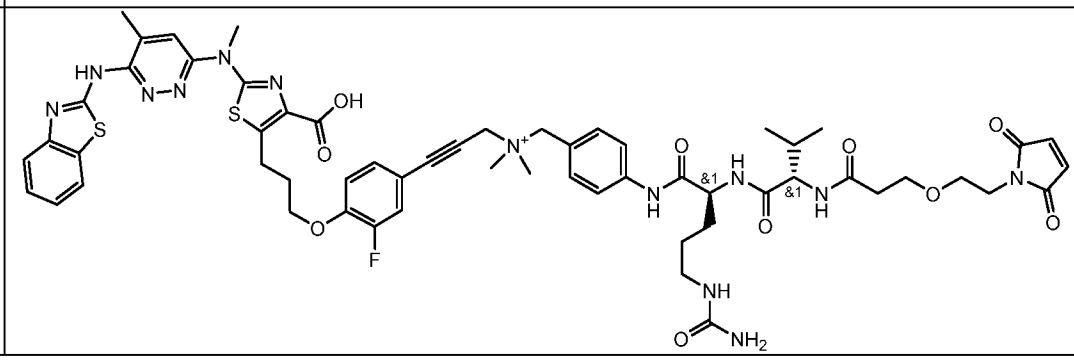
L8C-P7	
L9A-P8	
L9A-P9	

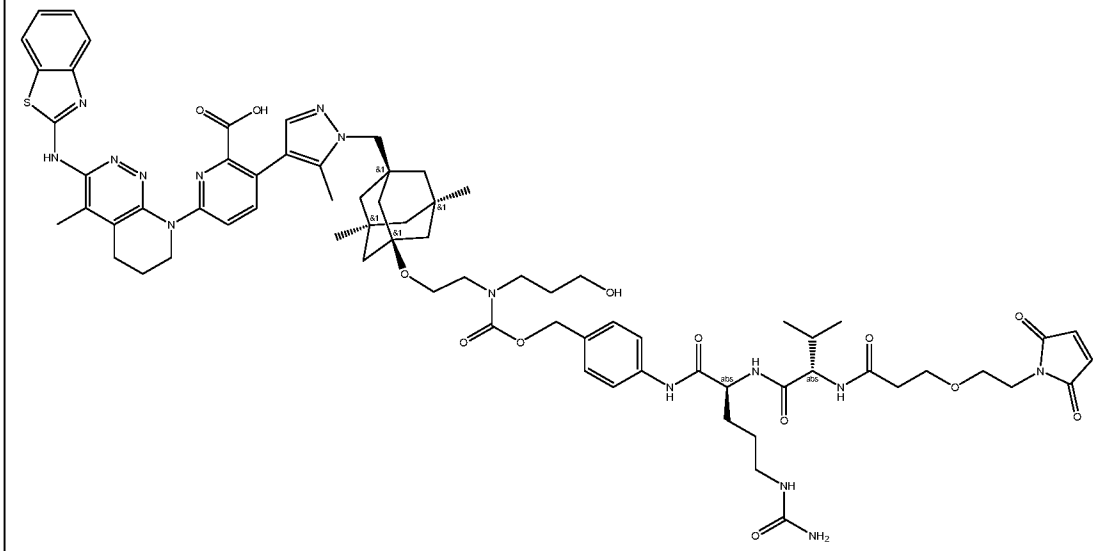
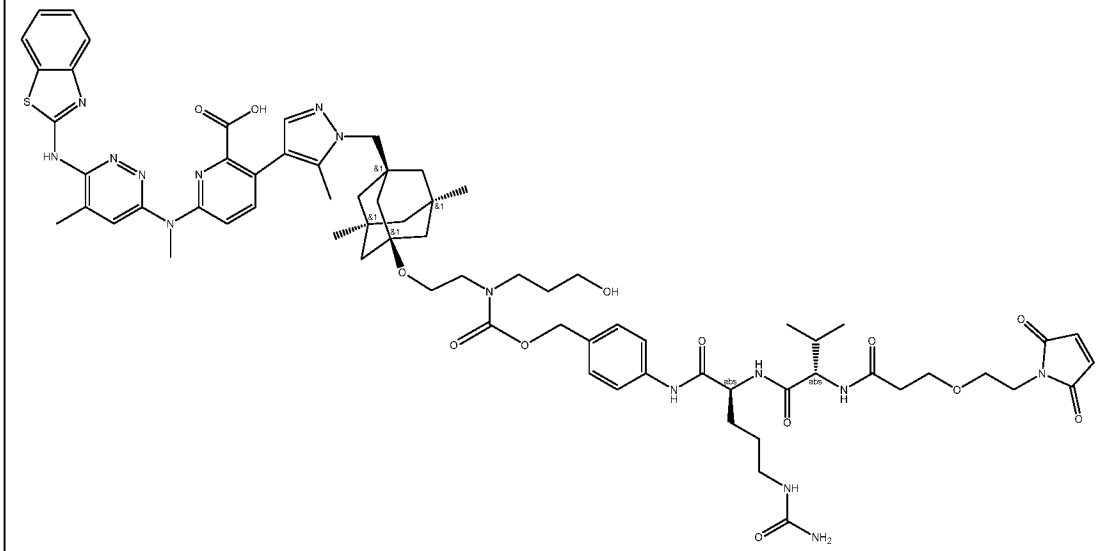
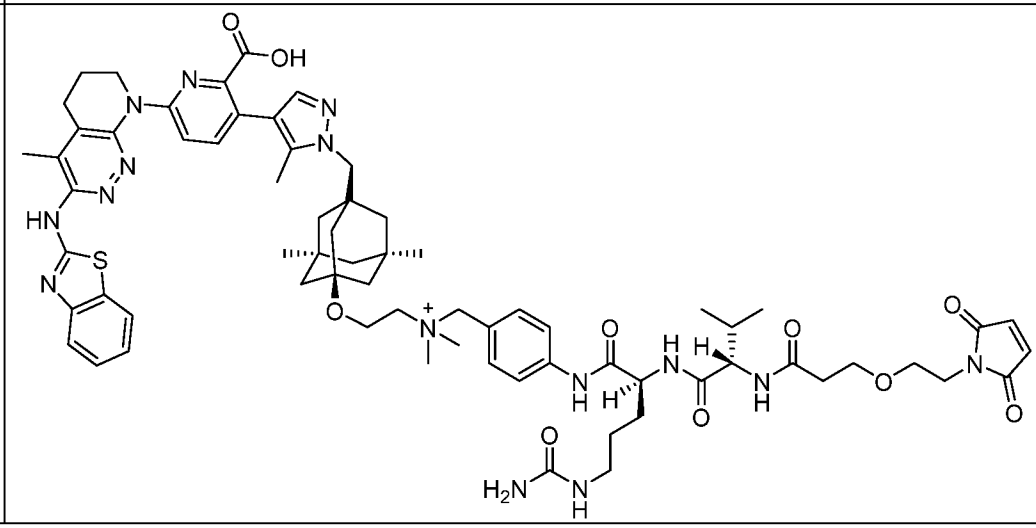
L9A-P10	
L9A-P11	
L9C-P12	

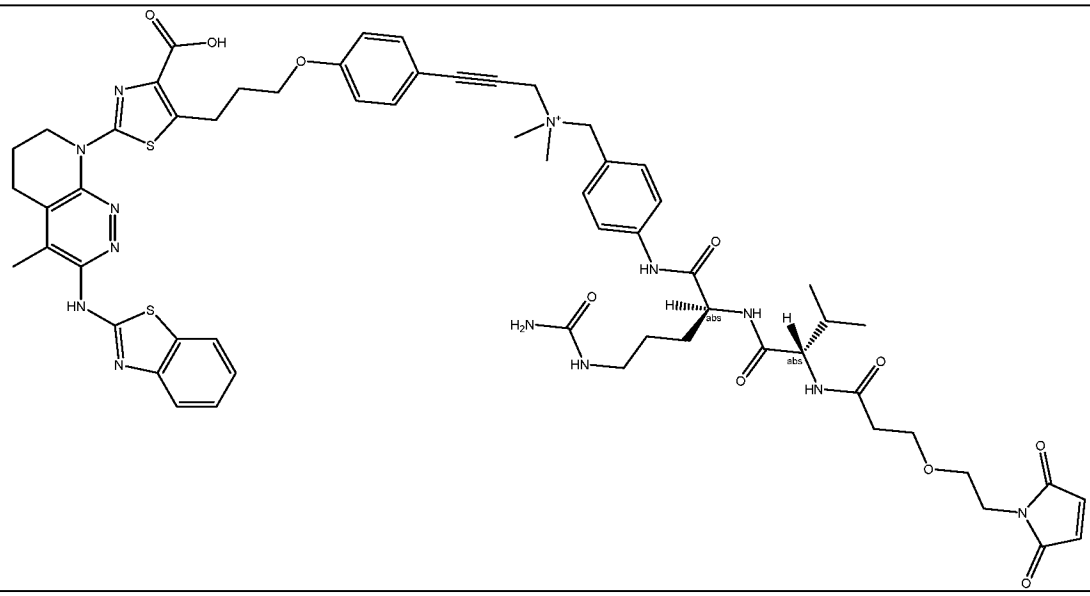
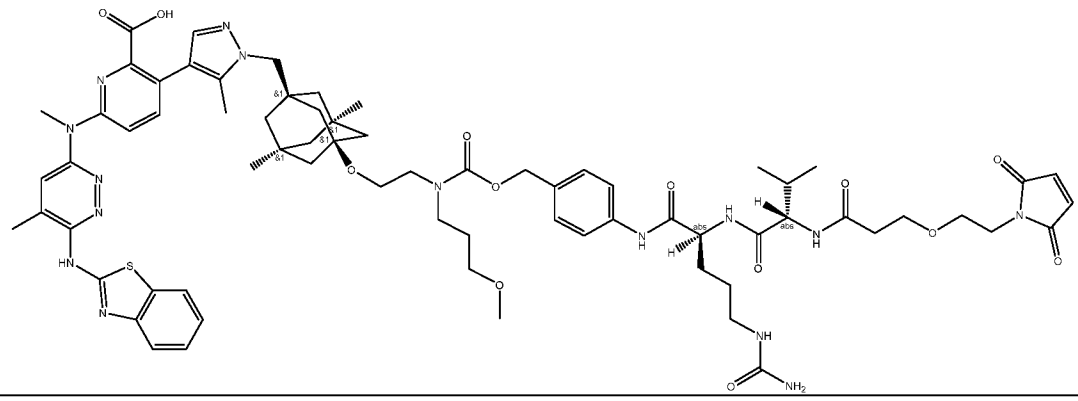
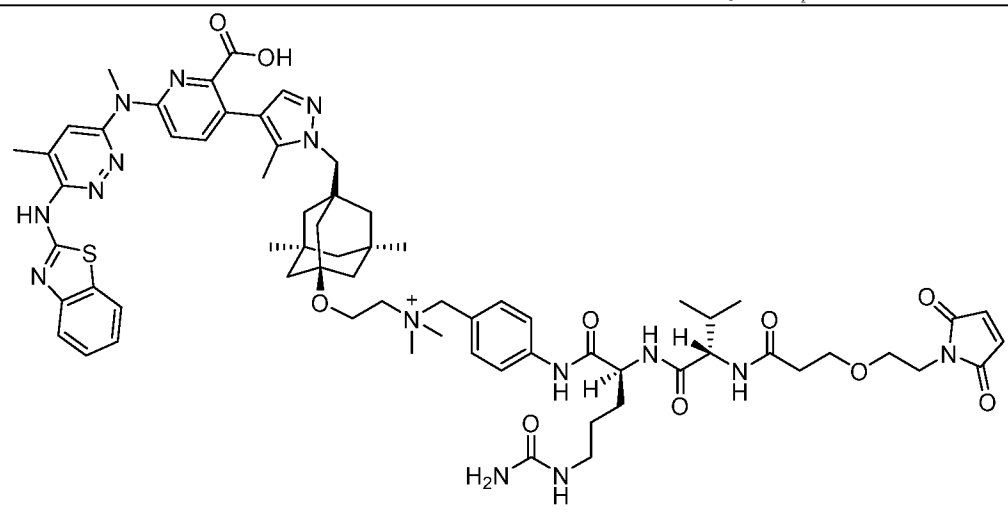
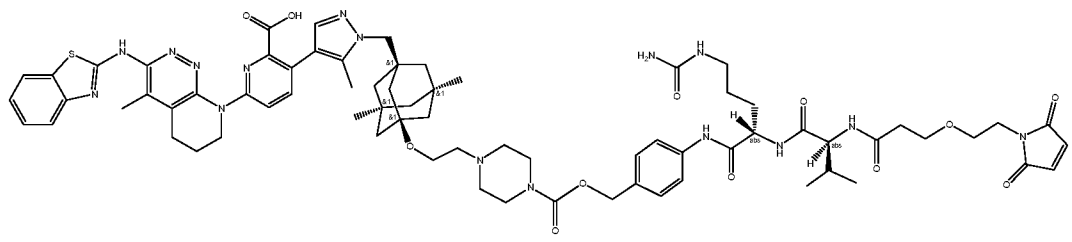
L9A-P13	
L9A-P14	
L9A-P15	

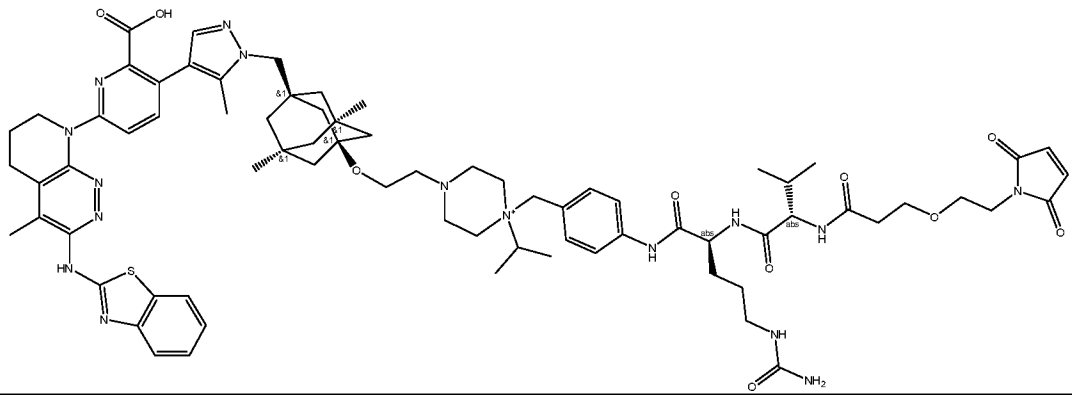
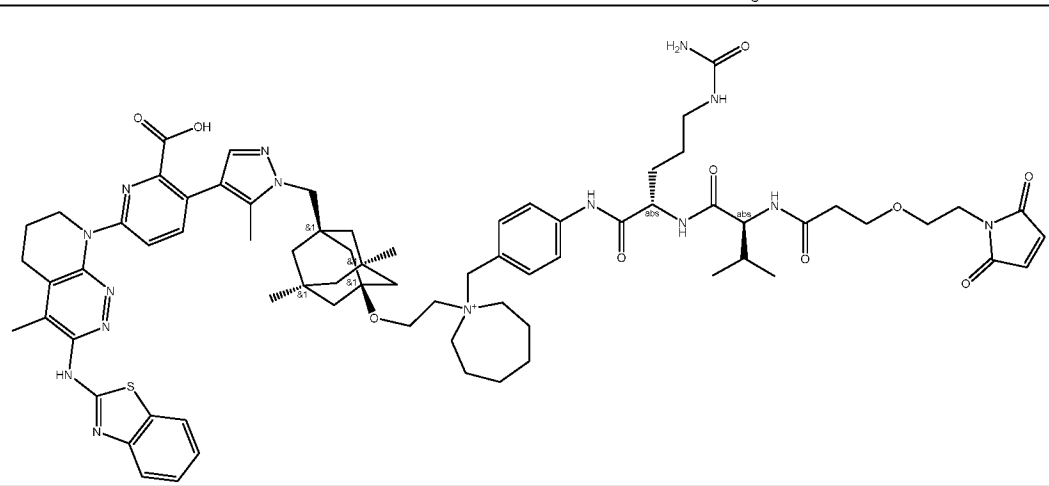
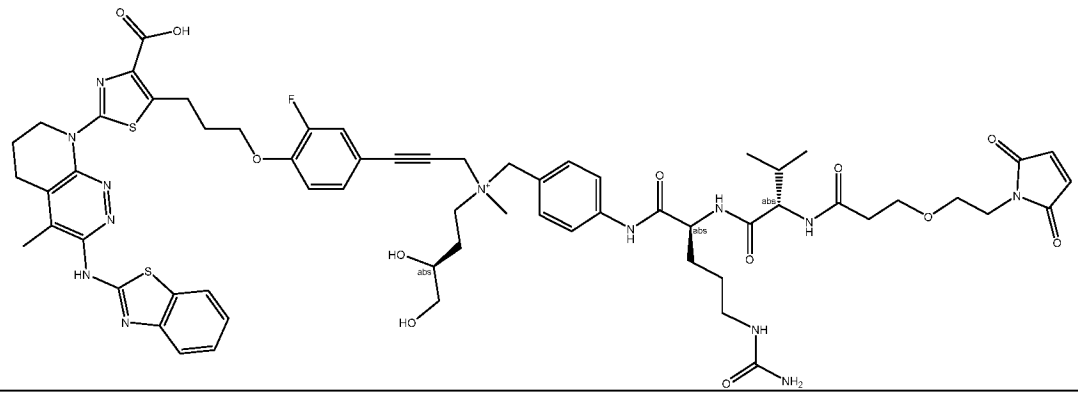
L9C-P16	
L9A-P1	
L9C-P17	

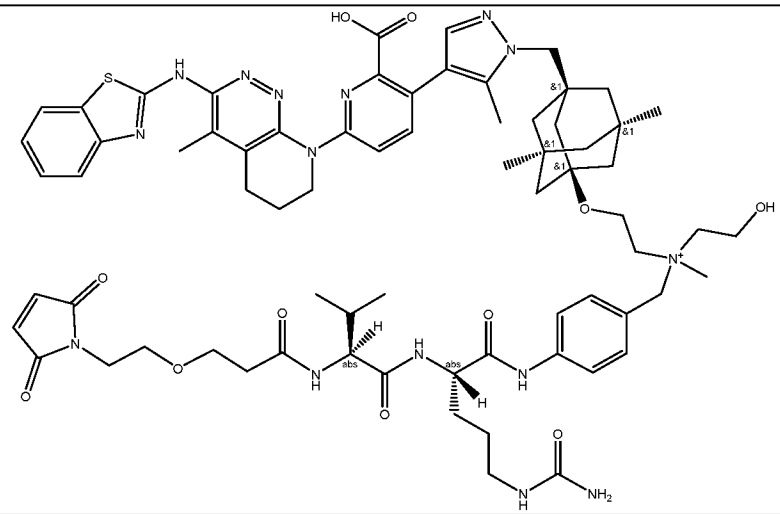
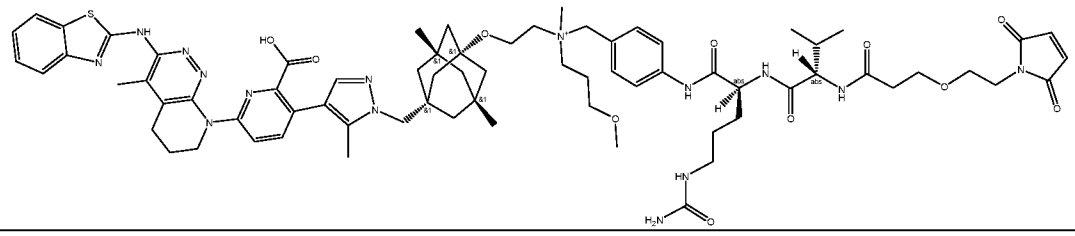
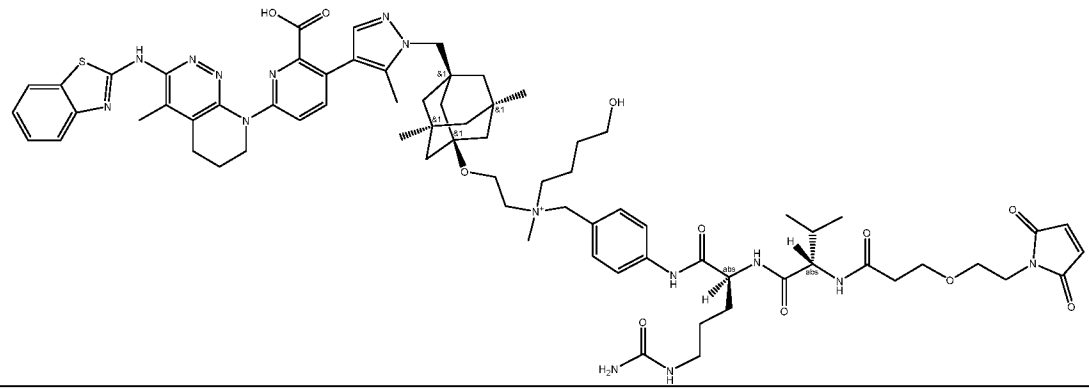
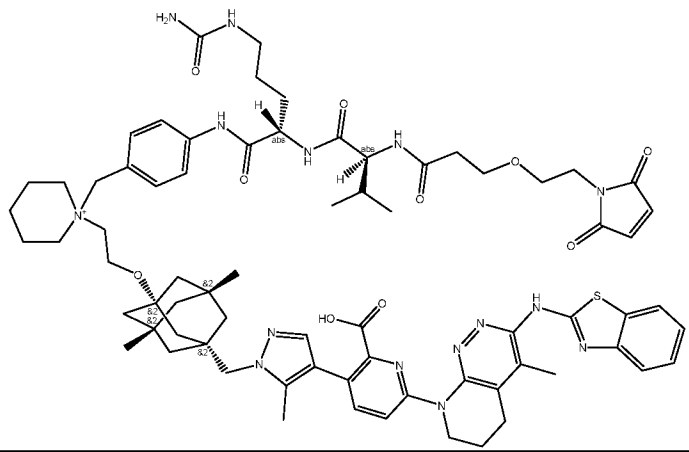
L9A-P18	
L9C-P19	
L9A-P20	
L9A-P21	

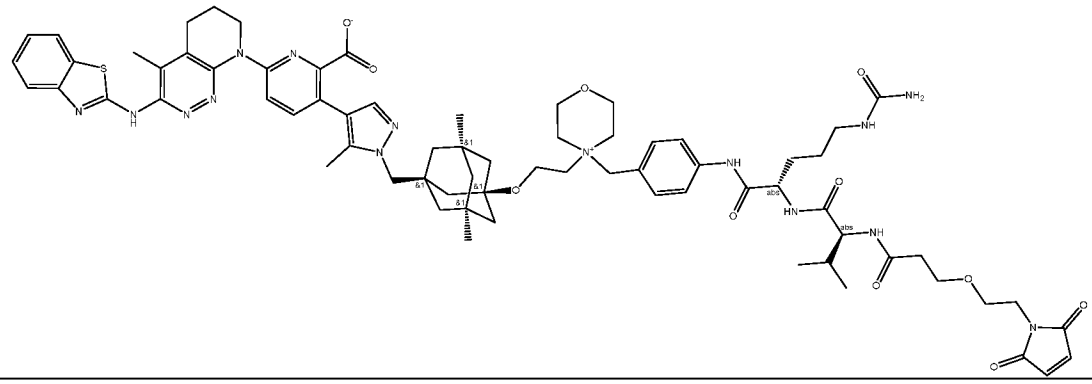
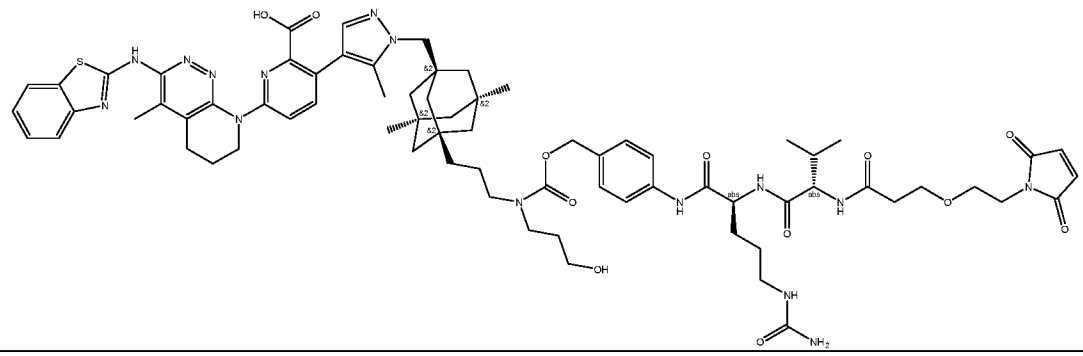
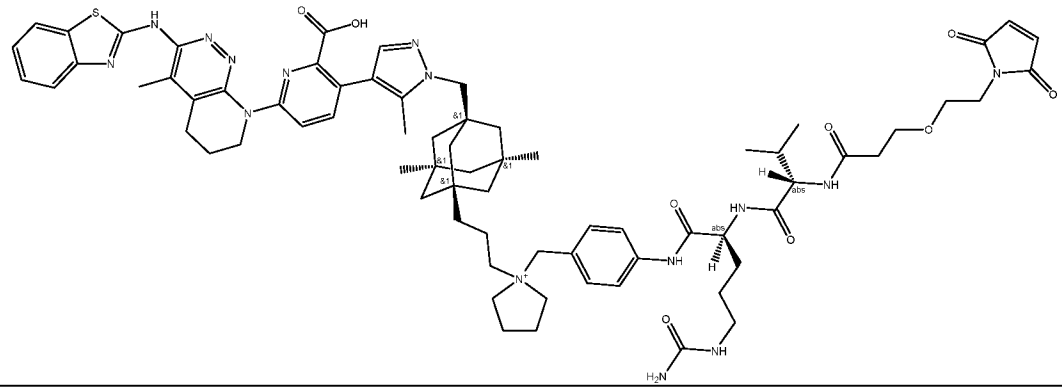
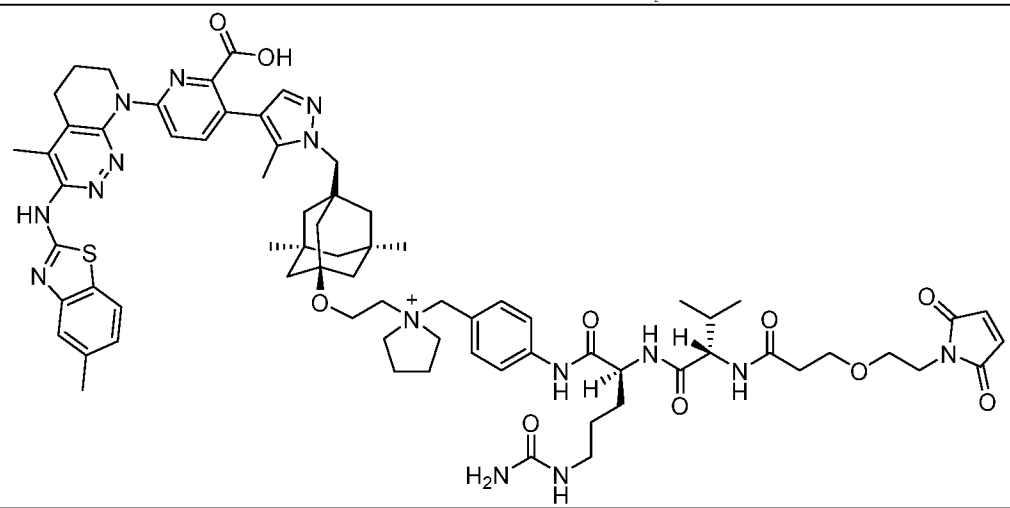
L9C-P22	
L9C-P23	
L9C-P24	
L9A-P2	

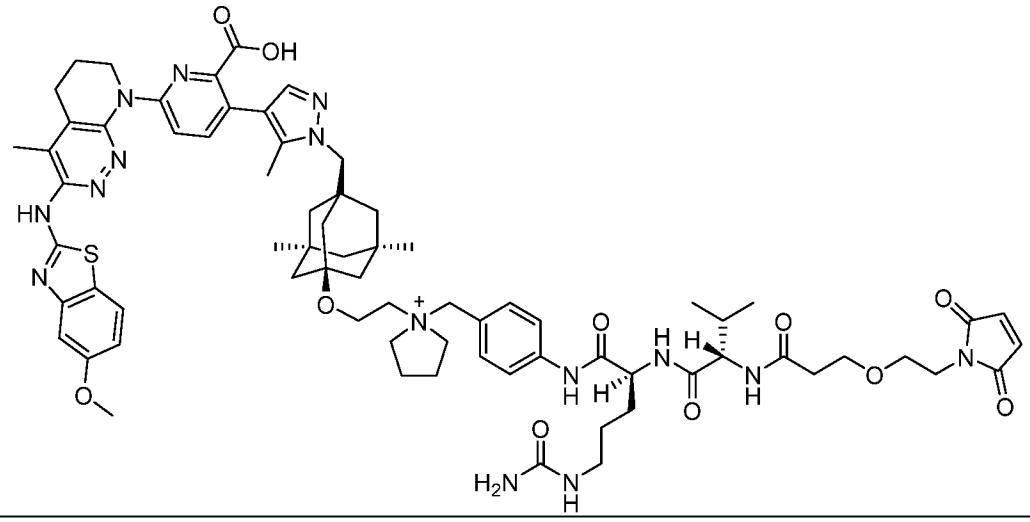
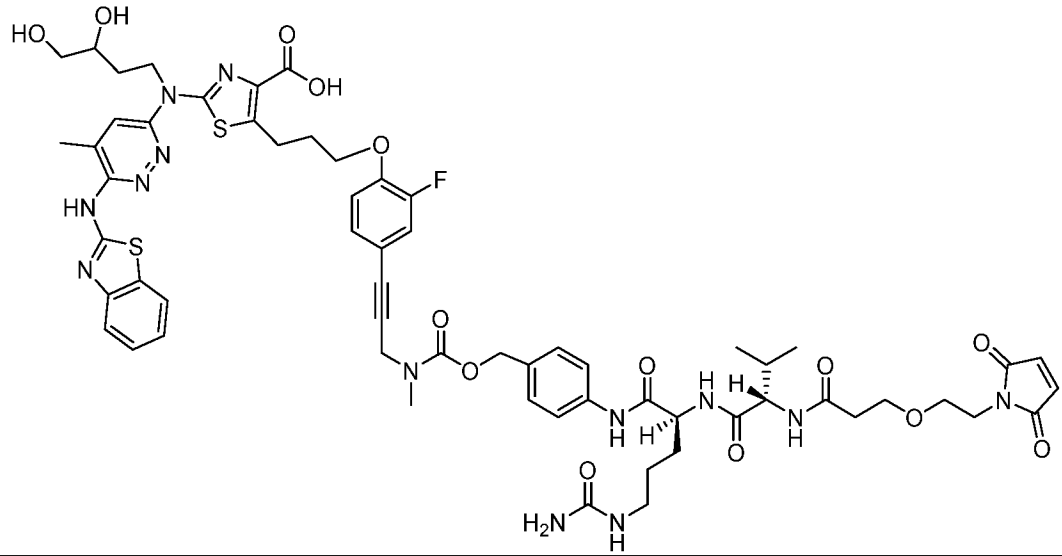
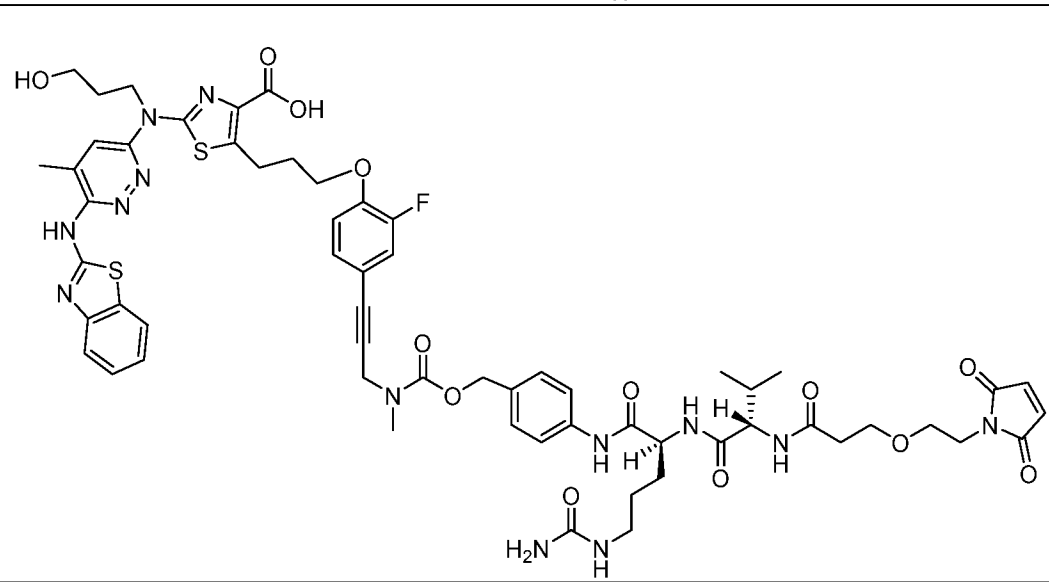
L9C-P25	
L9C-P26	
L9A-P27	

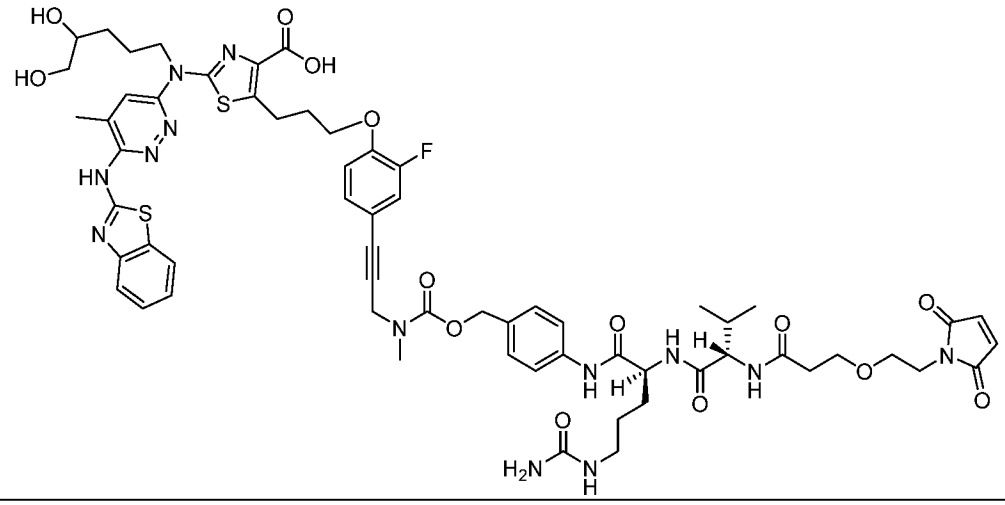
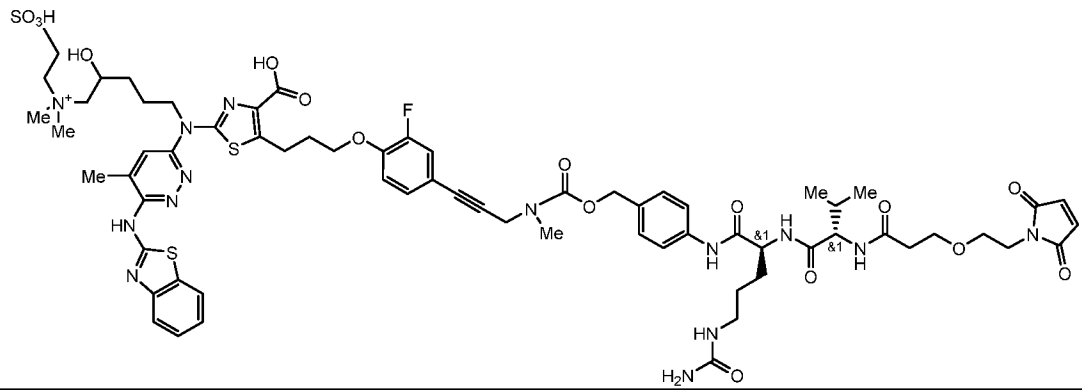
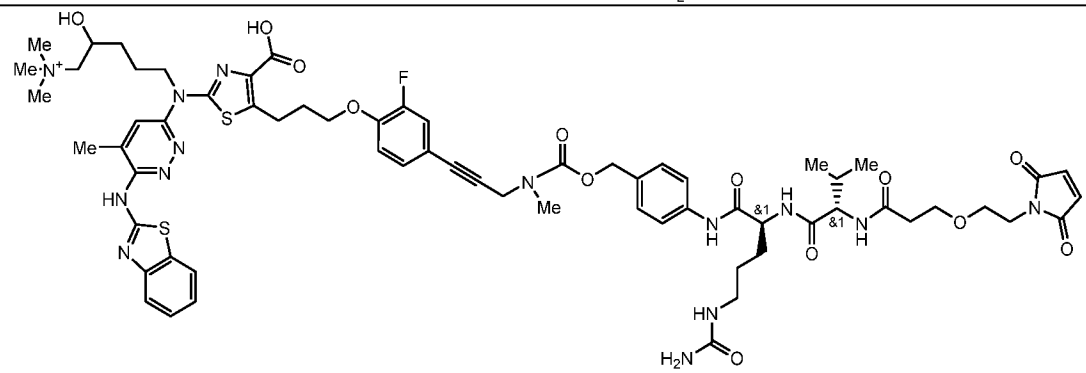
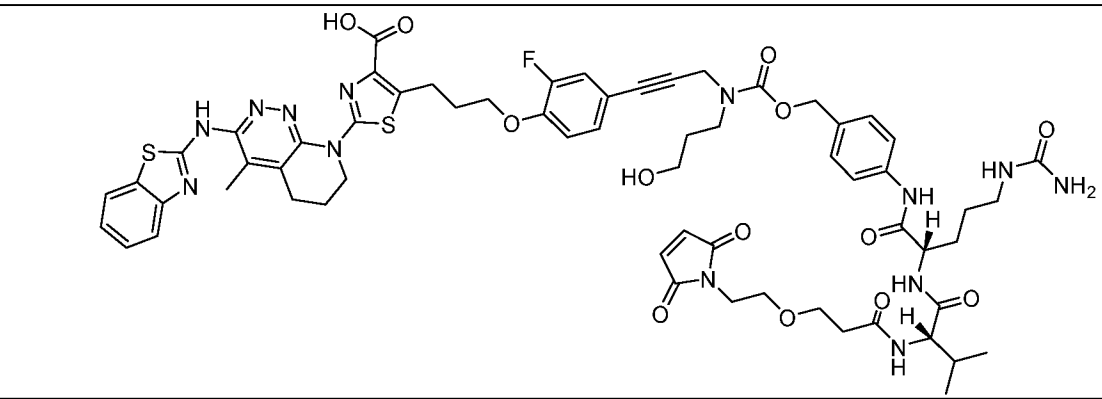
L9A-P28	
L9C-P29	
L9A-P30	
L9C-P31	

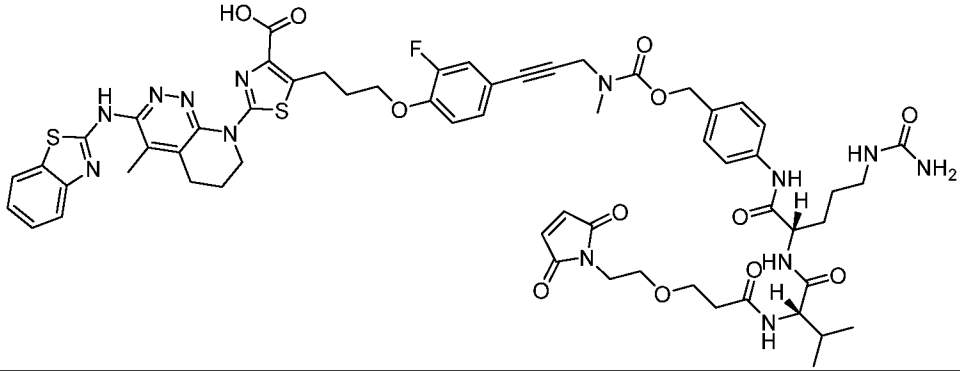
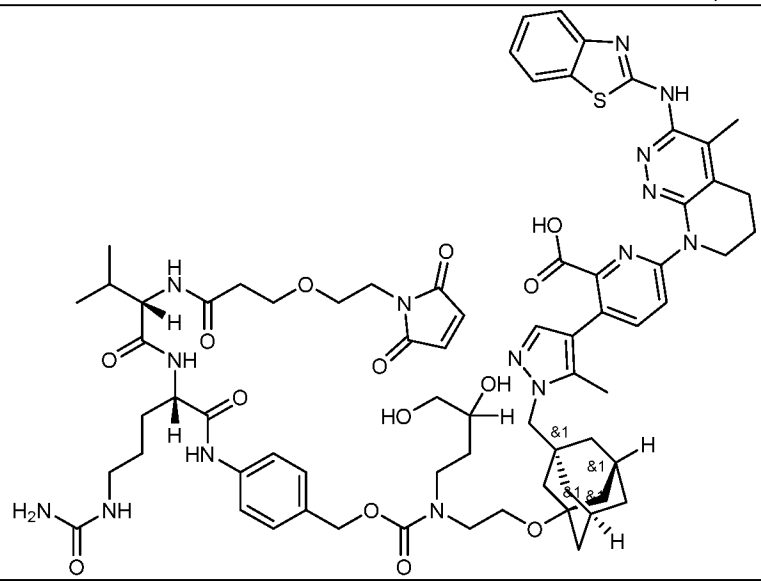
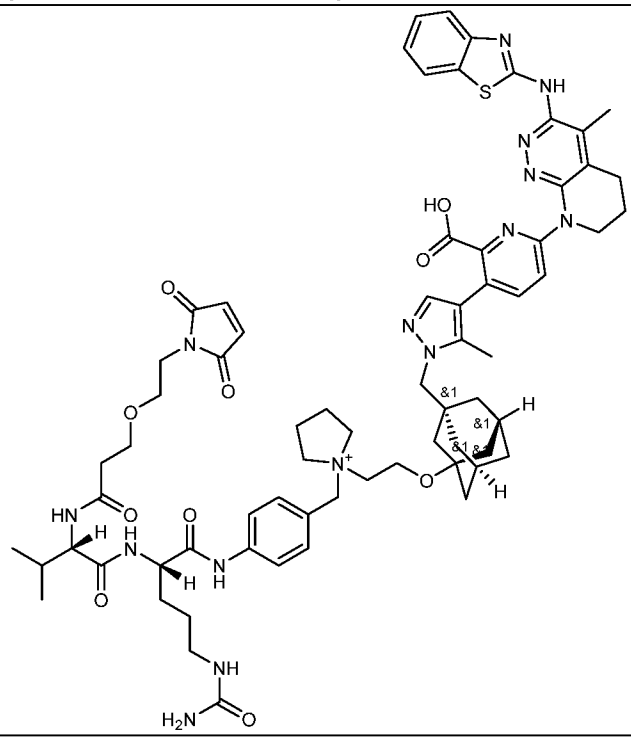
L9A-P32	
L9A-P33	
L9A-P34	

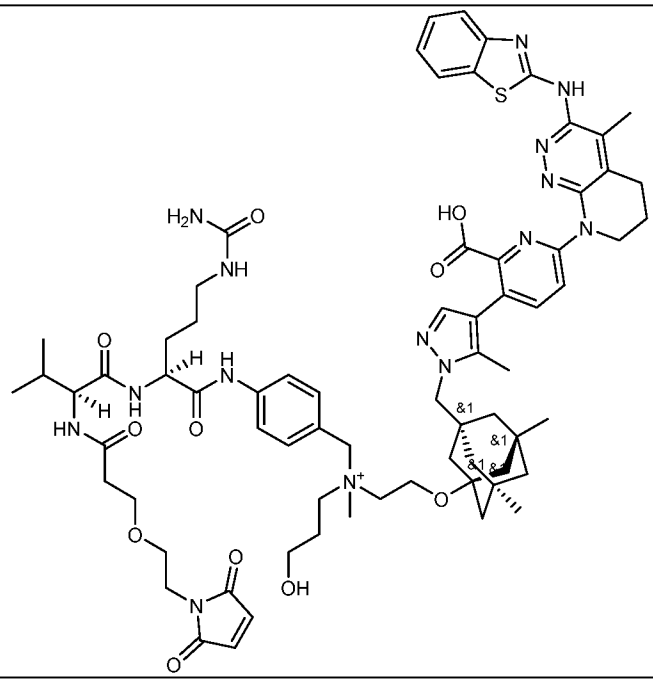
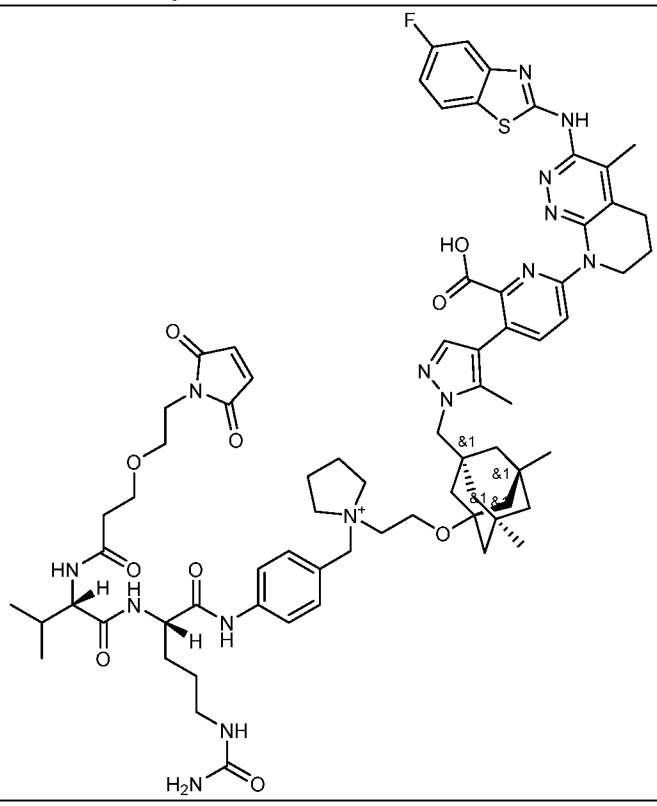
L9A-P35	
L9A-P36	
L9A-P37	
L9A-P38	

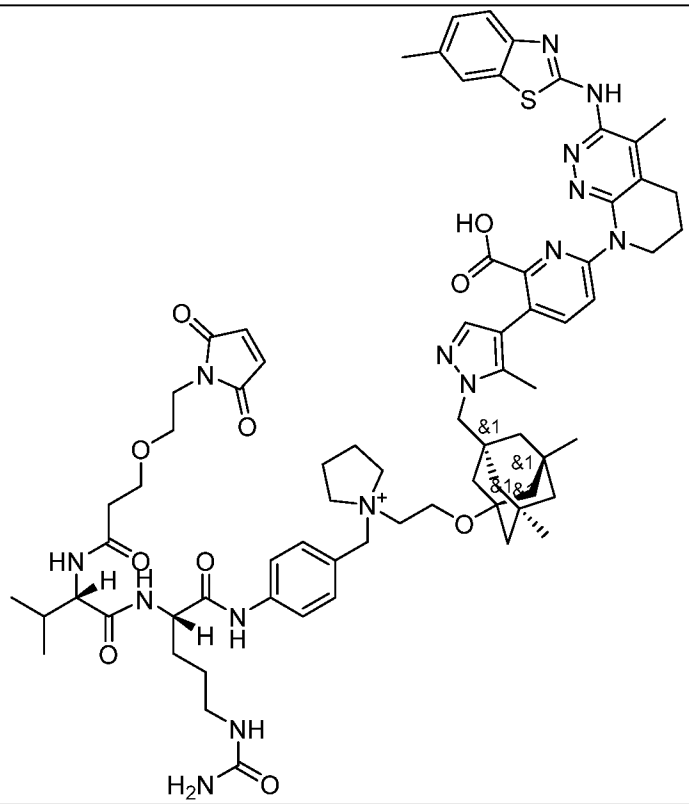
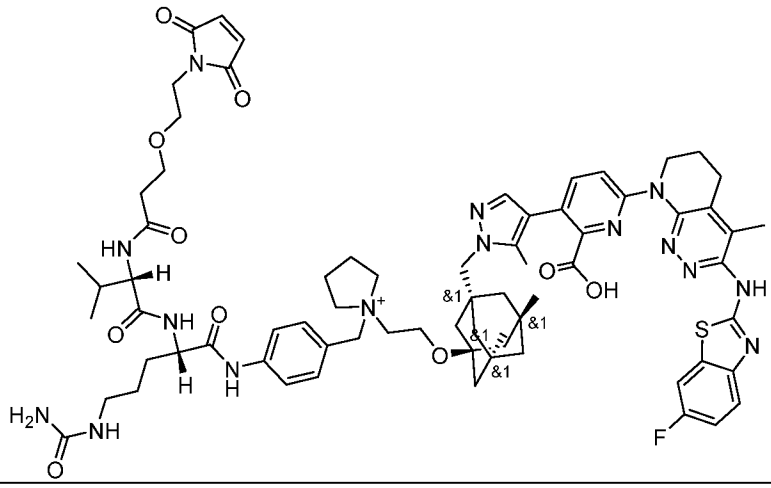
L9A-P39	
L9C-P40	
L9A-P41	
L9A-P42	

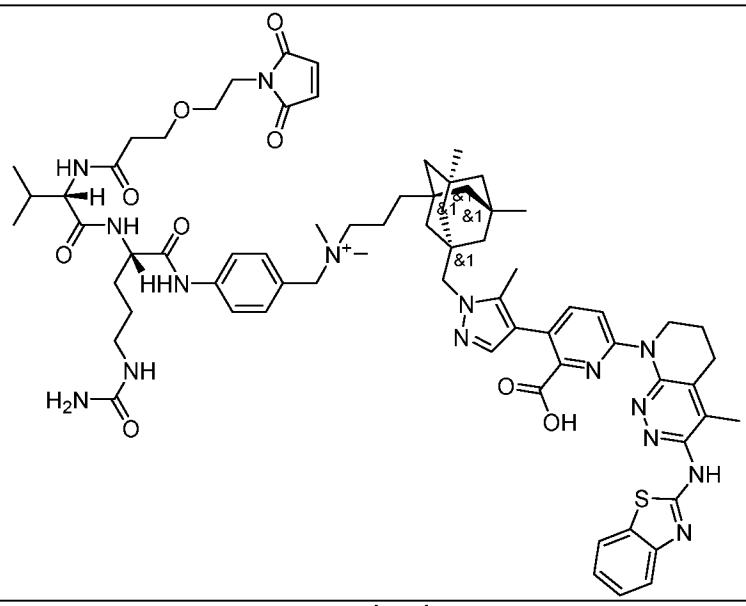
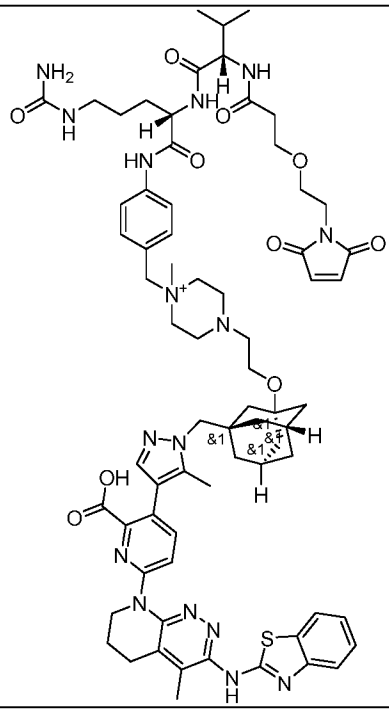
L9A-P43	
L9C-P44	
L9C-P45	

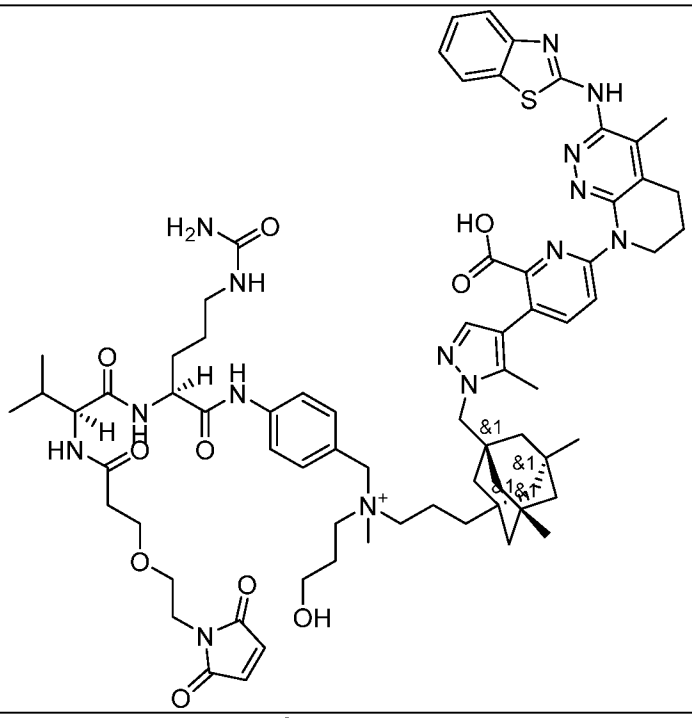
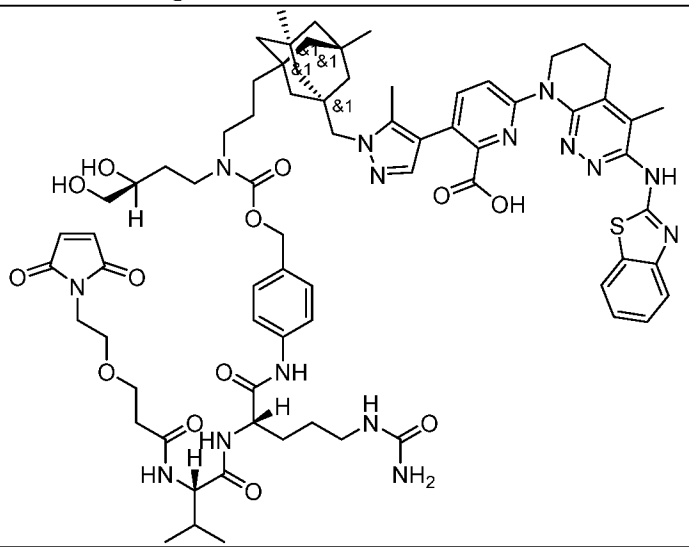
L9C-P46	
L9C-P4	
L9C-P5	
L9C-P59	

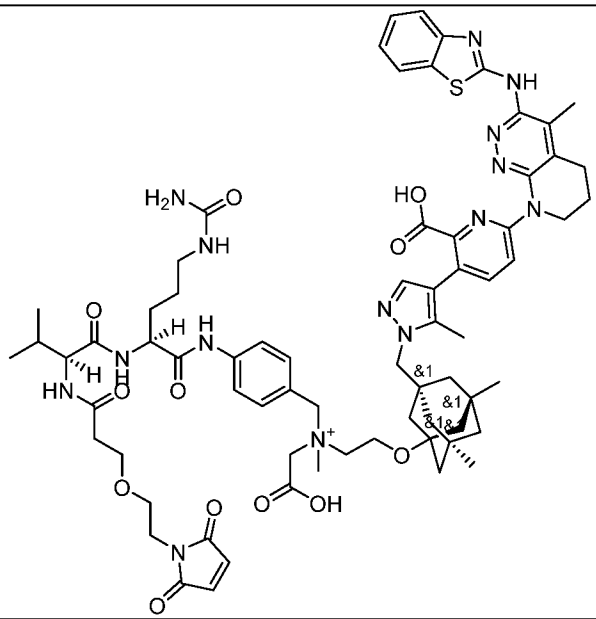
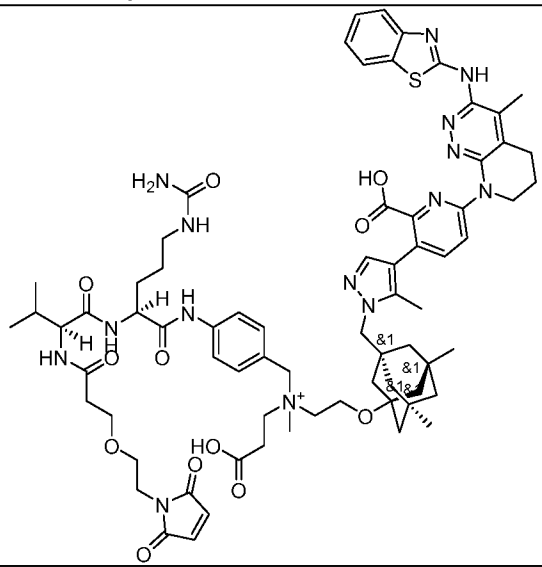
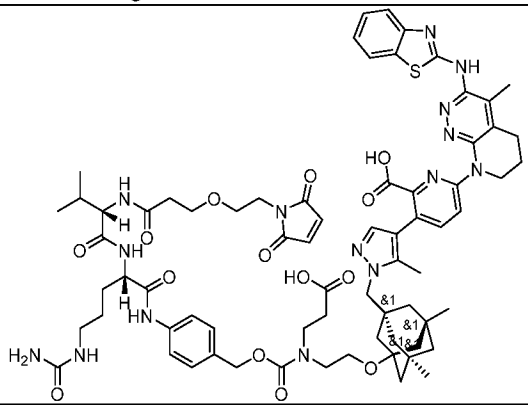
L9C-P3	
L9C-P60	
L9A-P61	

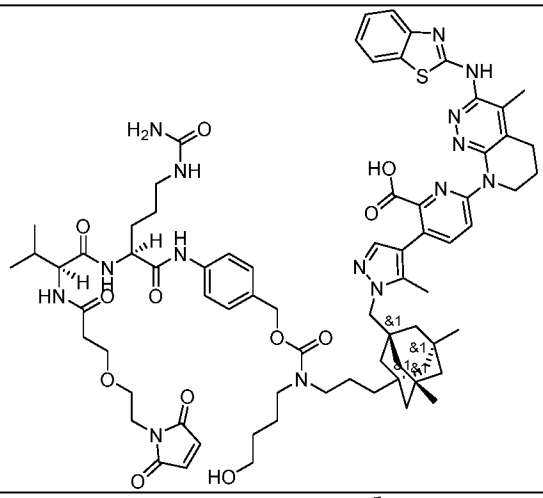
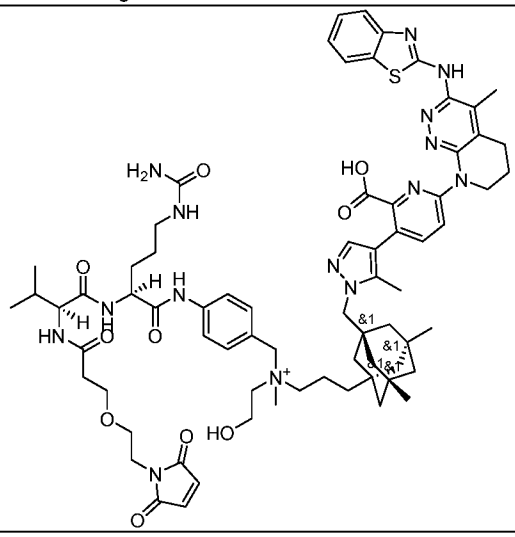
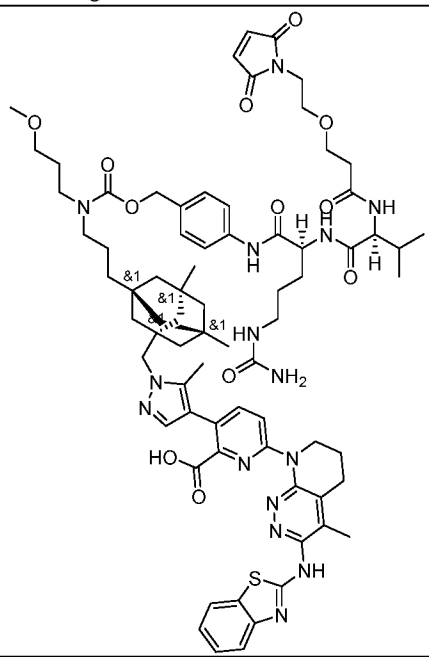
L9A-P62	
L9A-P63	

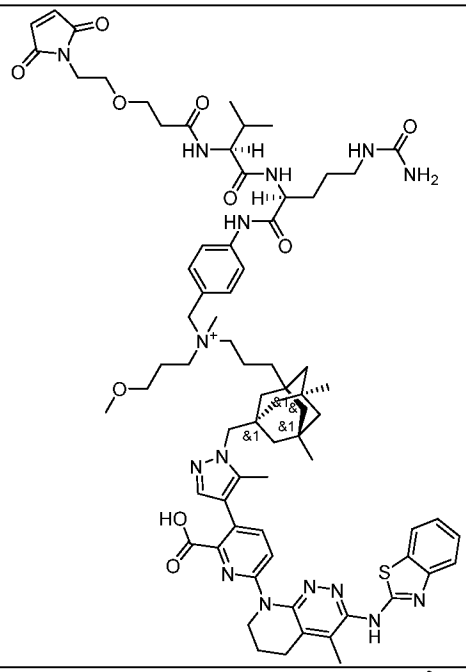
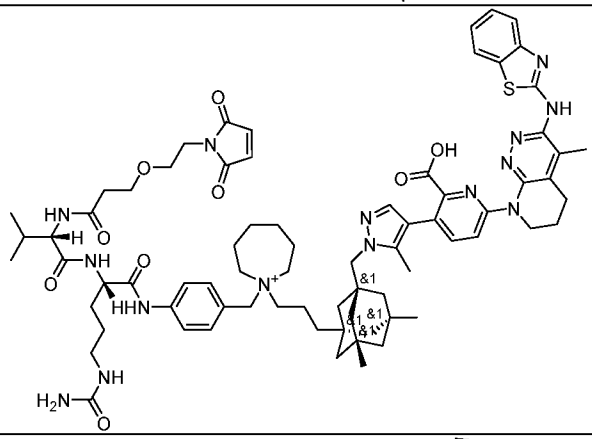
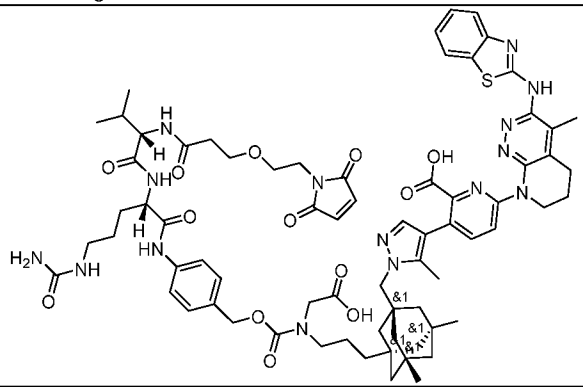
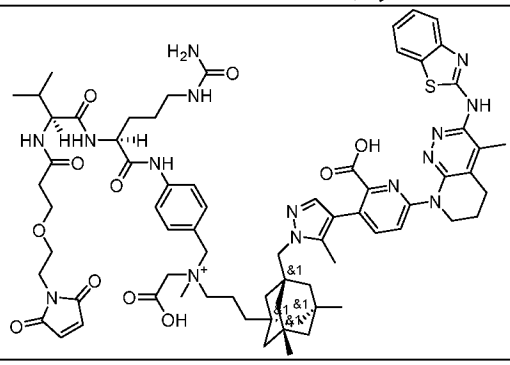
L9A-P64	
L9A-P65	

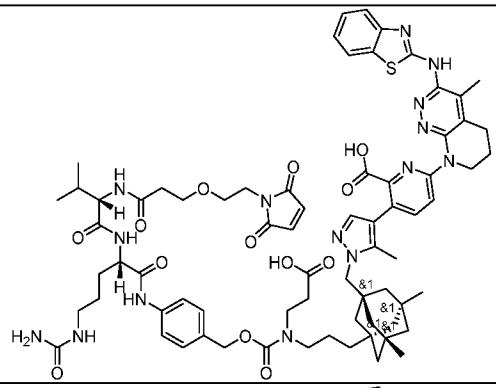
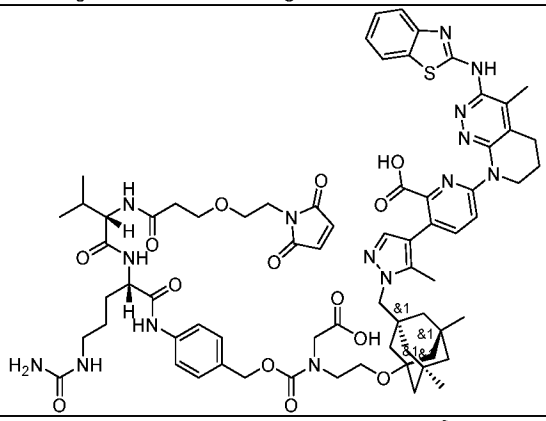
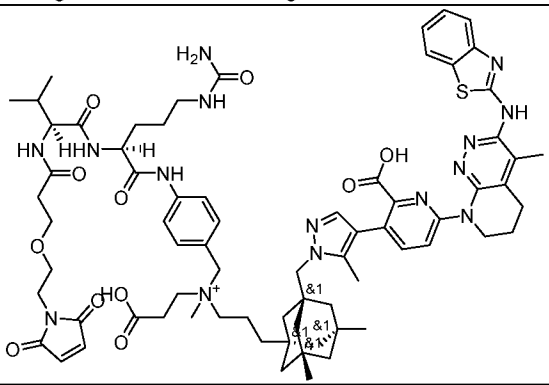
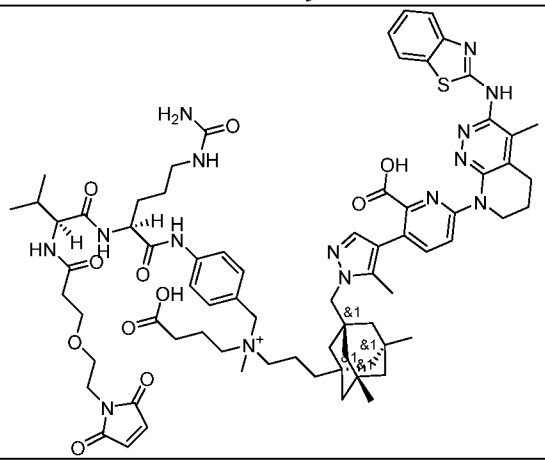
L9A-P66	
L9A-P67	

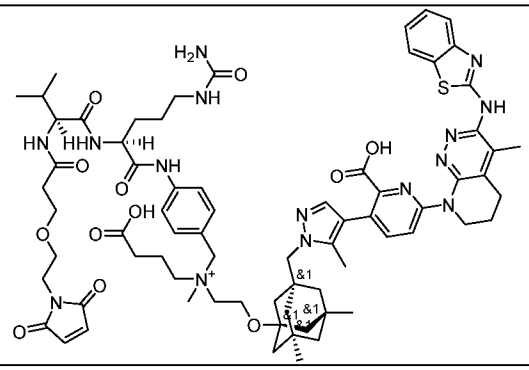
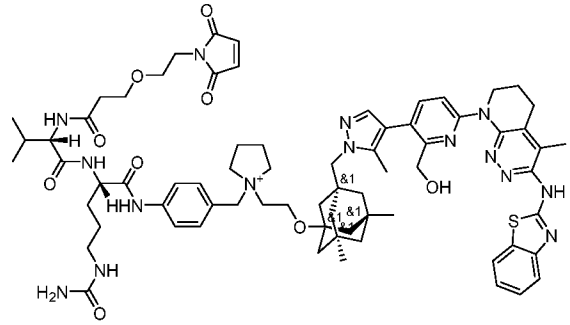
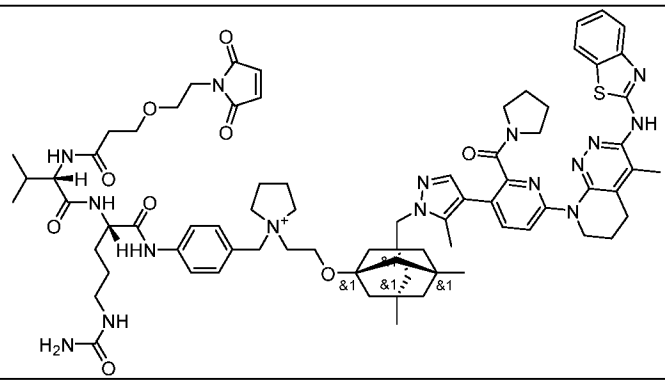
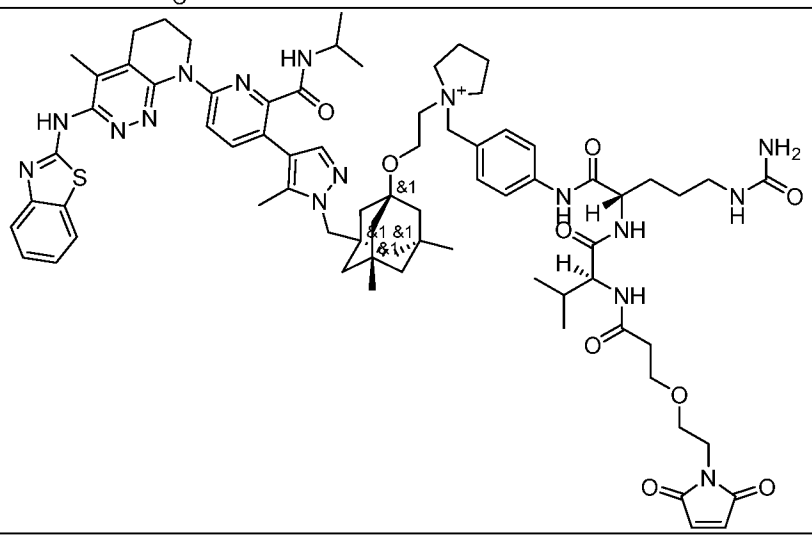
L9A-P68	
L9C-P69	

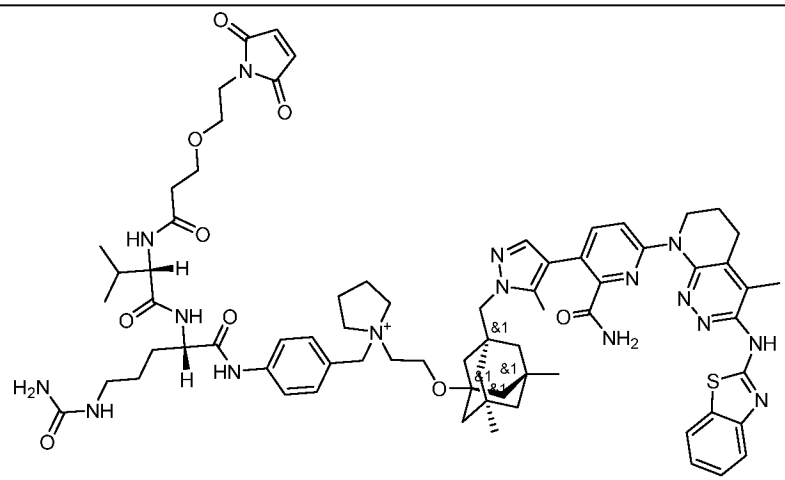
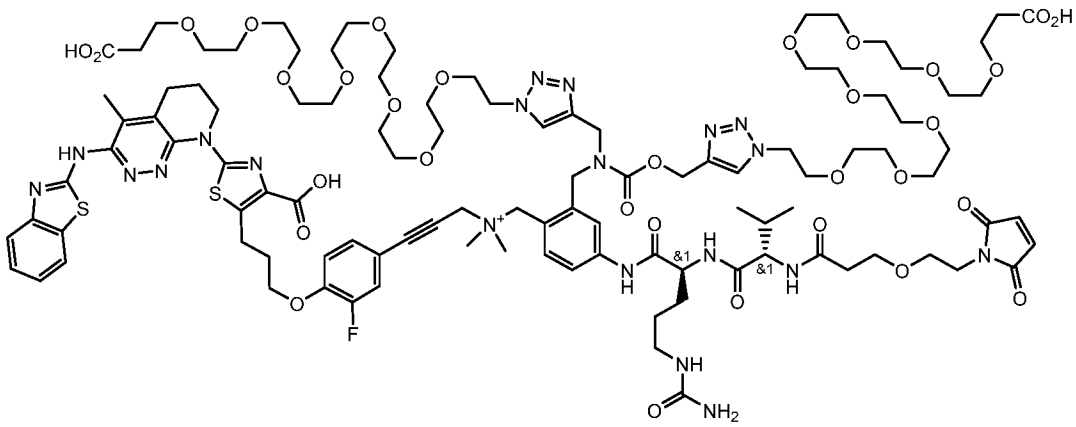
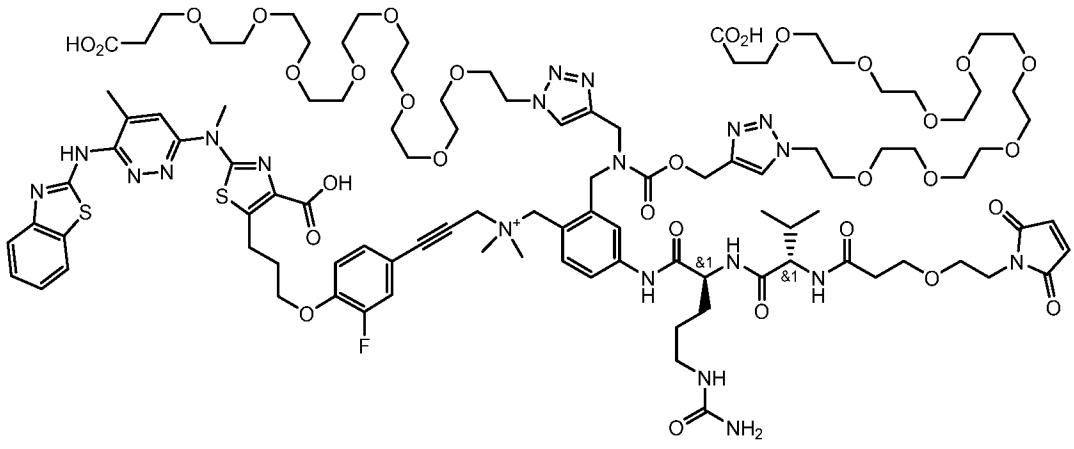
L9A-P48	
L9A-P70	
L9C-P71	

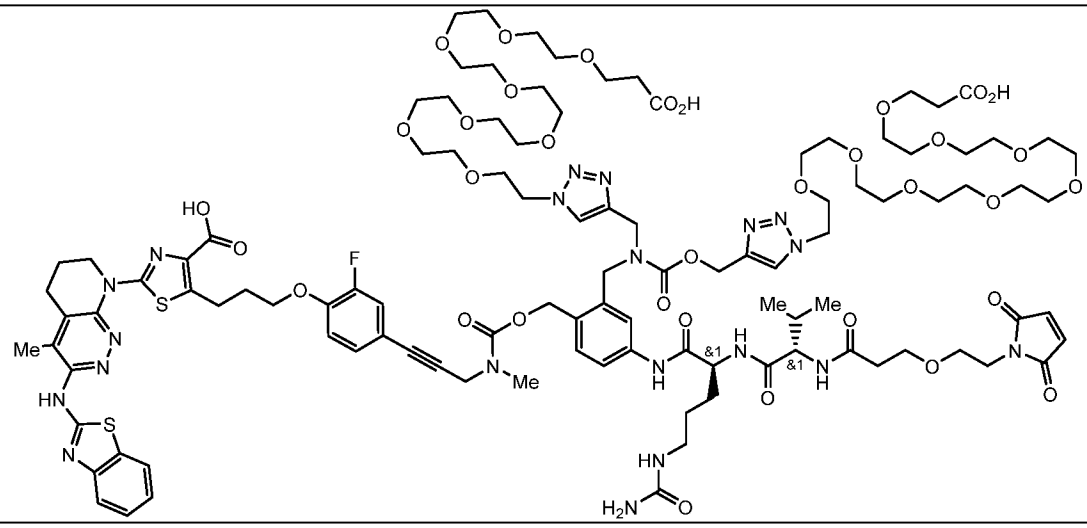
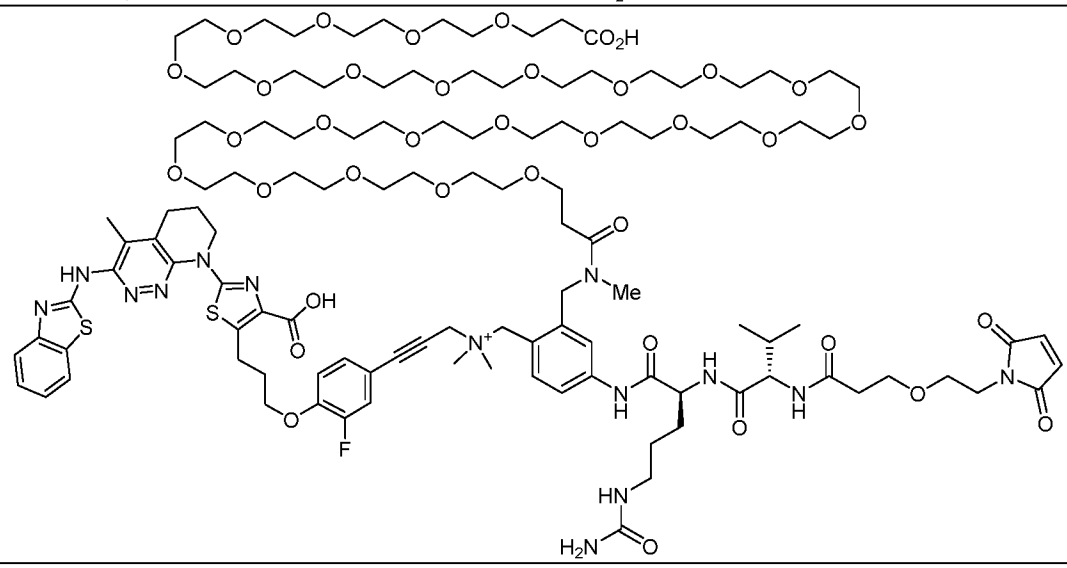
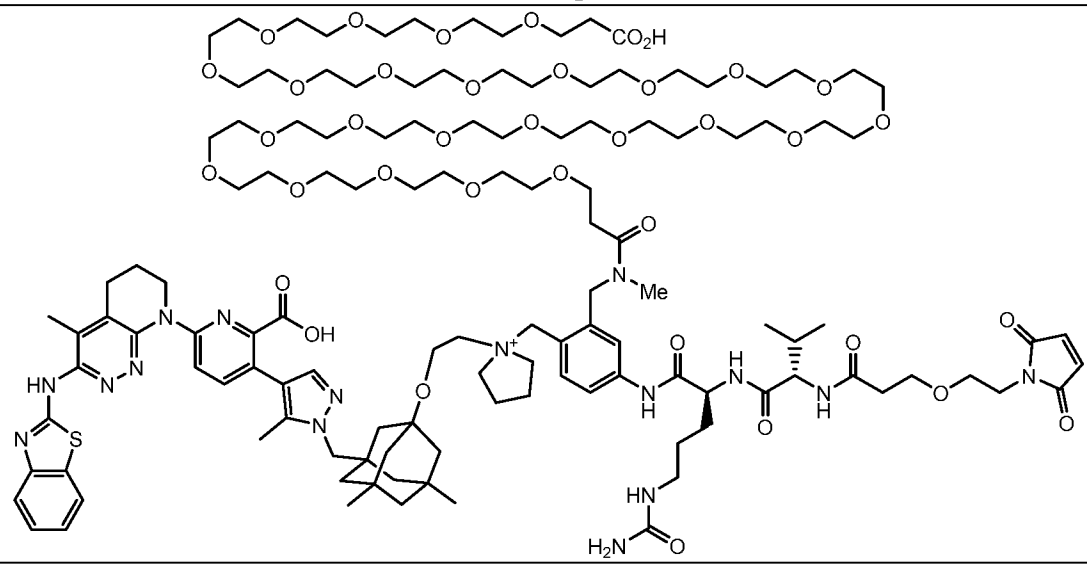
L9C-P72	
L9A-P49	
L9C-P51	

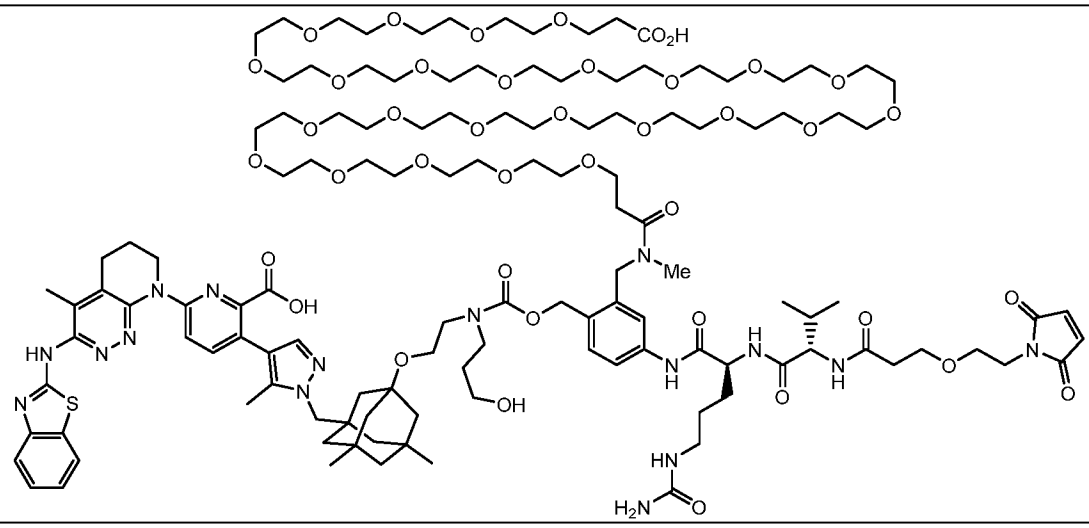
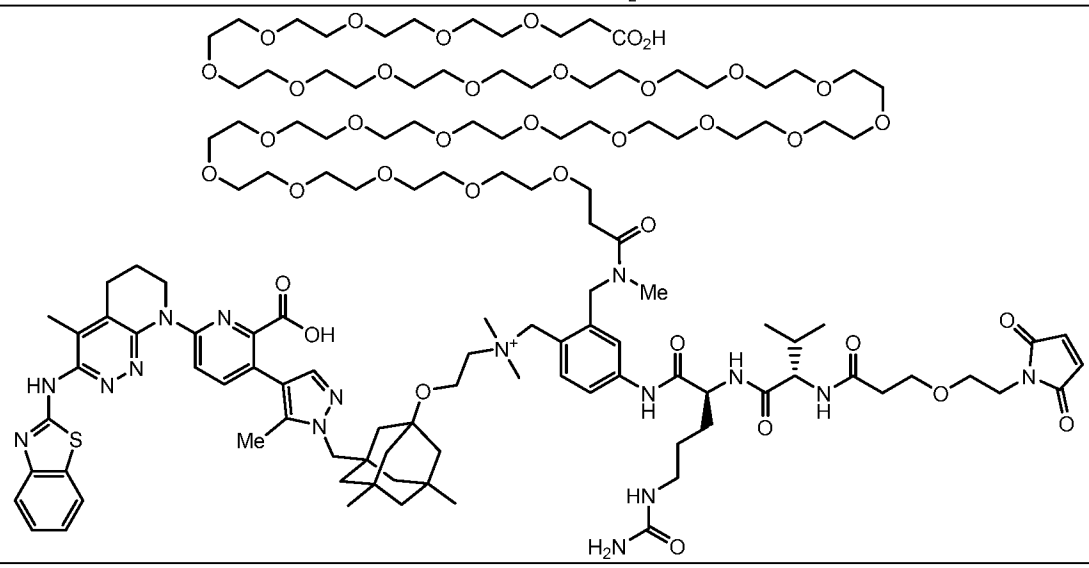
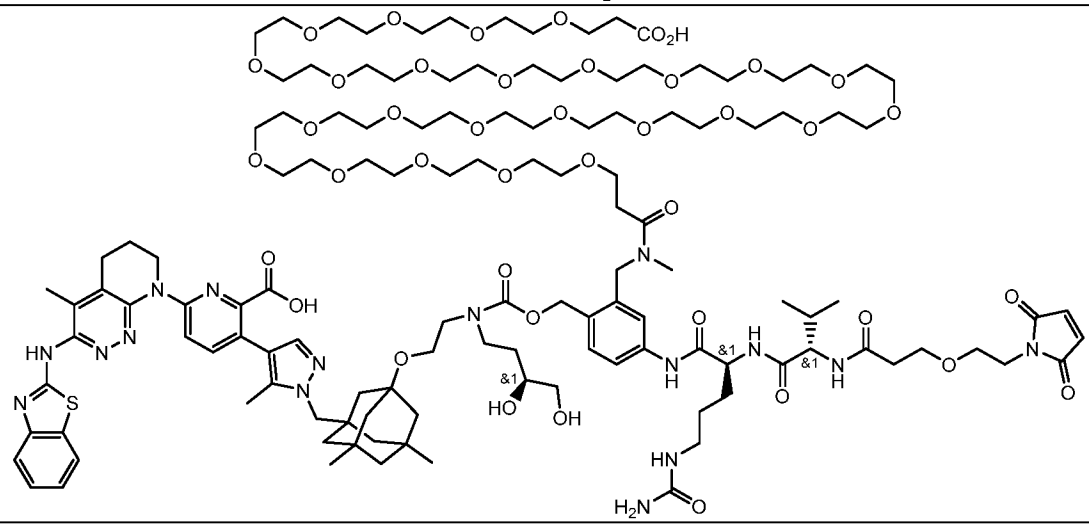
L9A-P50	
L9A-P52	
L9C-P53	
L9A-P55	

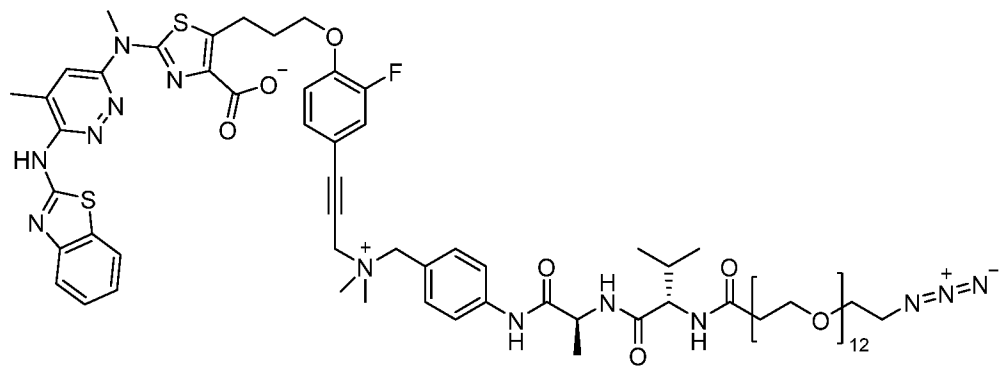
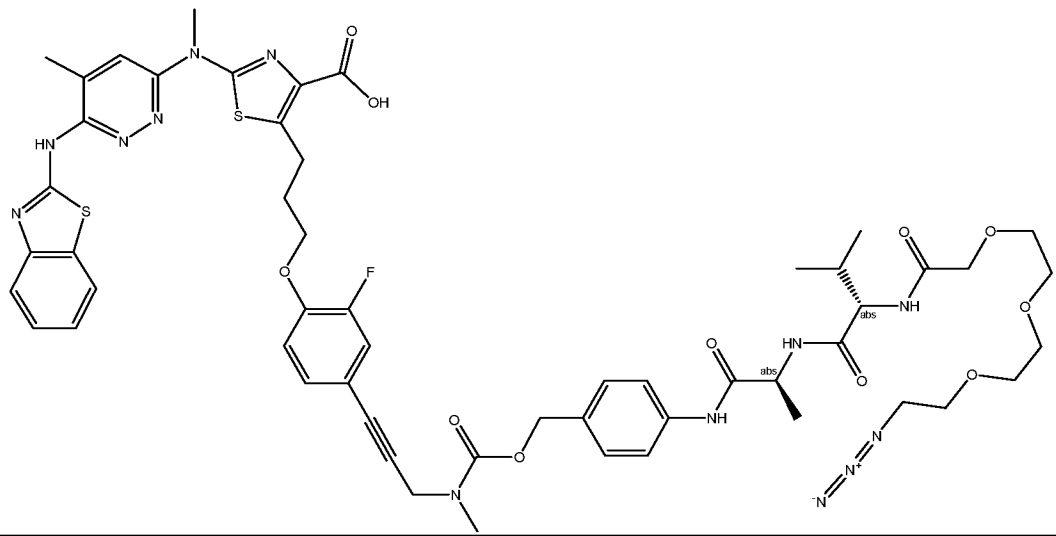
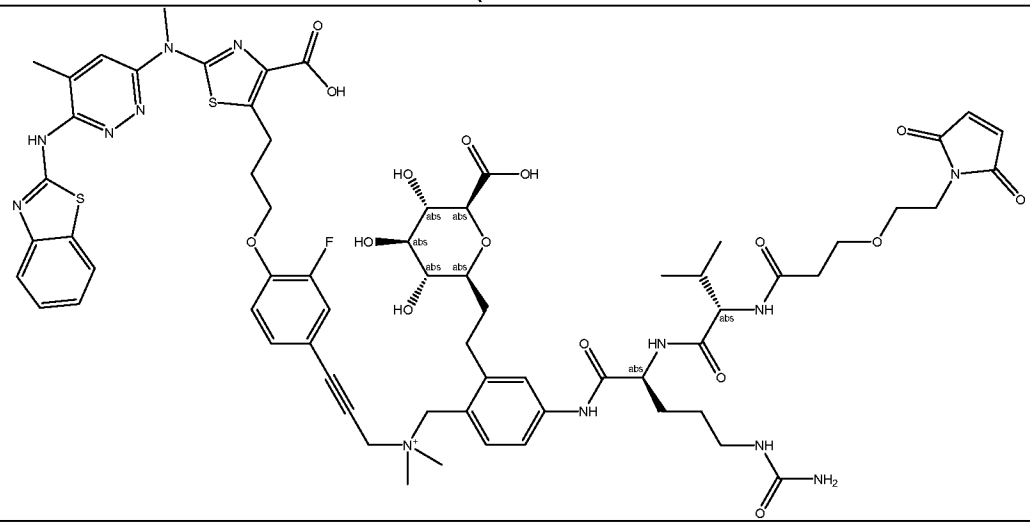
L9C-P54	
L9C-P47	
L9A-P56	
L9A-P58	

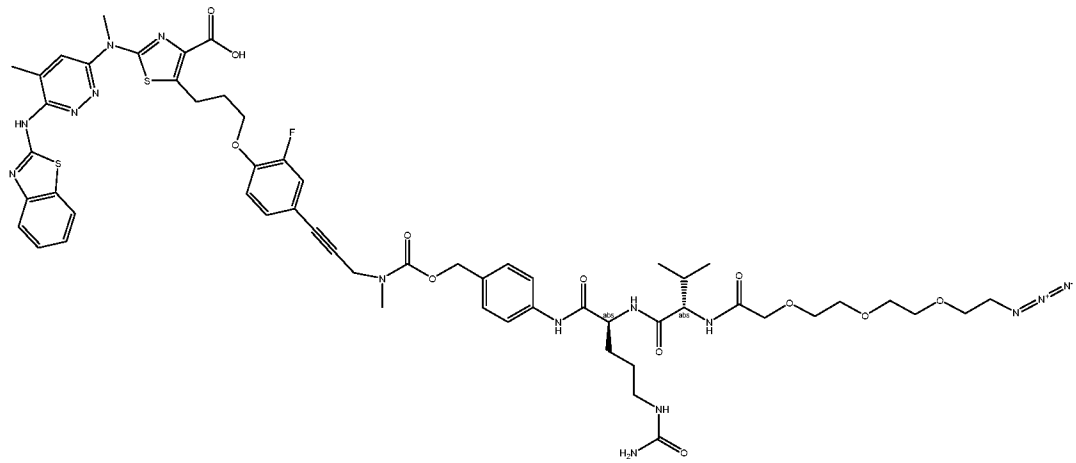
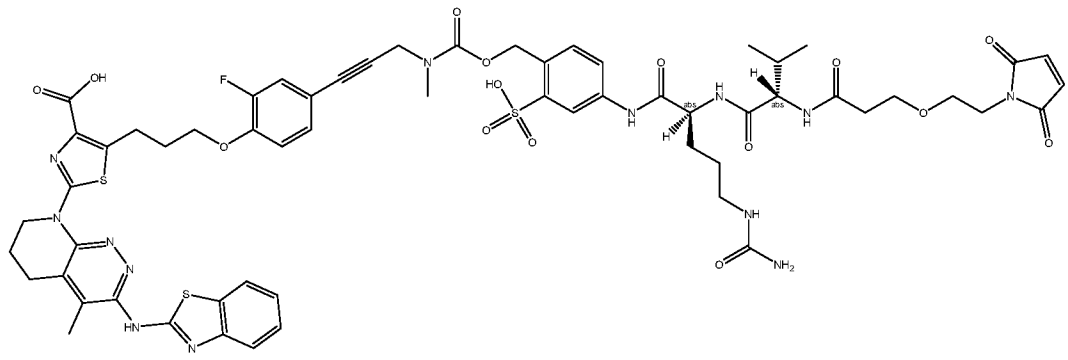
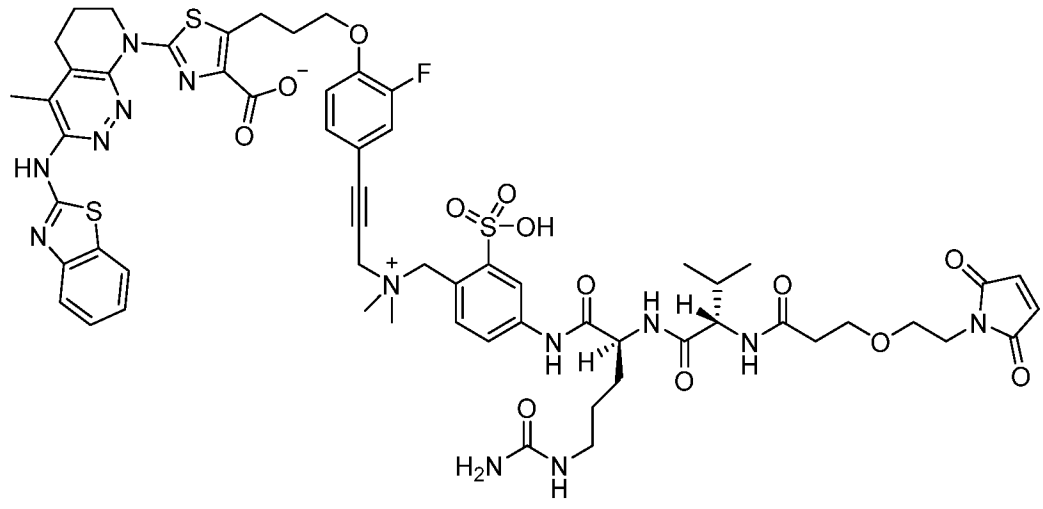
L9A-P57	
L9A-P73	
L9A-P74	
L9A-P75	

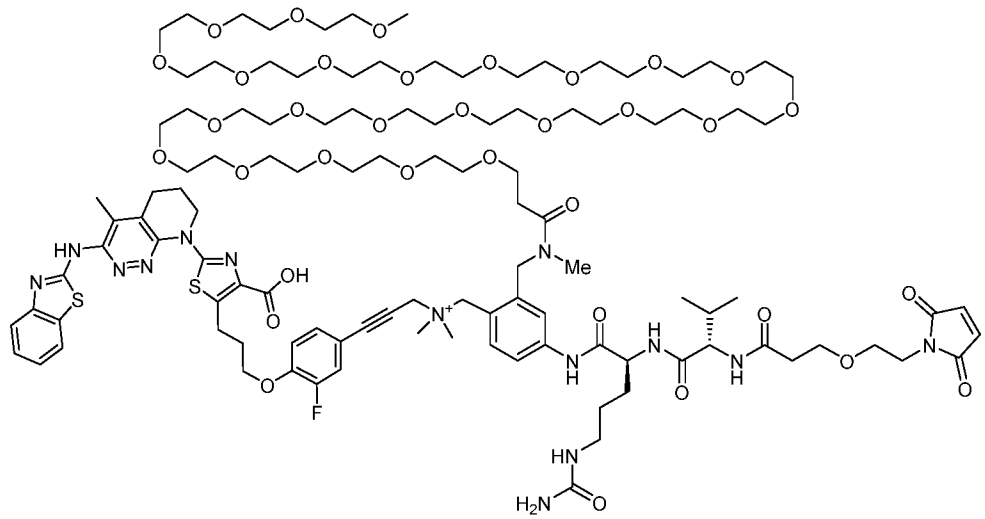
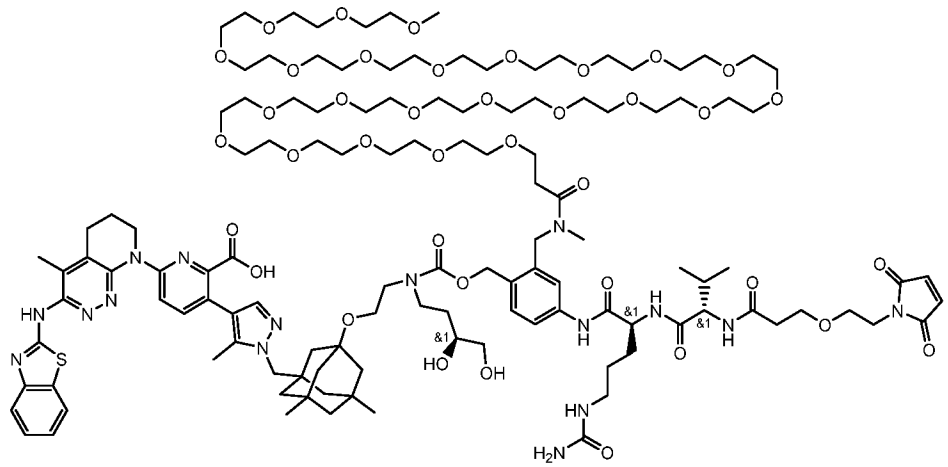
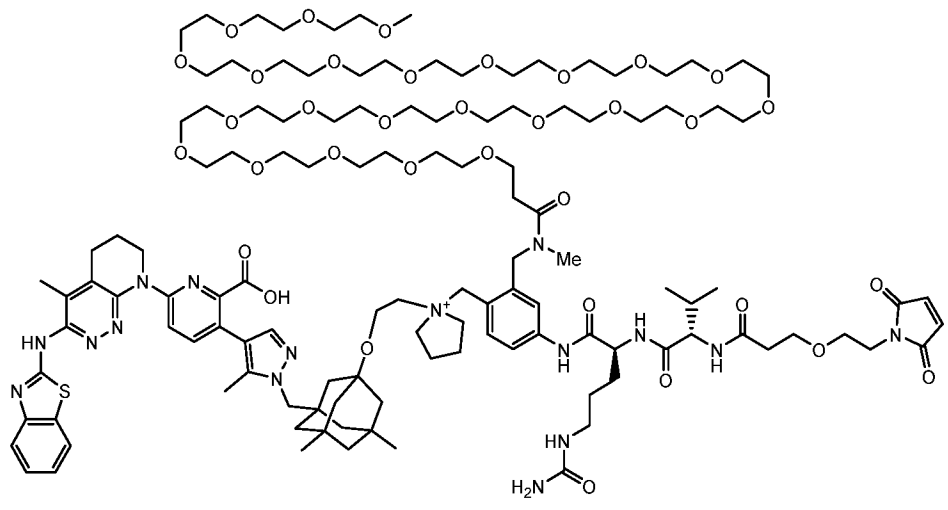
L9A-P76	
L10A-P1	
L10A-P2	

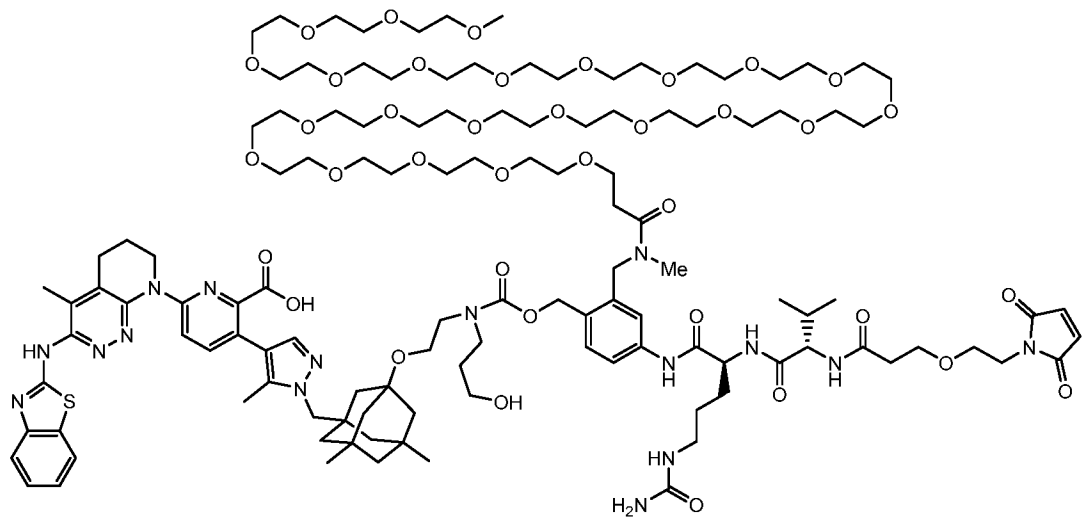
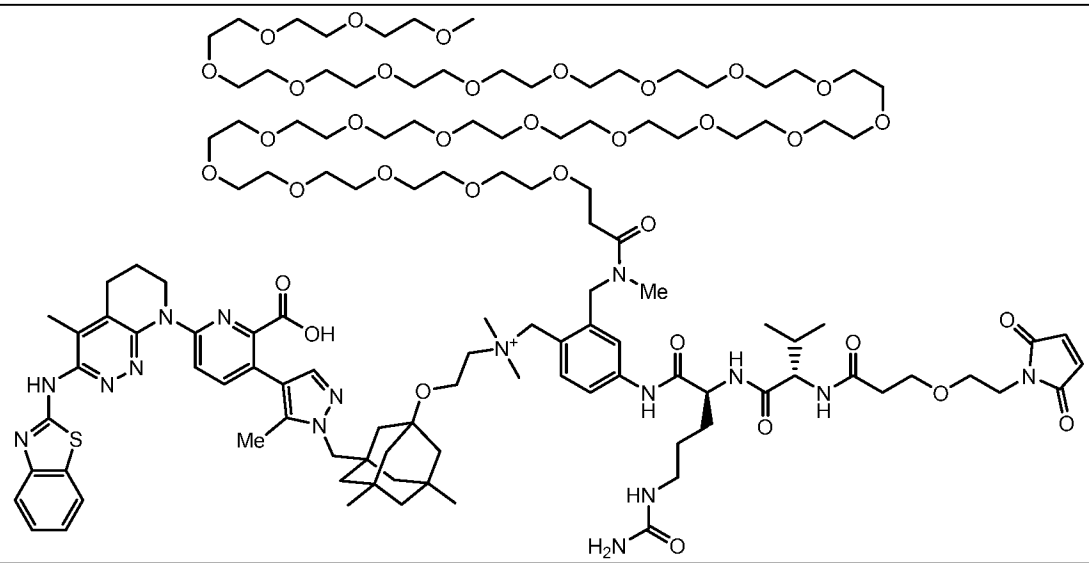
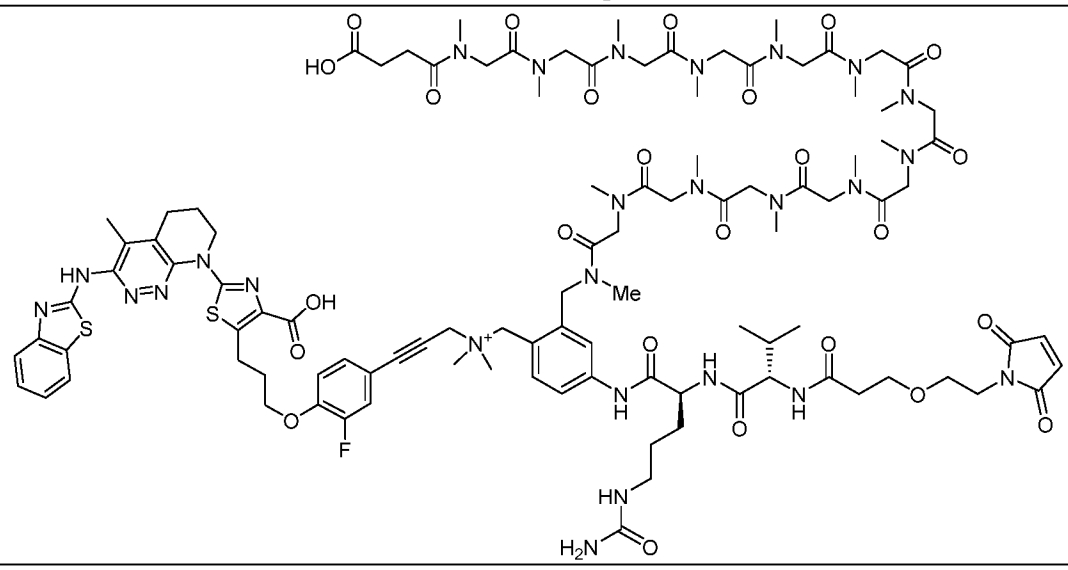
L10C-P3	
L11A-P1	
L11A-P21	

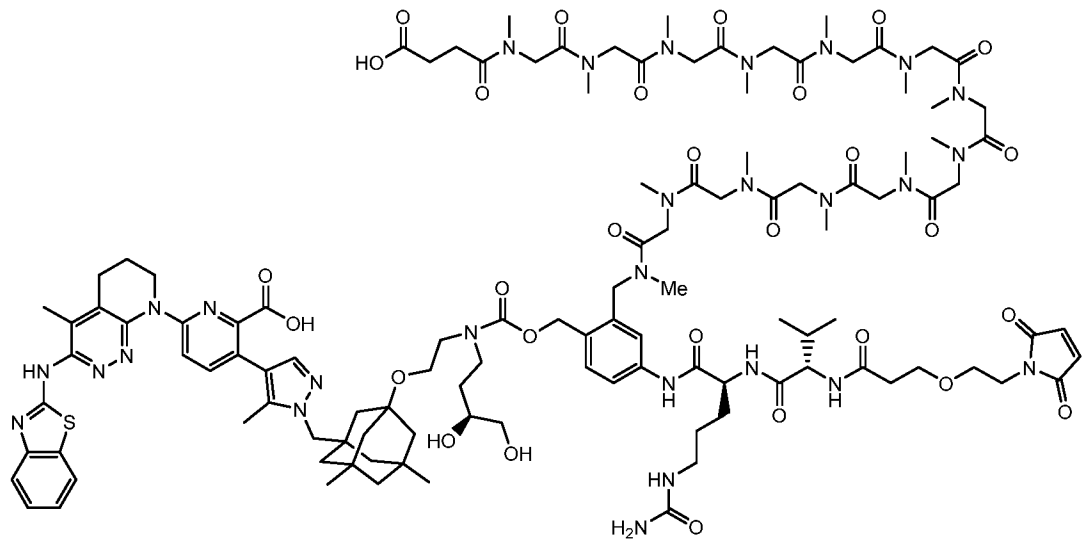
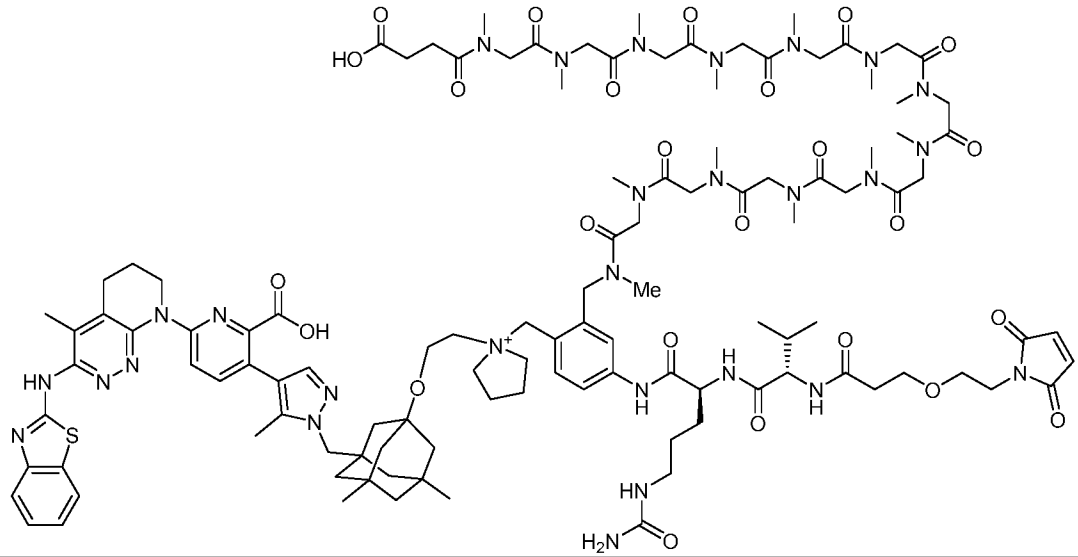
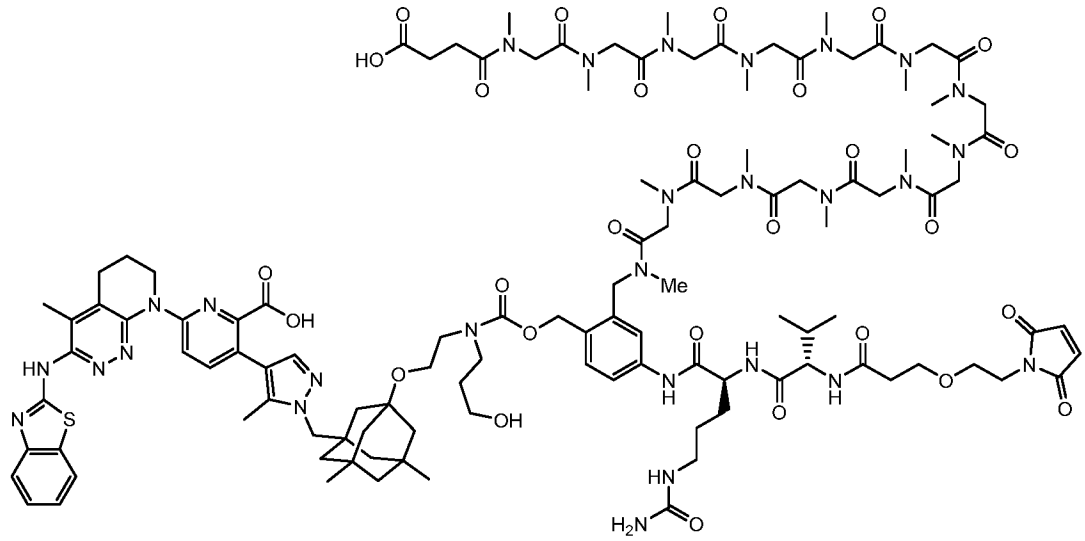
L11C-P25	
L11A-P27	
L11C-P19	

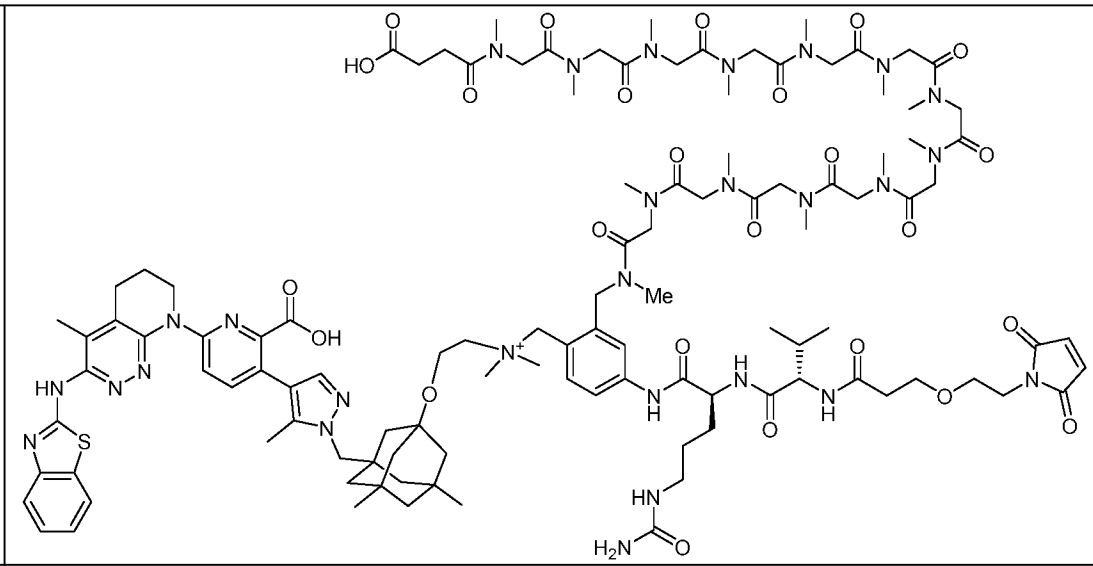
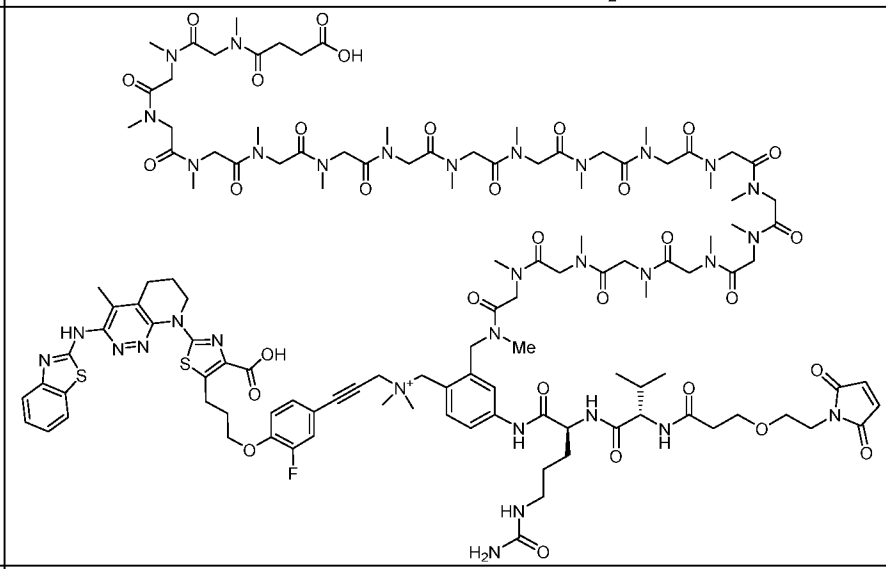
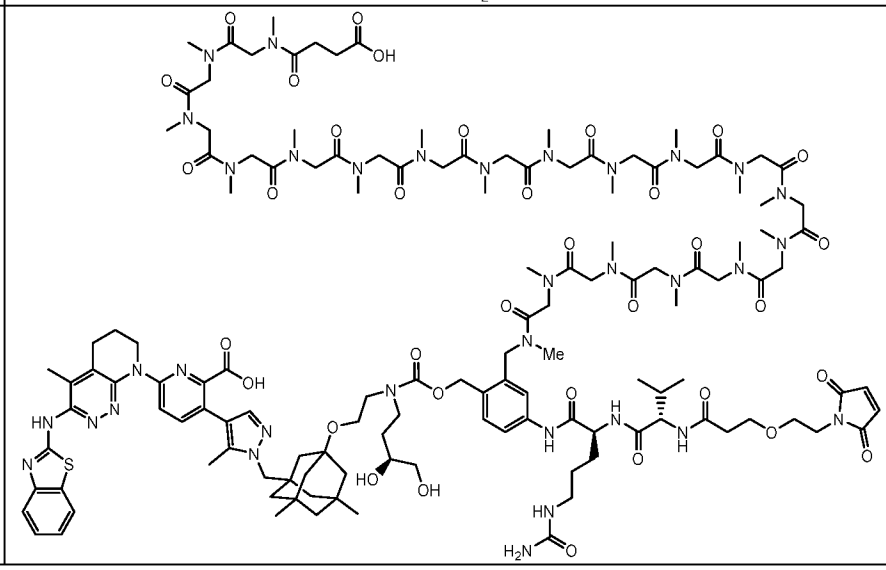
L13A-P2	
L19C-P7	
L21A-P2	

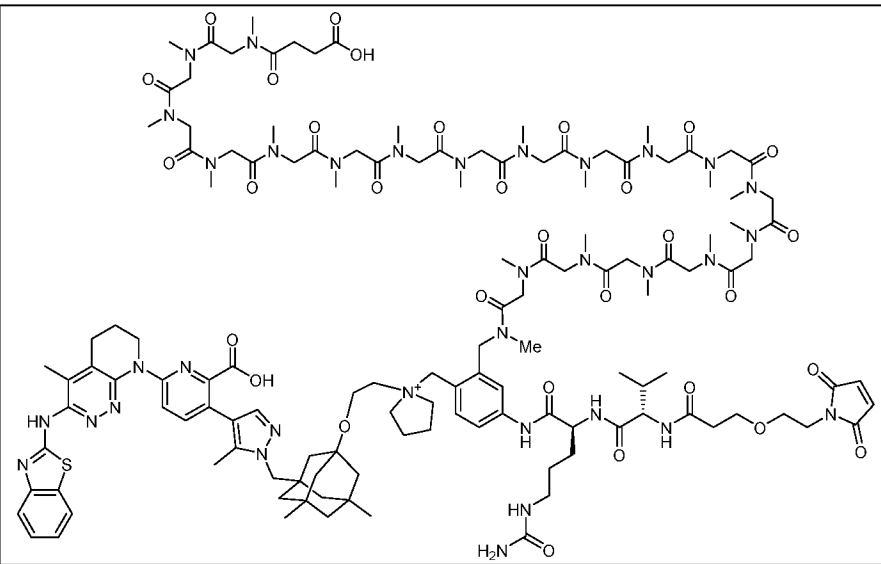
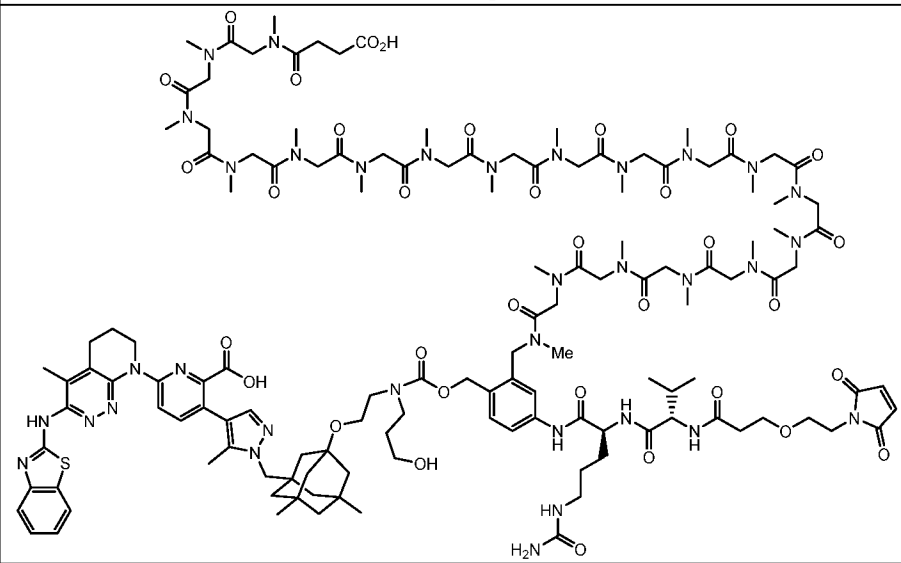
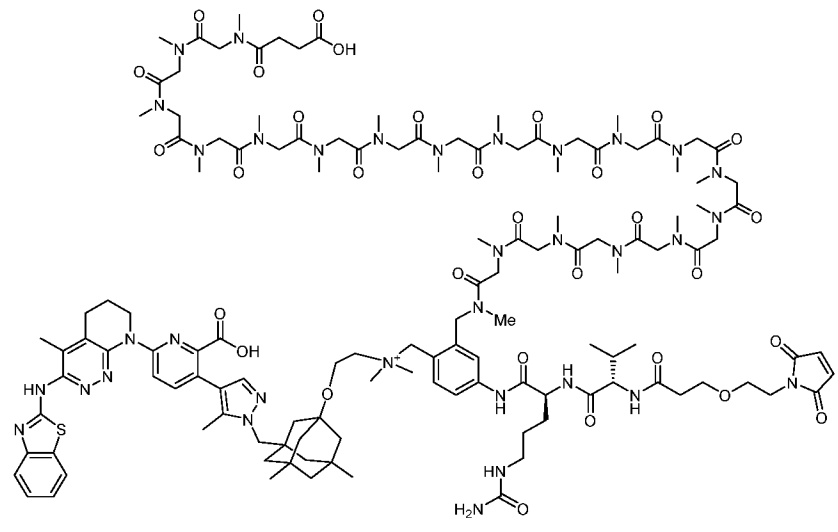
L23C-P7	
L27C-P3	
L27A-P1	

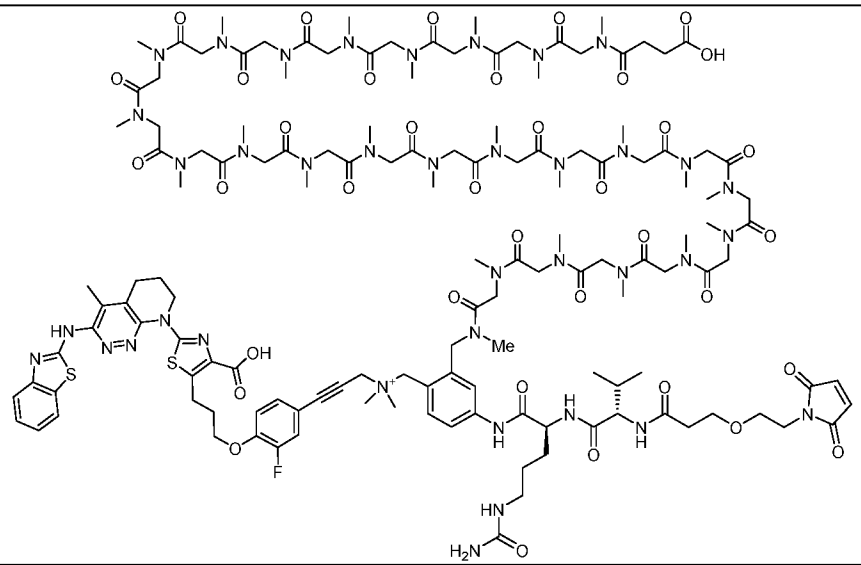
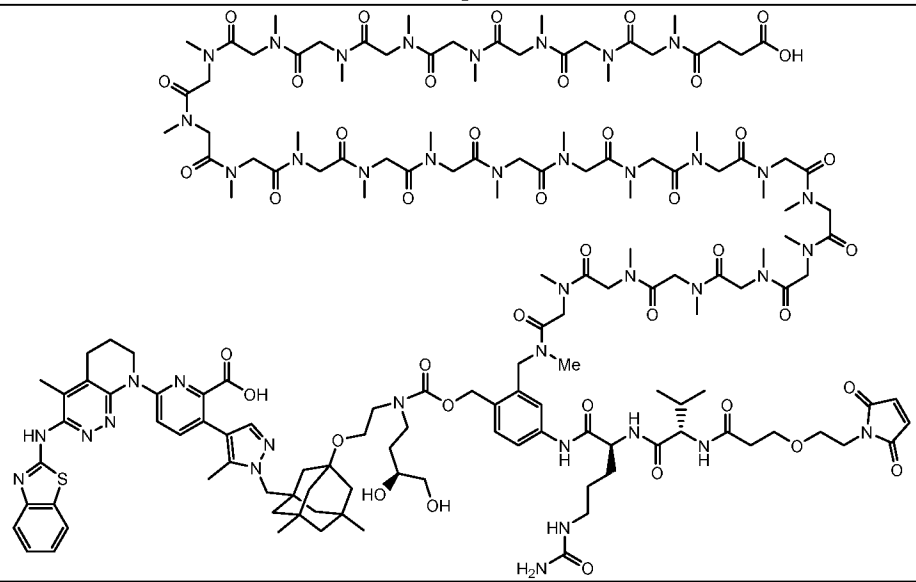
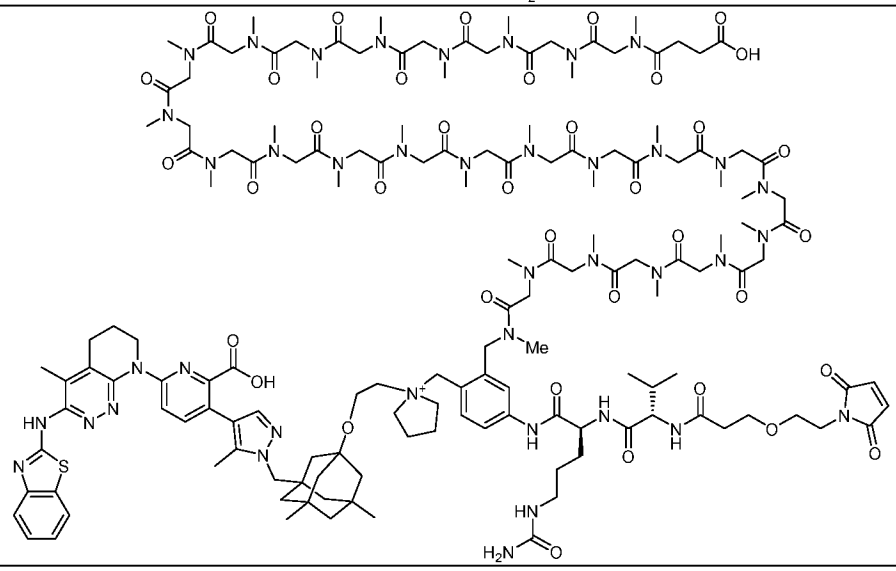
L30A-P1	
L30C-P19	
L30A-P21	

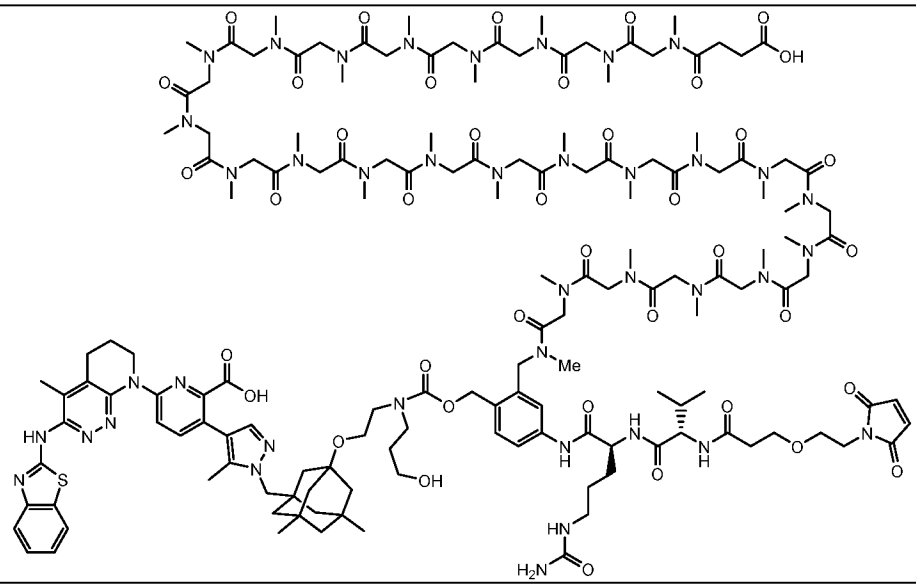
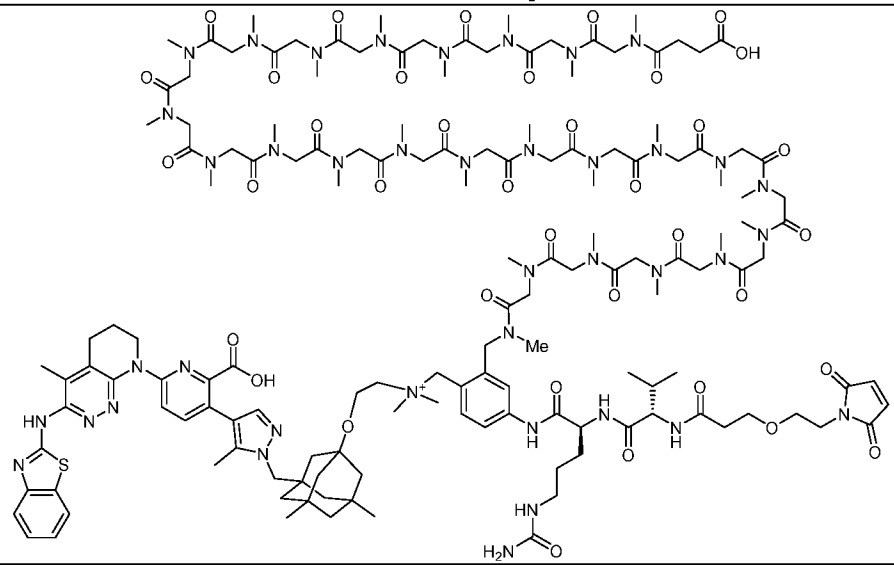
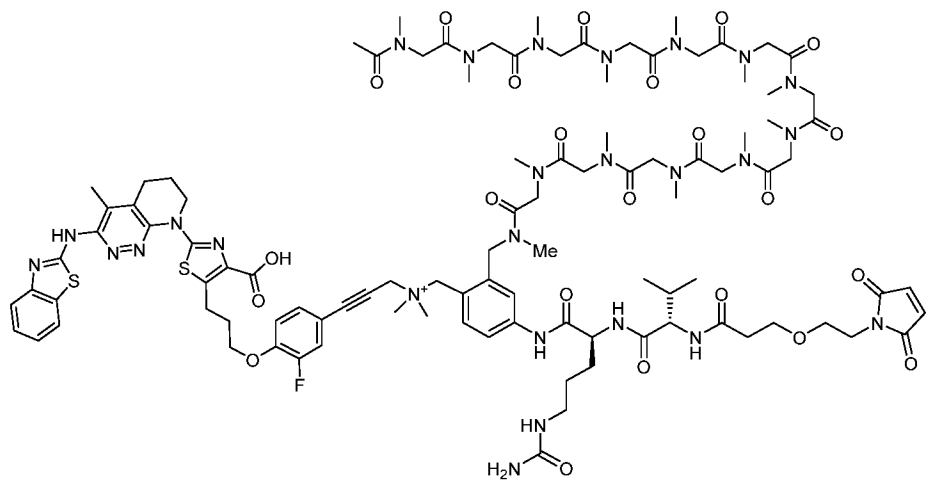
L30C-P25	
L30A-P27	
L35A-P1	

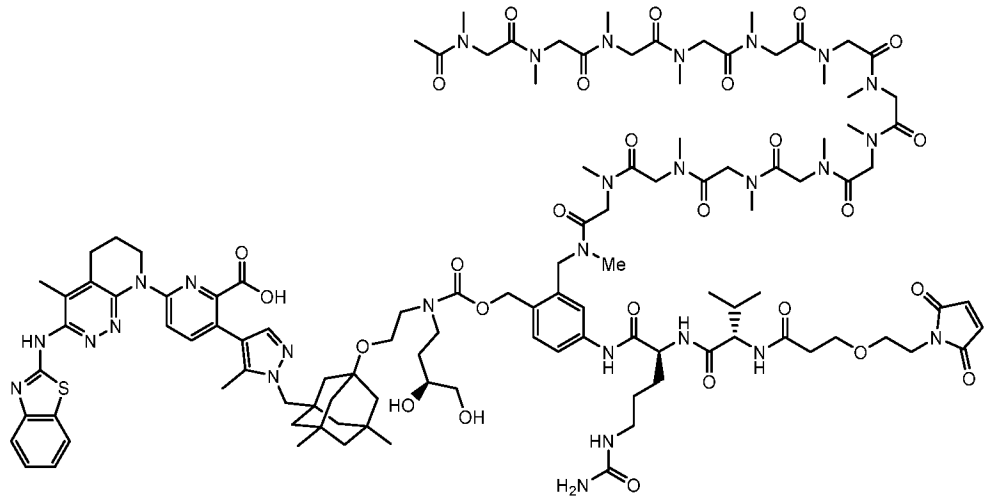
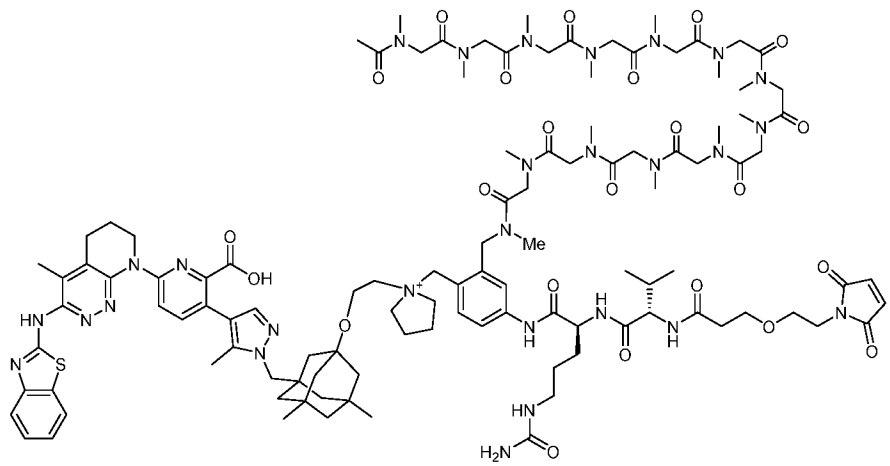
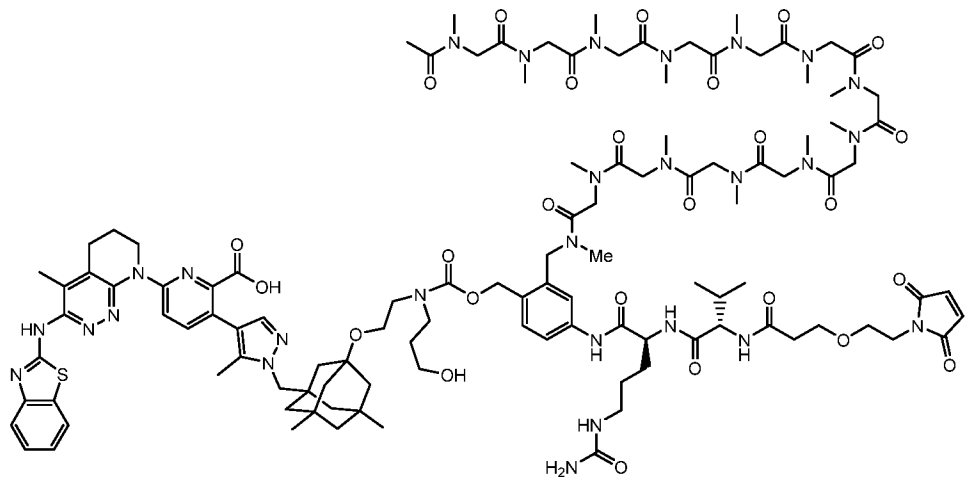
L35C-P19	
L35A-P21	
L35C-P25	

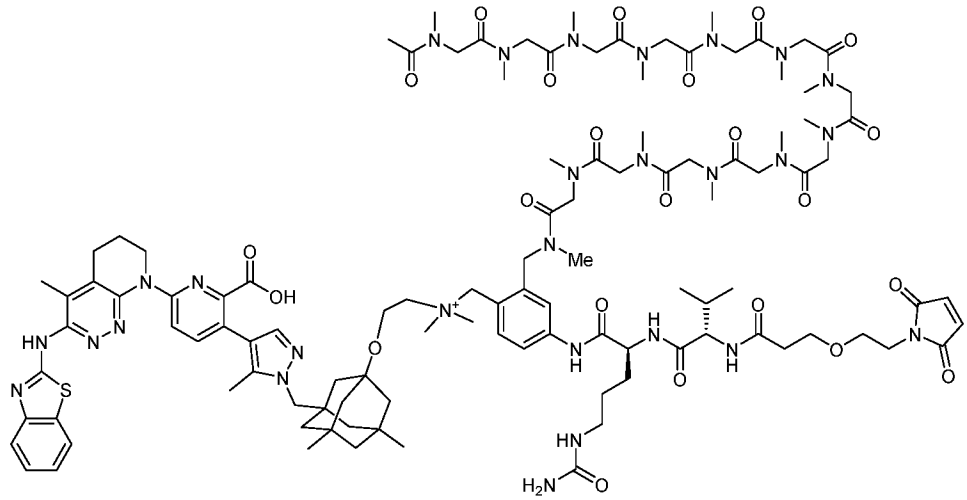
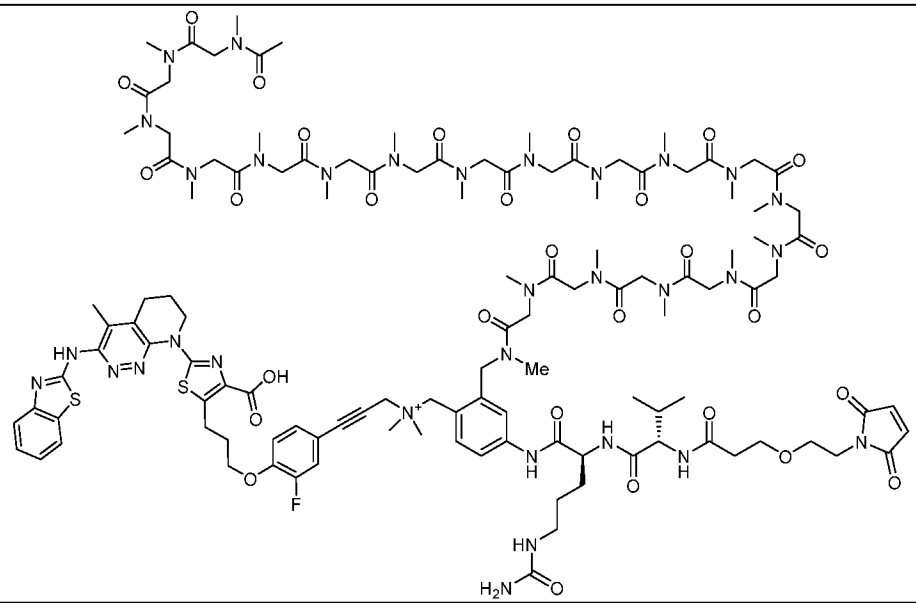
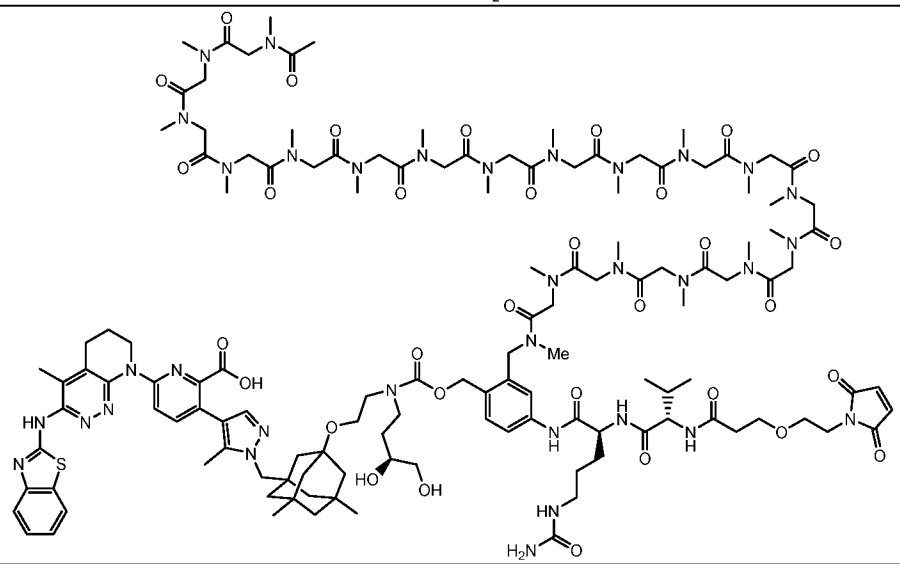
L35A-P27	
L36A-P1	
L36C-P19	

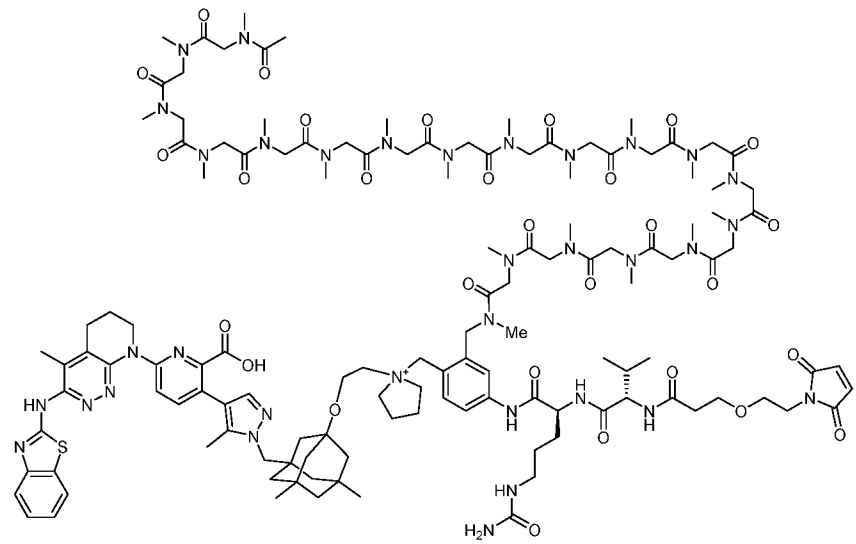
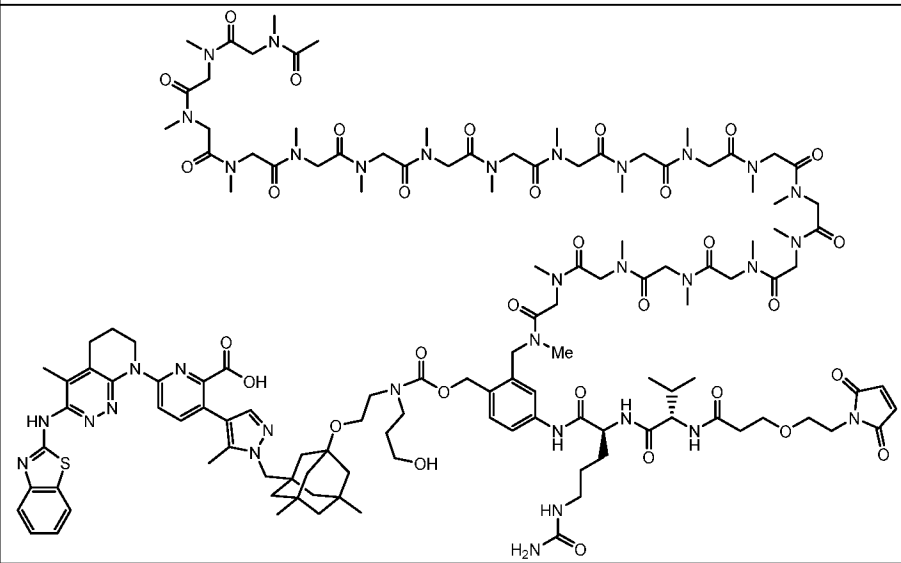
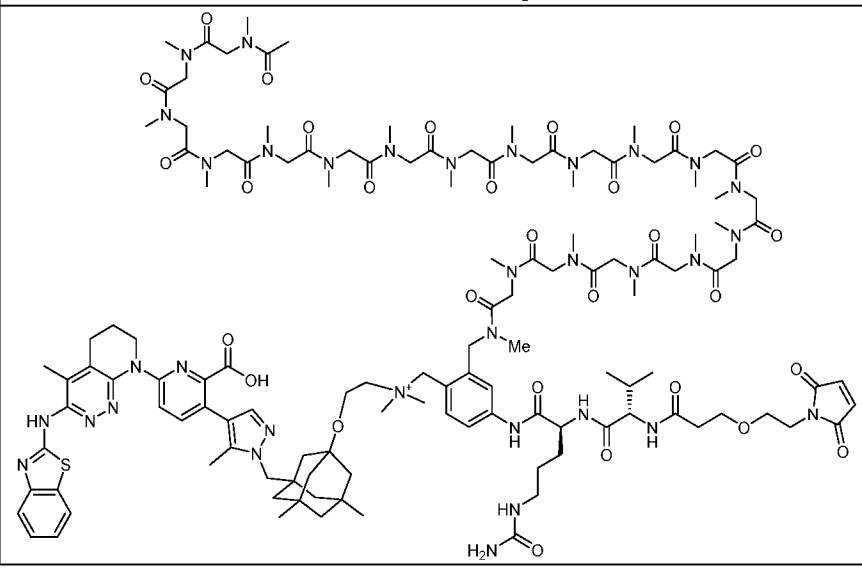
L36A-P21	
L36C-P25	
L36A-P27	

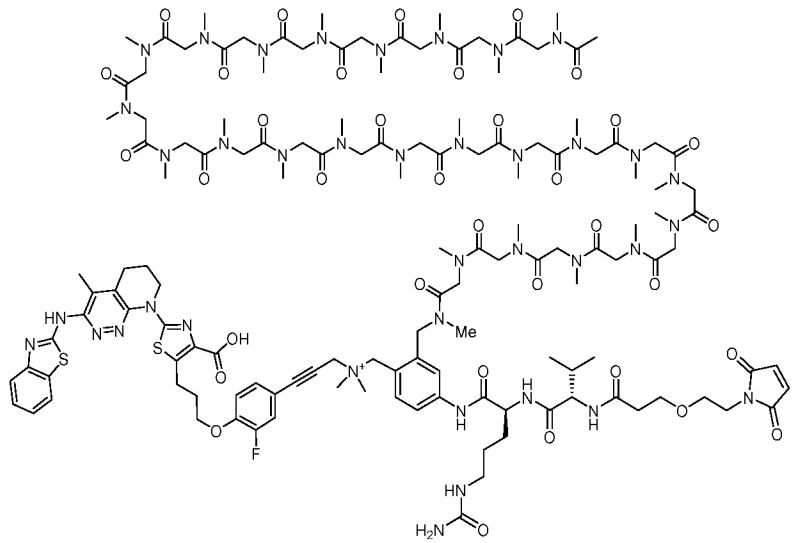
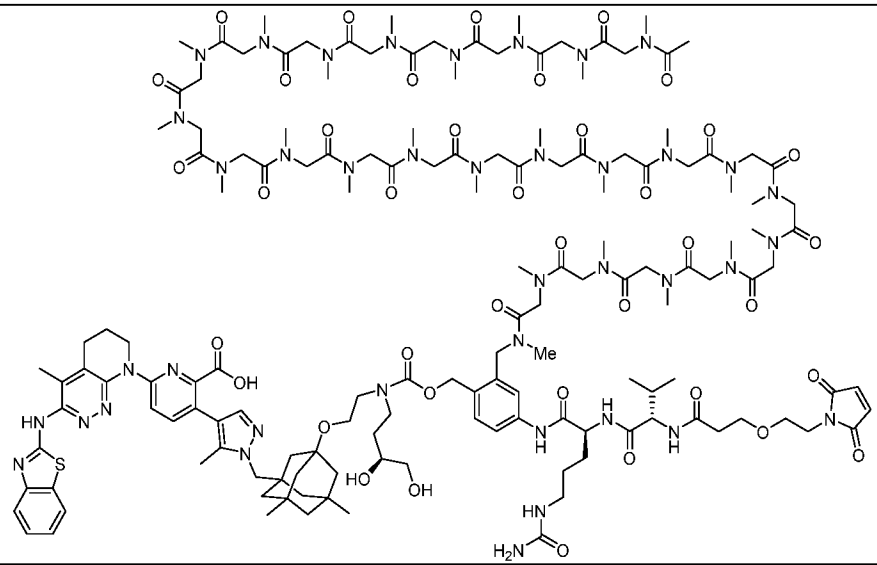
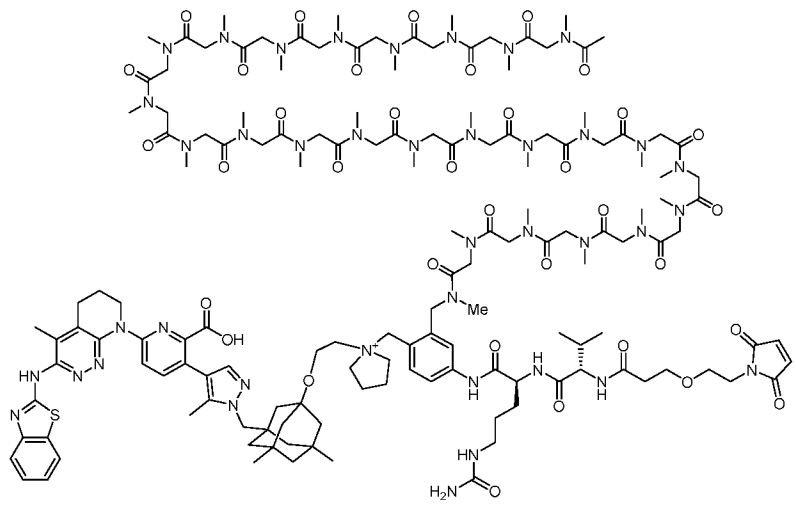
L37A-P1	
L37C-P19	
L37A-P21	

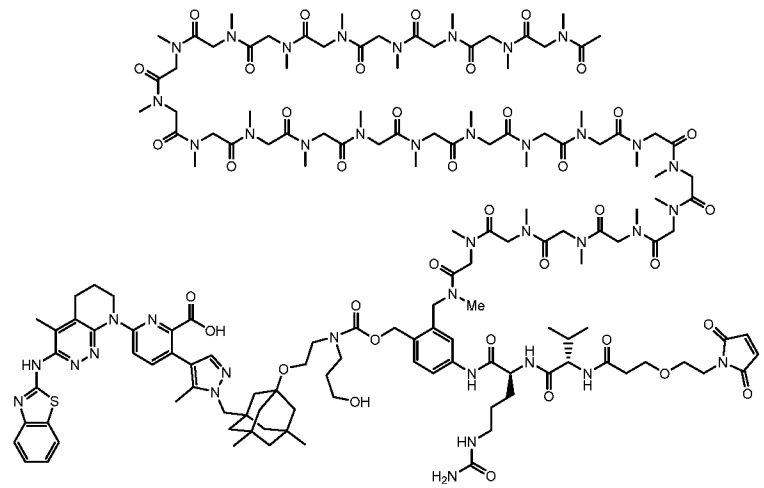
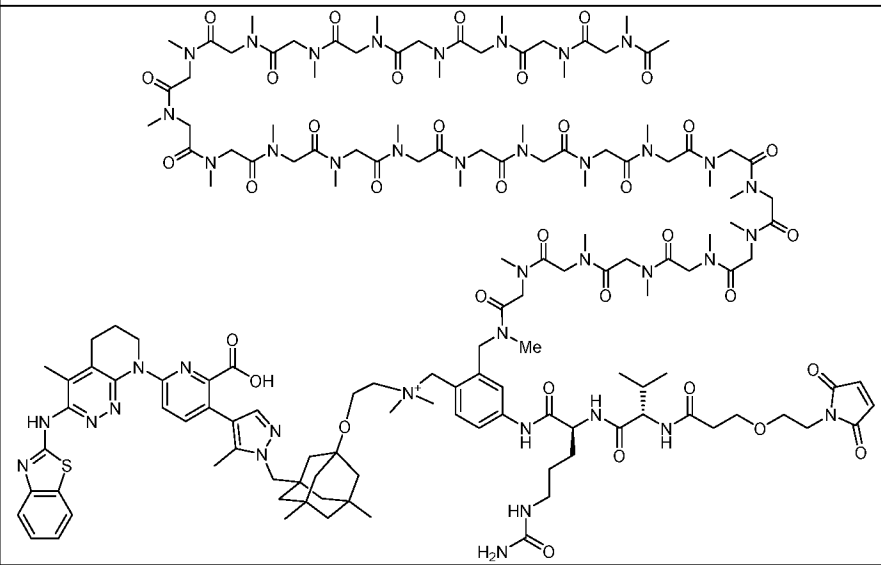
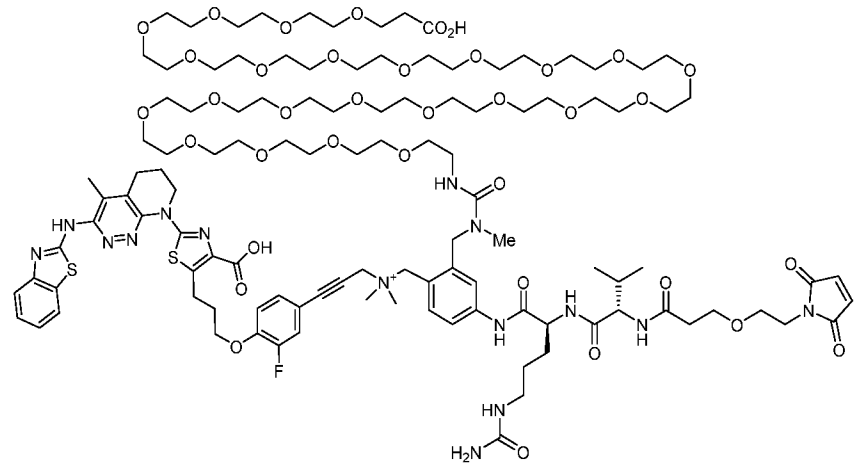
L37C-P25	
L37A-P27	
L38A-P1	

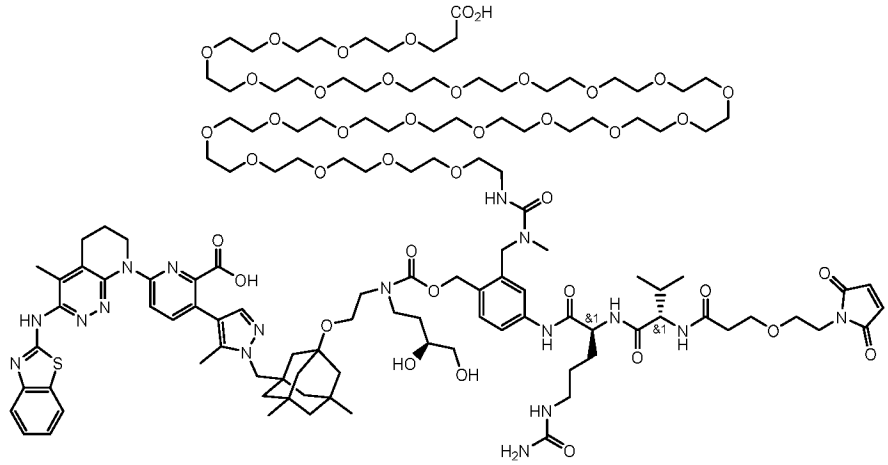
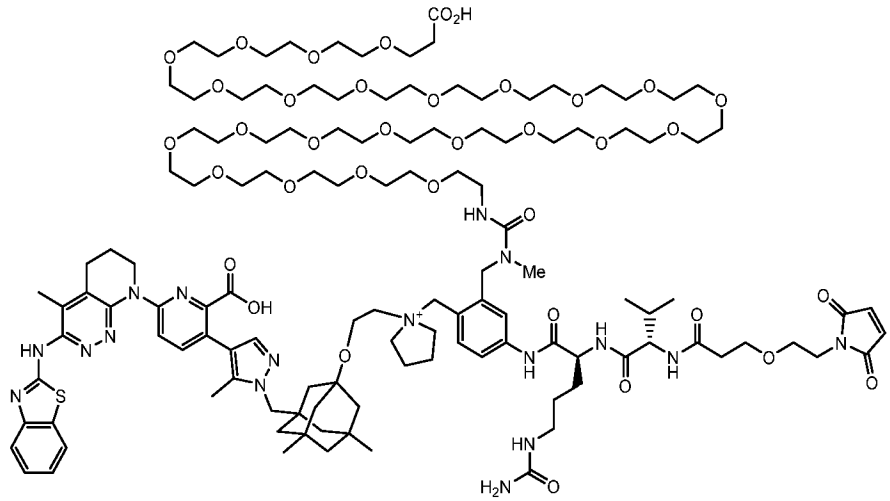
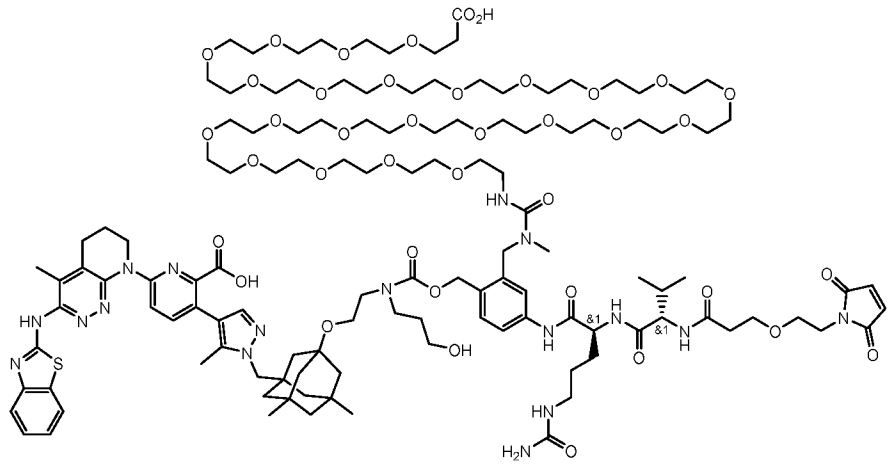
L38C-P19	
L38A-P21	
L38C-P25	

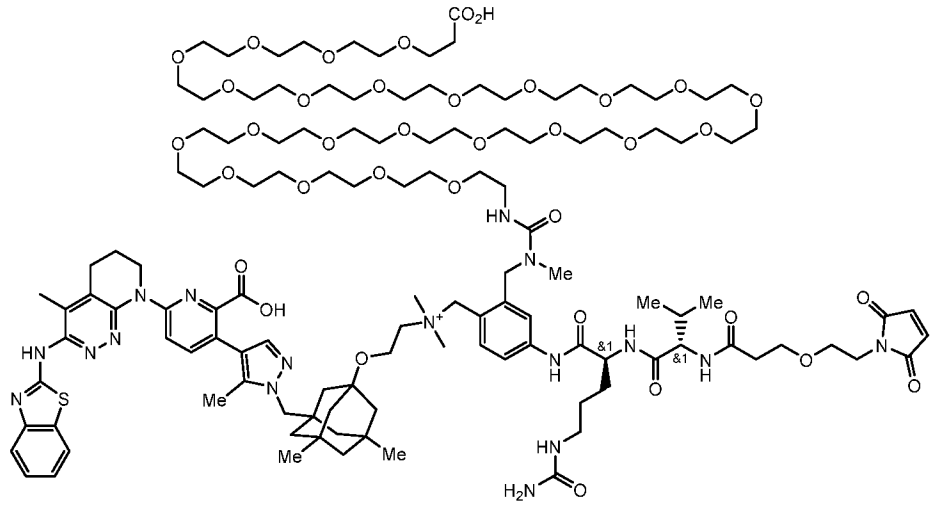
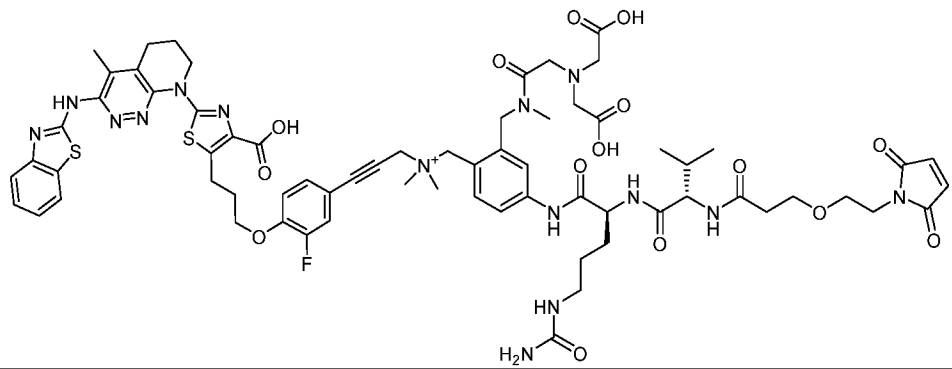
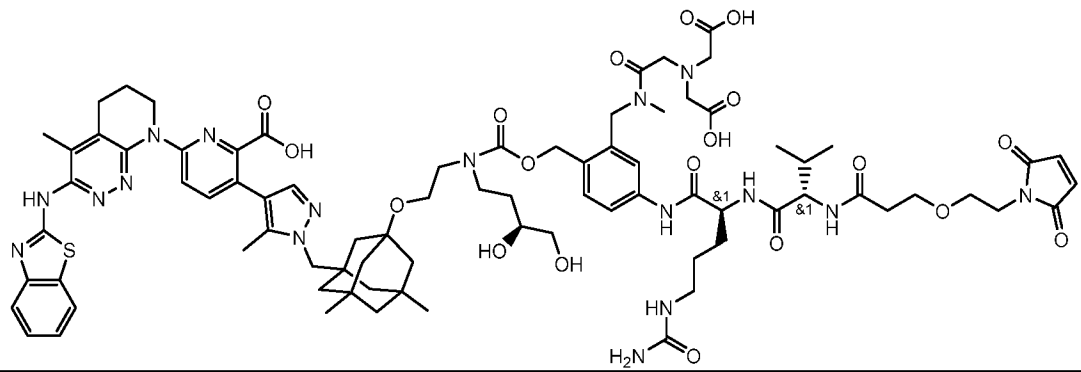
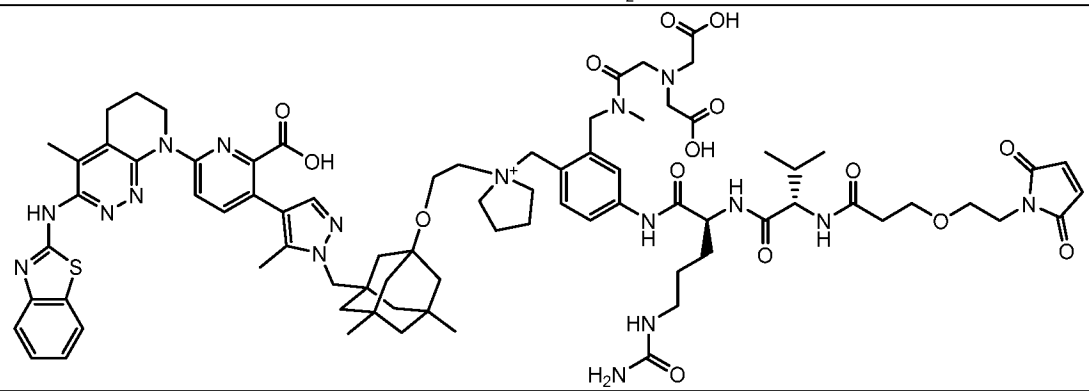
L38A-P27	
L39A-P1	
L39C-P19	

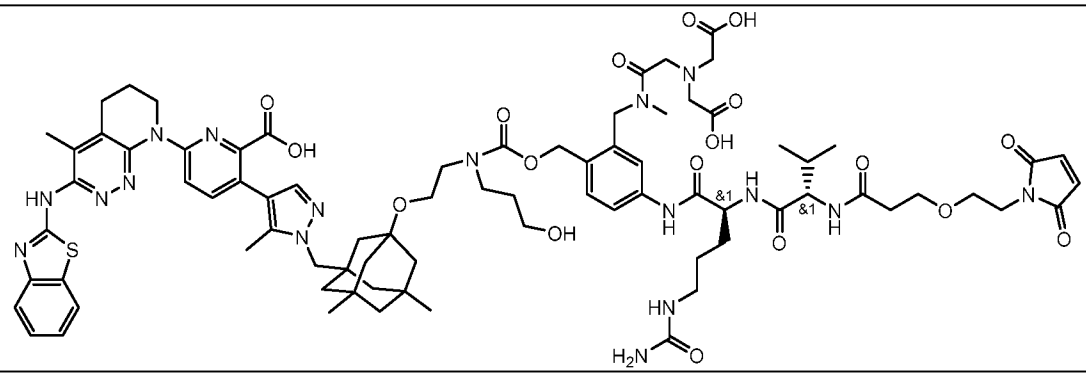
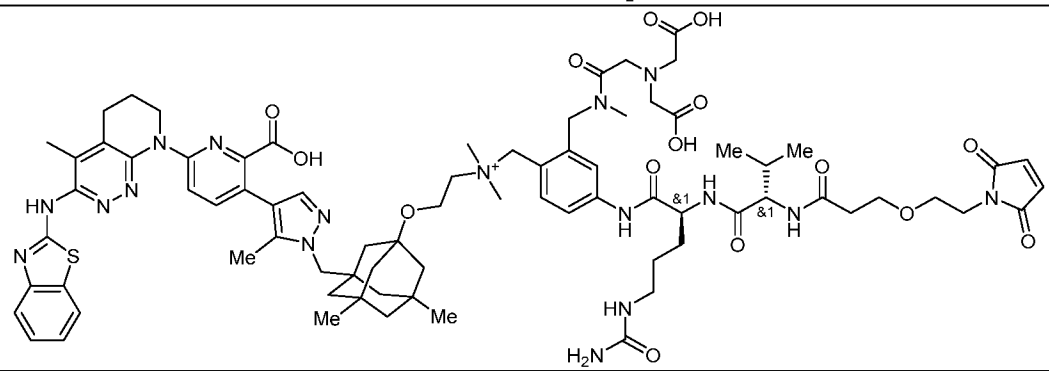
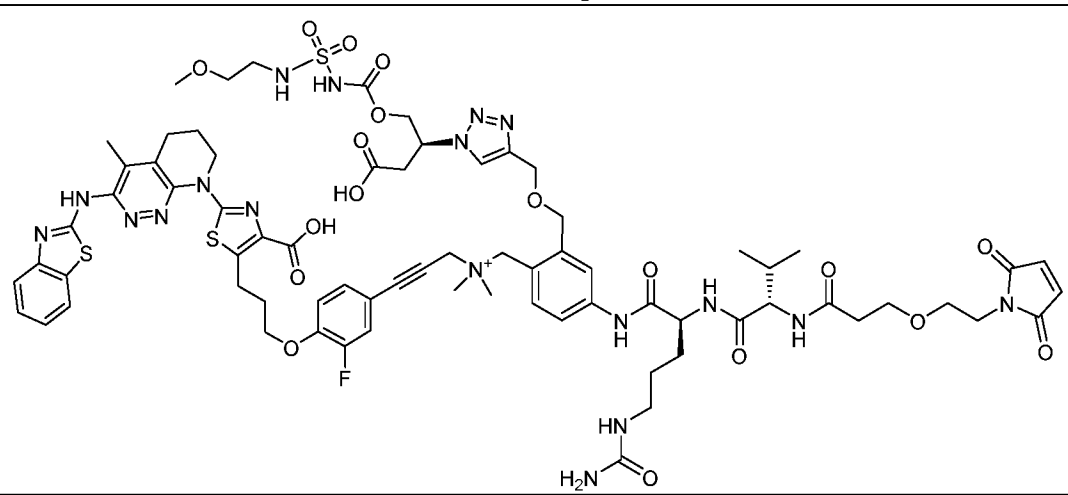
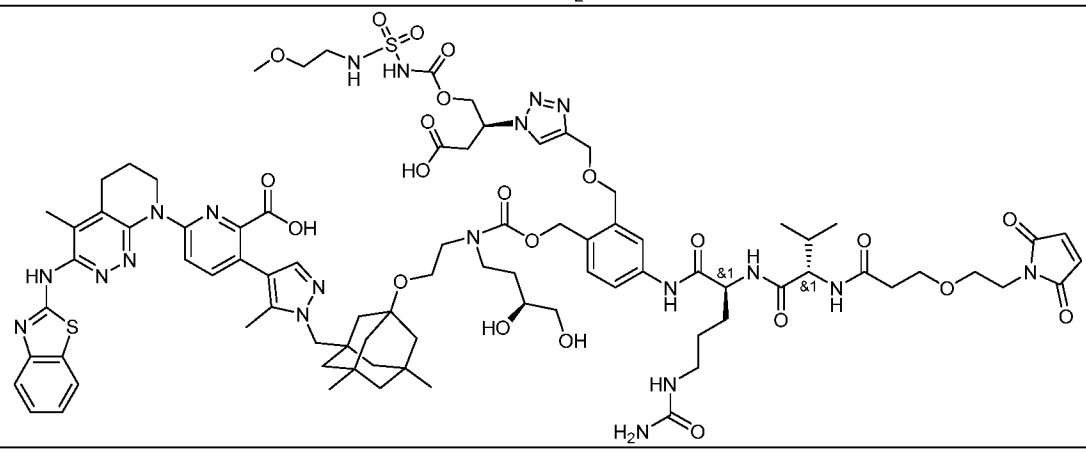
L39A-P21	
L39C-P25	
L39A-P27	

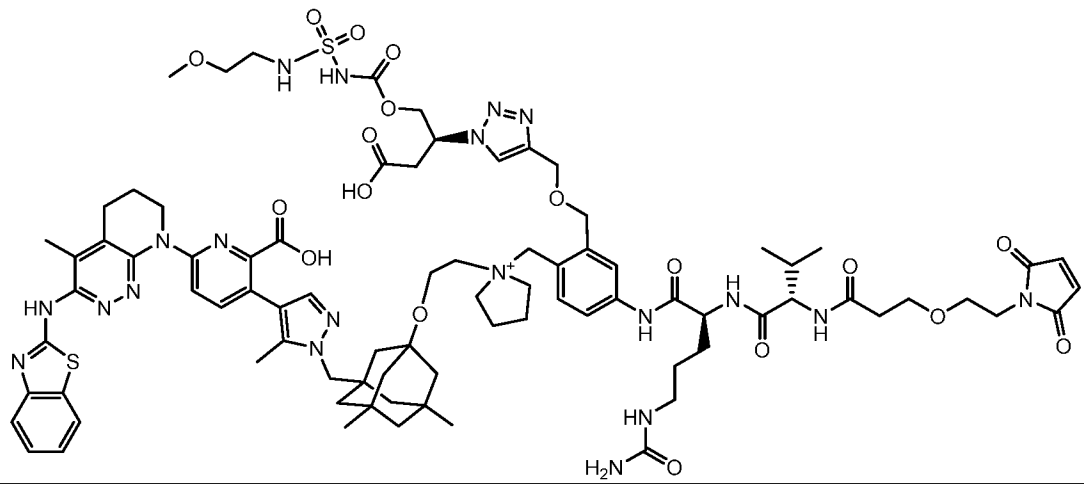
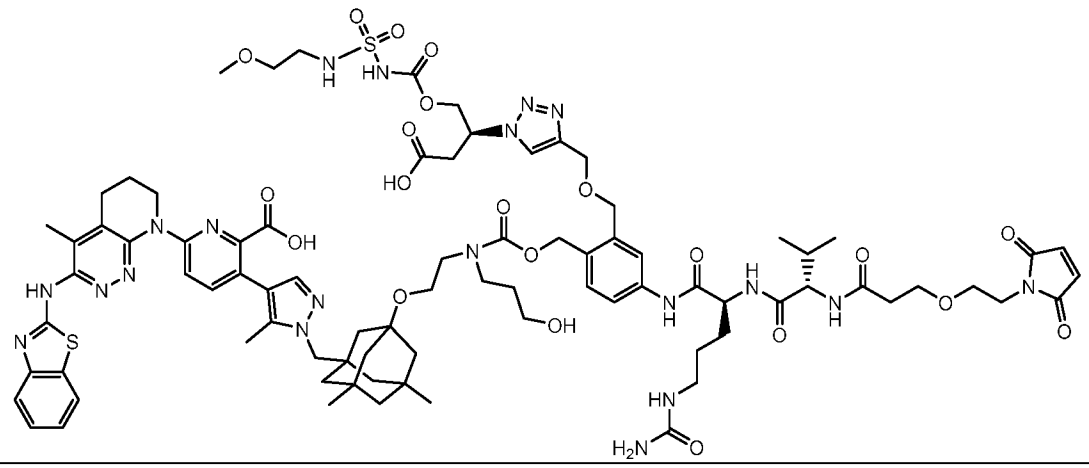
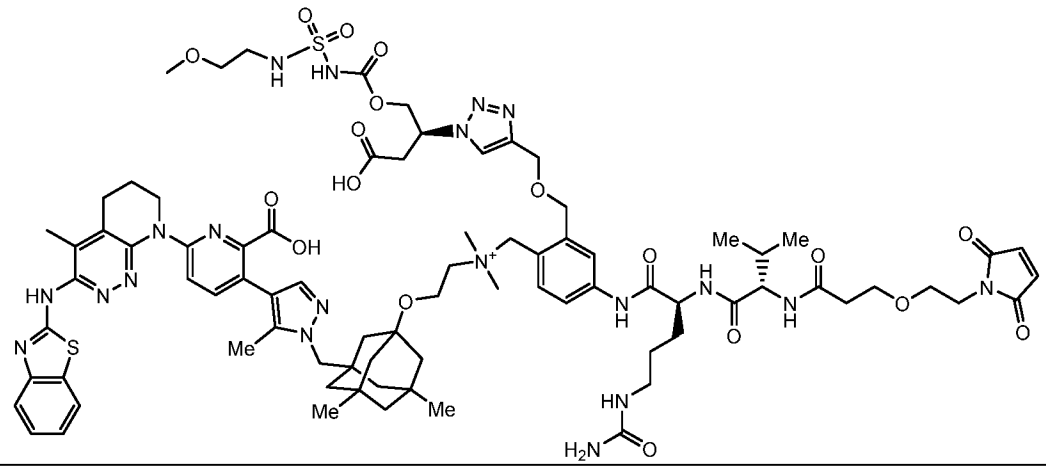
L40A-P1	
L40C-P19	
L40A-P21	

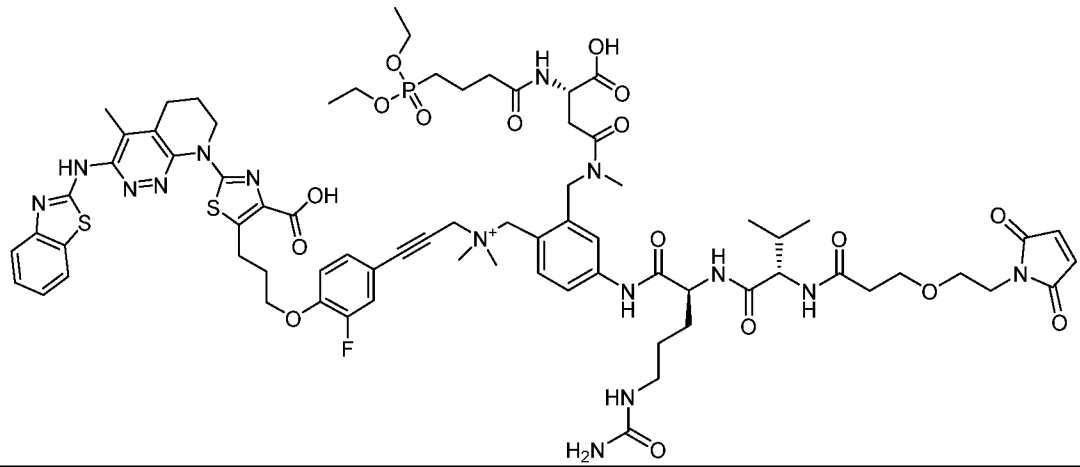
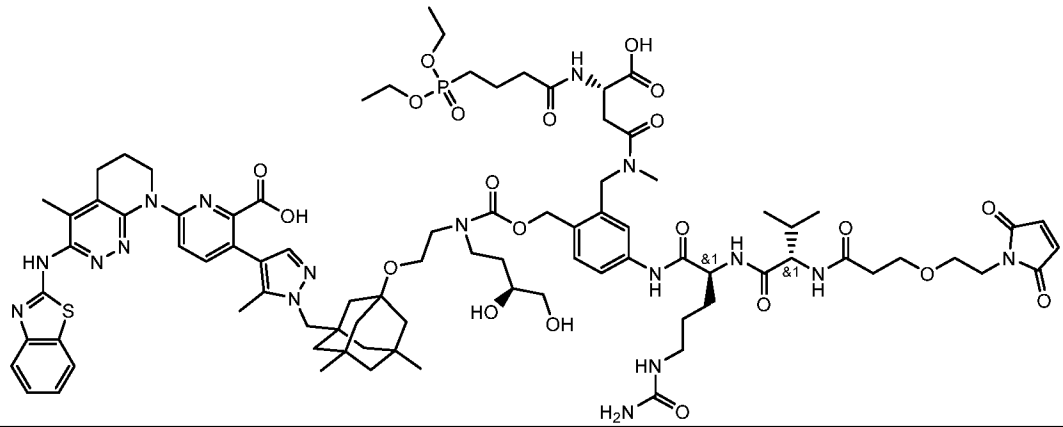
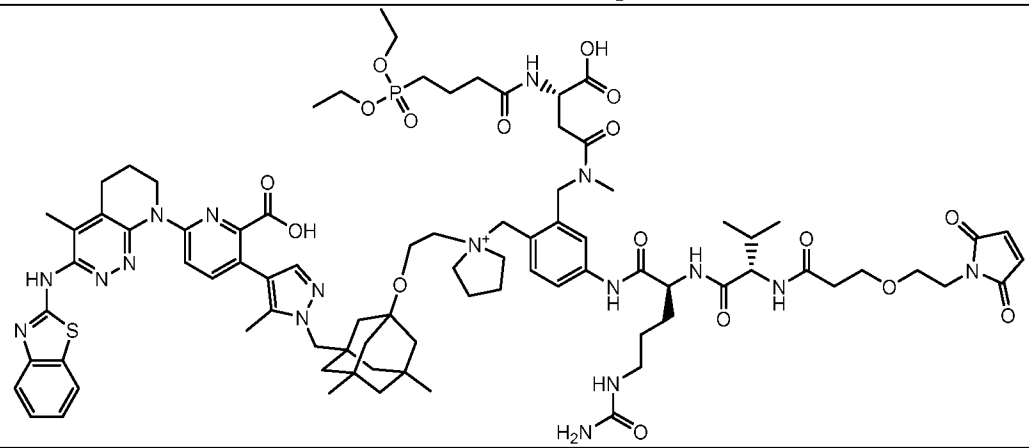
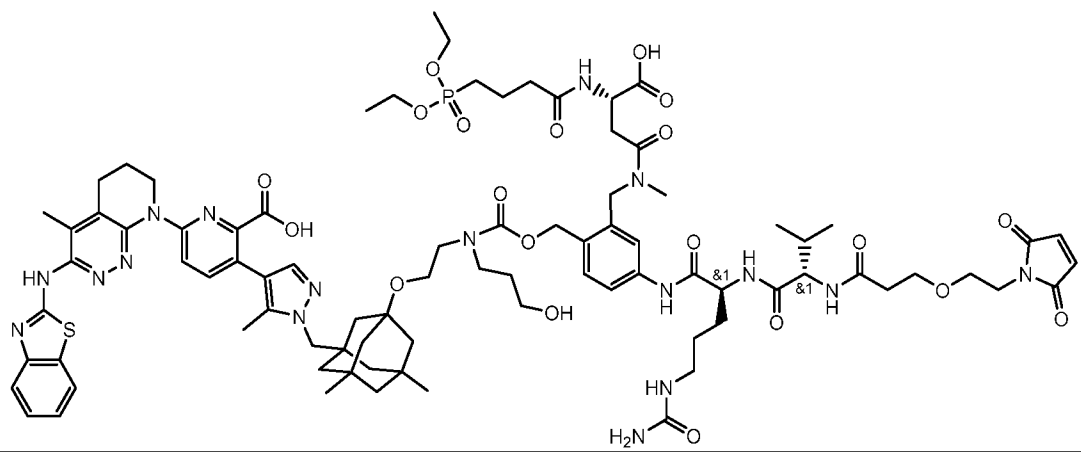
L40C-P25	
L40A-P27	
L42A-P1	

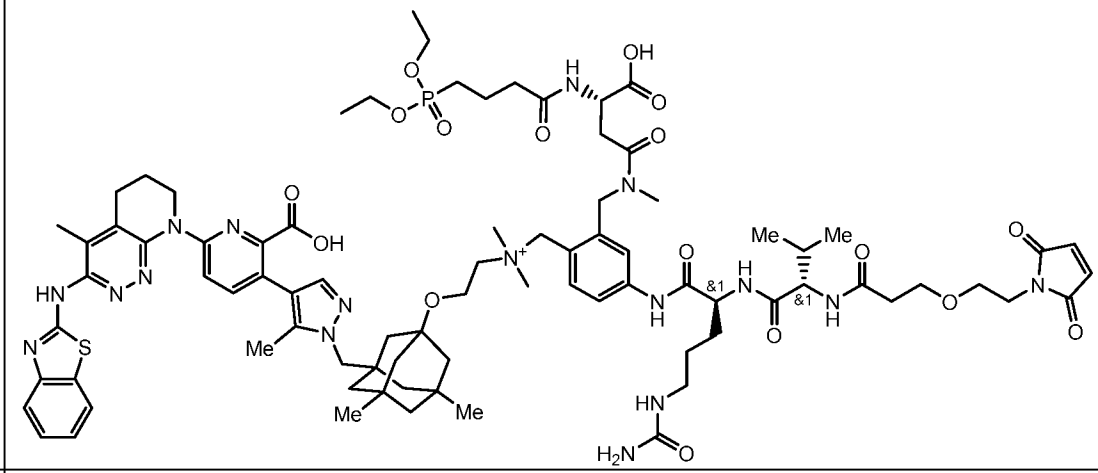
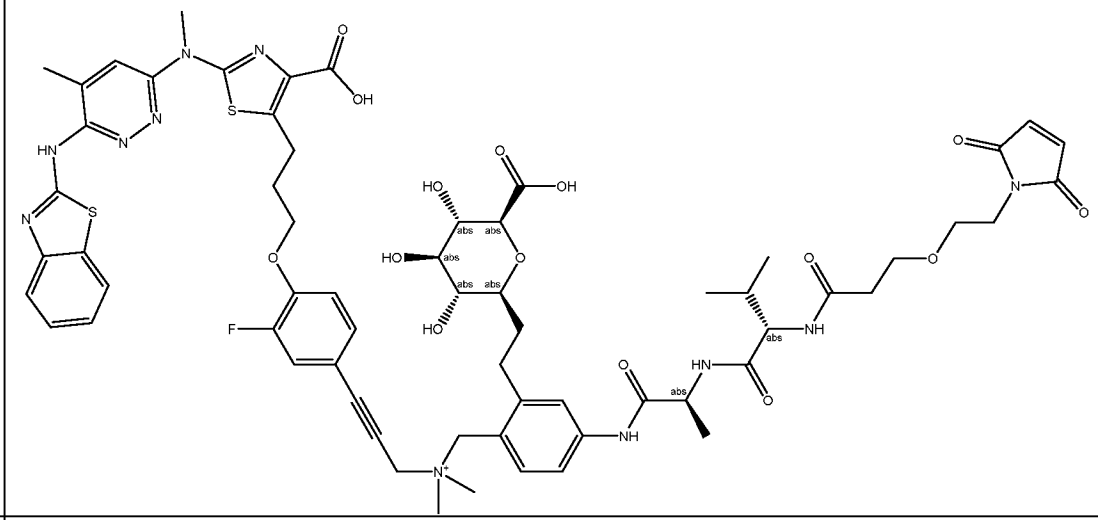
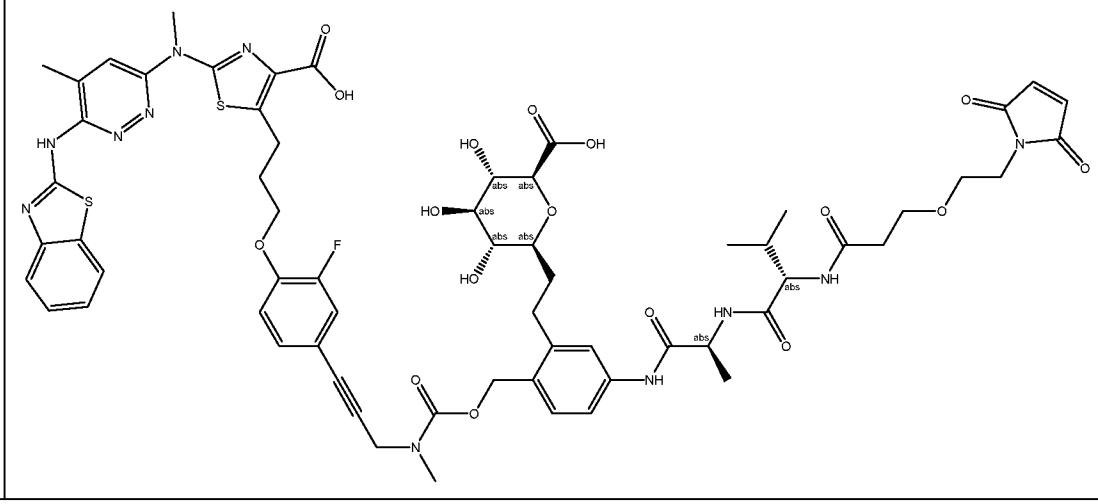
L42C-P19	
L42A-P21	
L42C-P25	

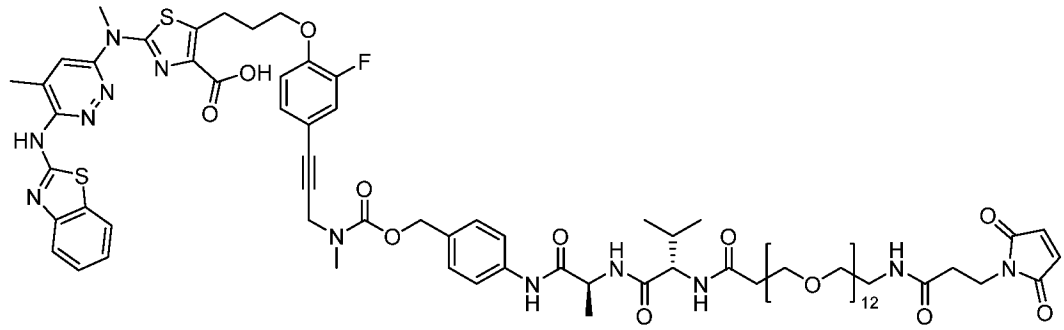
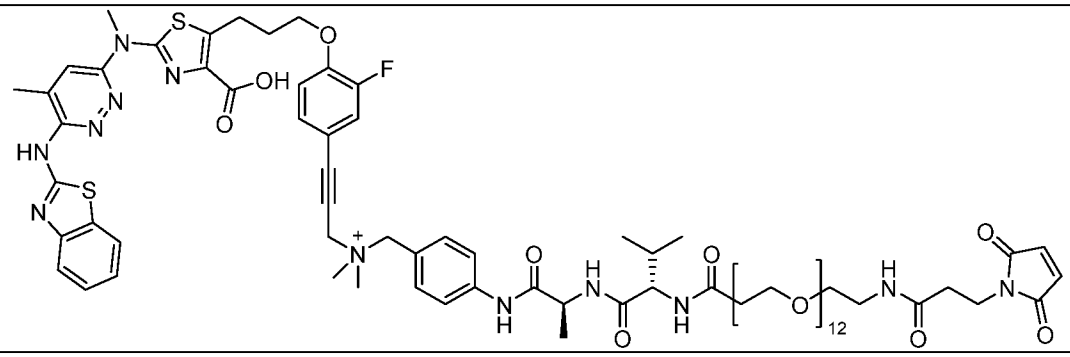
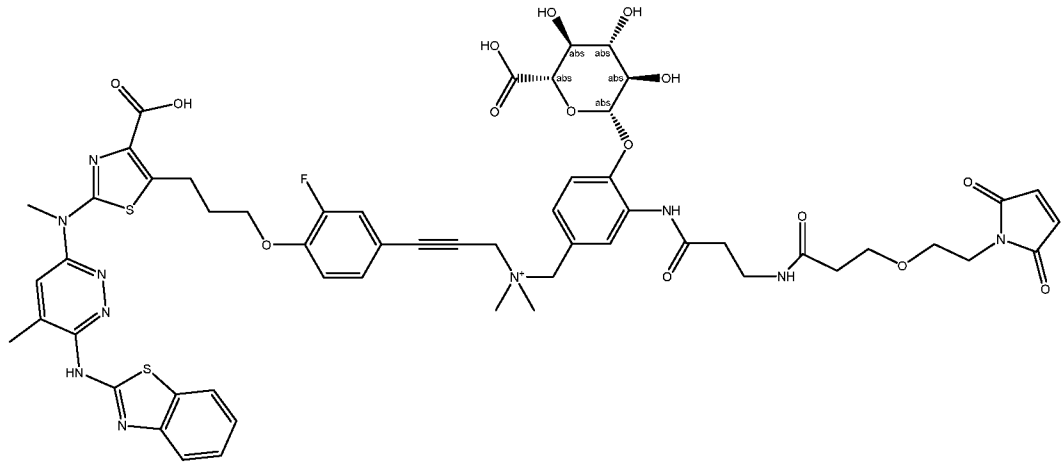
L42A-P27	
L67A-P1	
L67C-P19	
L67A-P21	

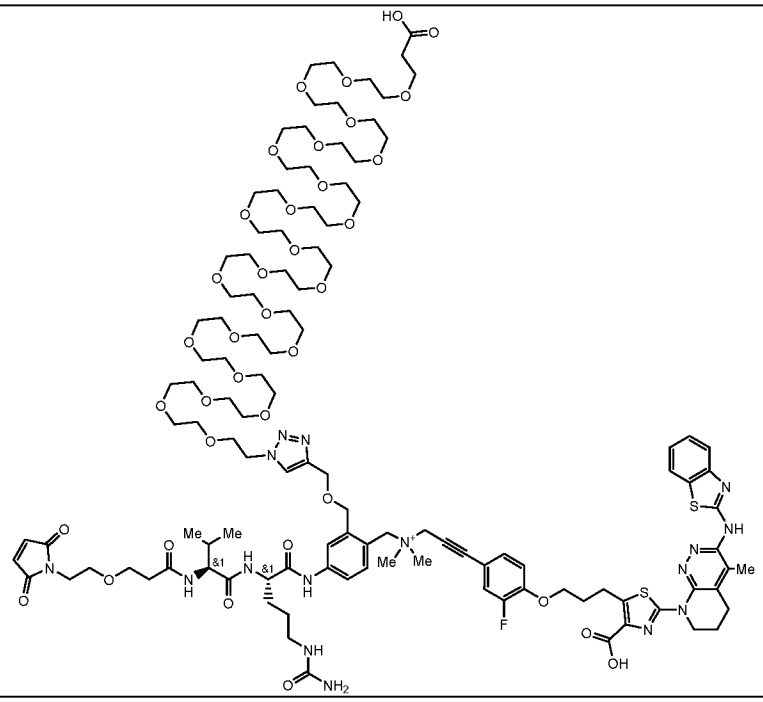
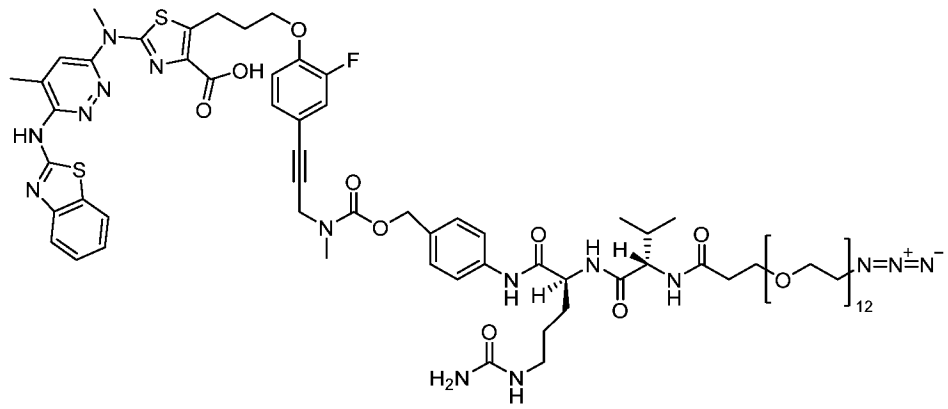
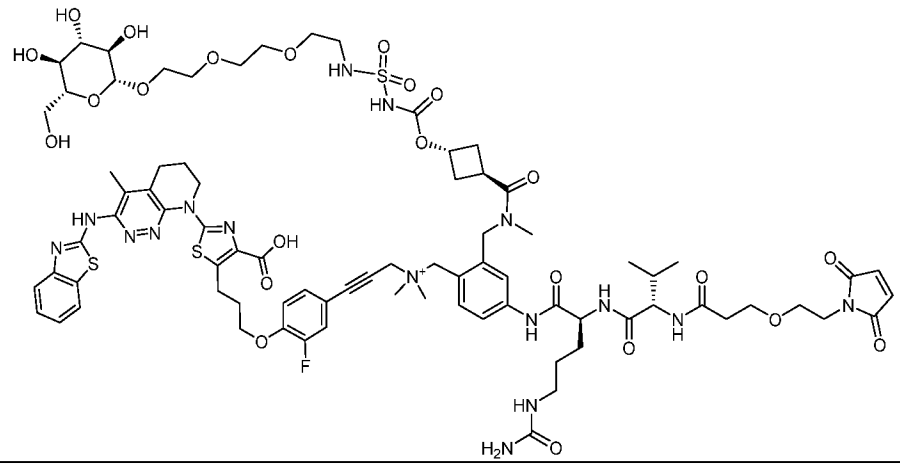
L67C-P25	
L67A-P27	
L100A-P1	
L100C-P19	

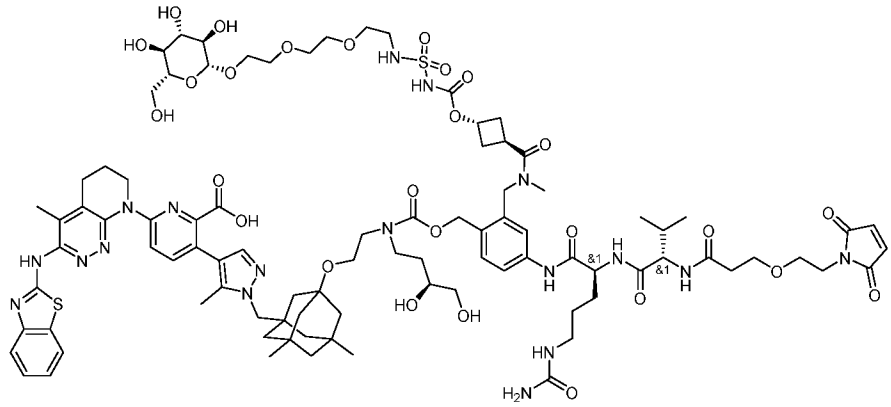
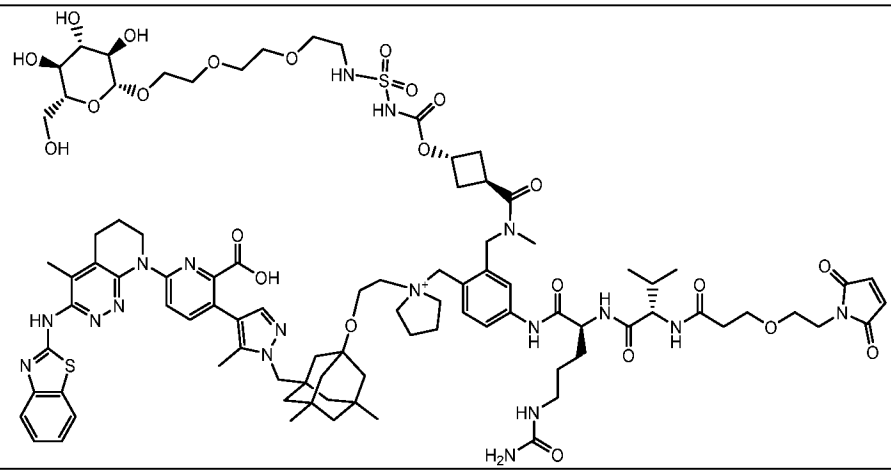
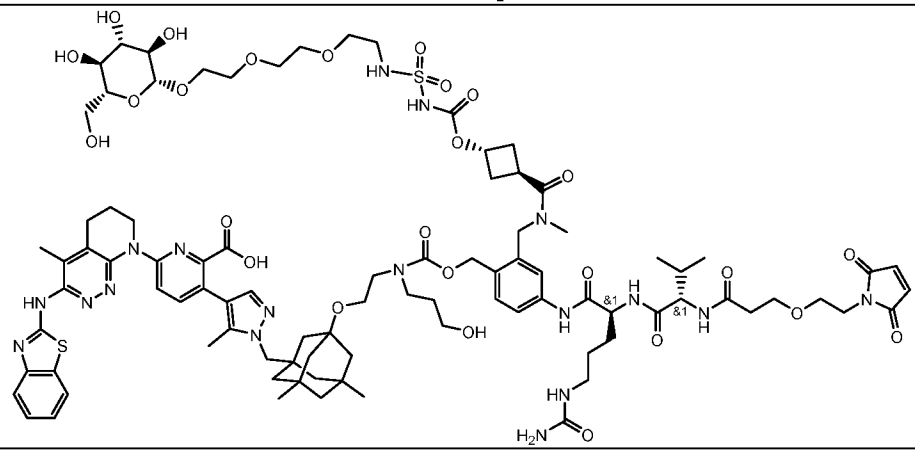
L100A-P21	
L100C-P25	
L100A-P27	

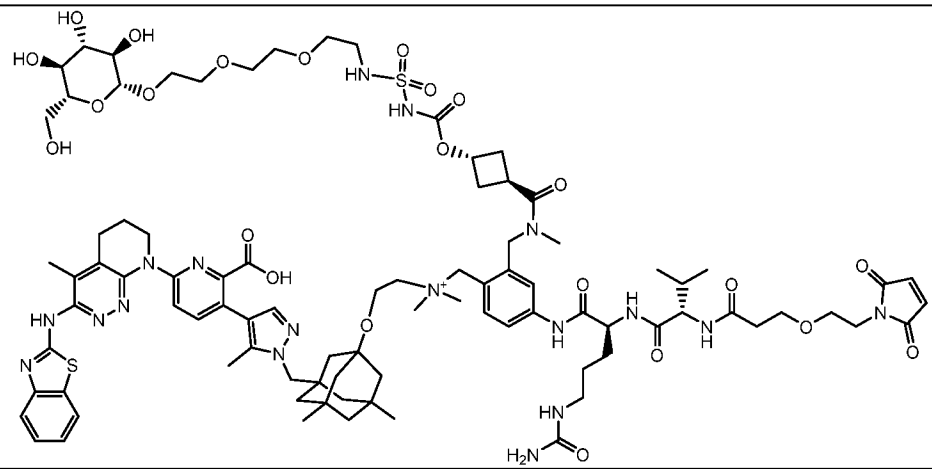
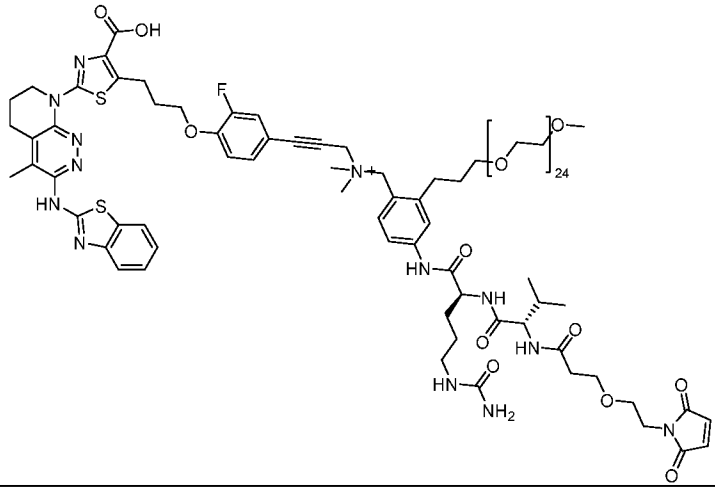
L103A-P1	
L103C-P19	
L103A-P21	
L103C-P25	

L103A-P27	
L106A-P2	
L106C-P7	

L107C-P7	
L107A-P2	
L108A-P2	

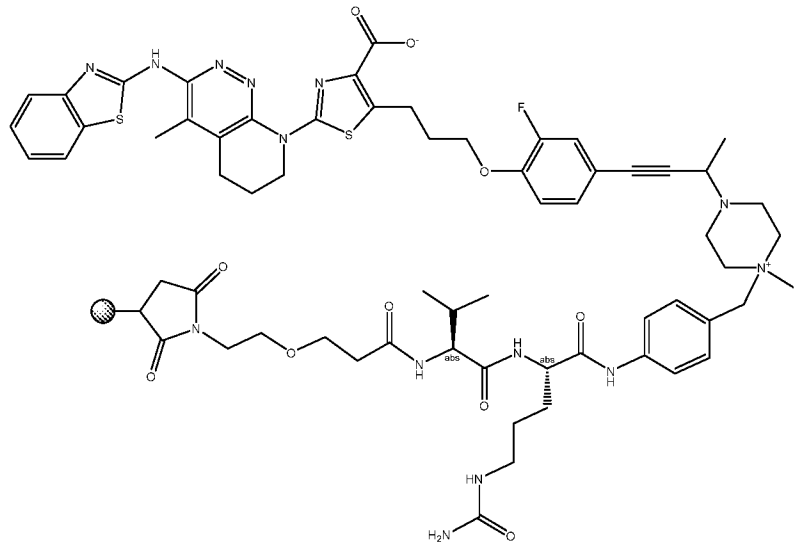
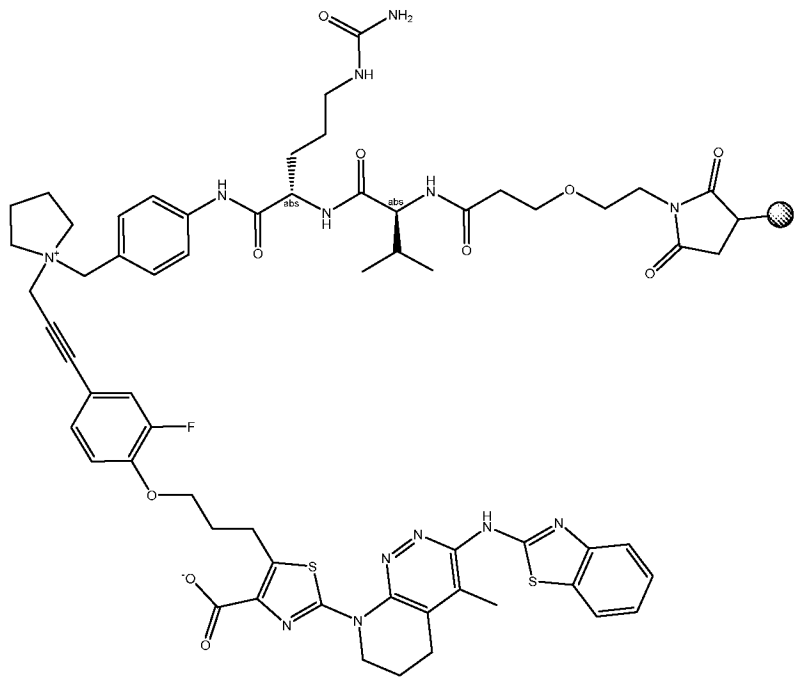
L109A-P1	
L110C-P7	
L111A-P1	

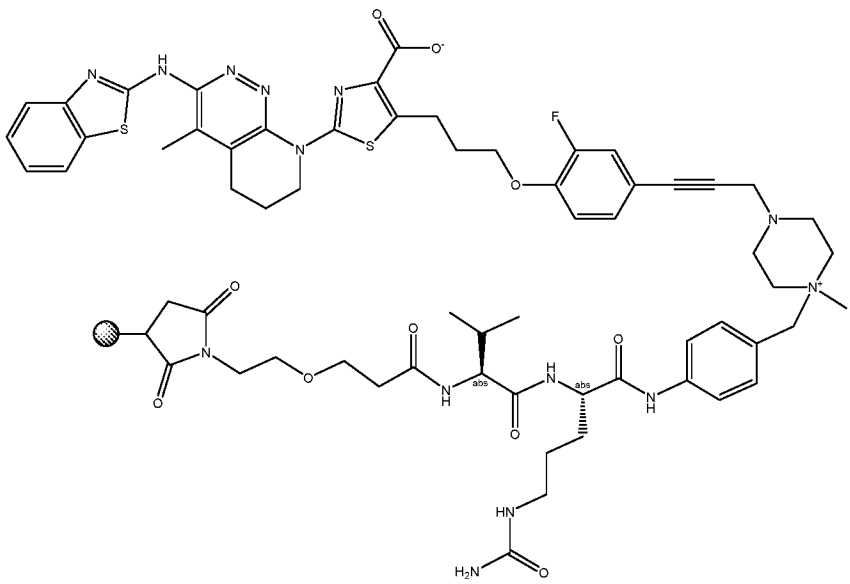
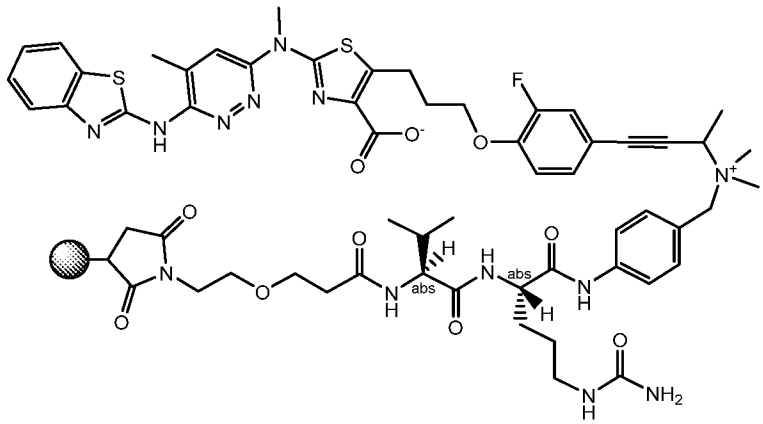
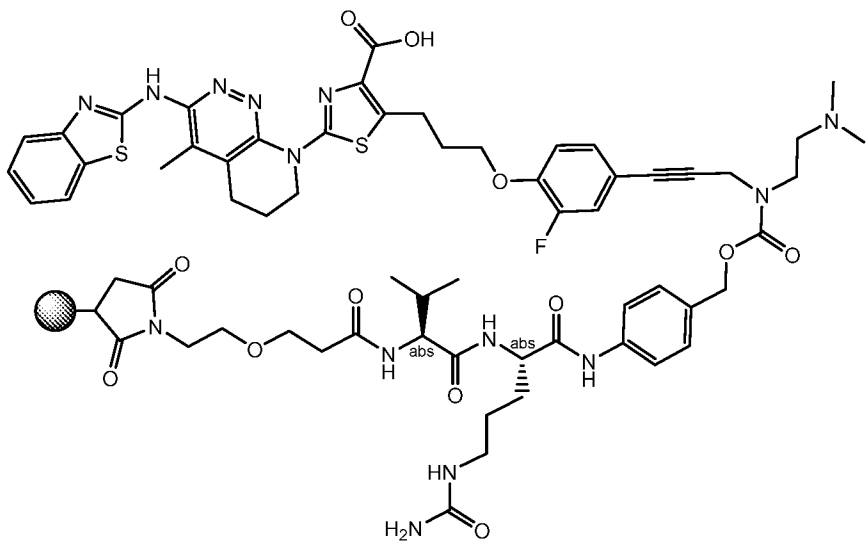
L111C-P19	
L111A-P21	
L111C-P25	

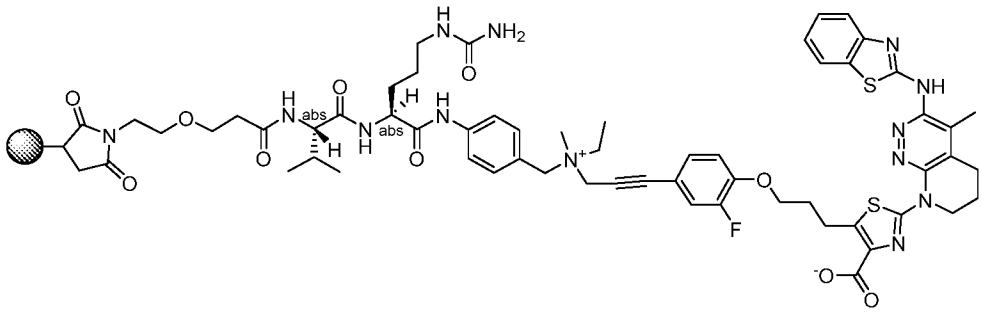
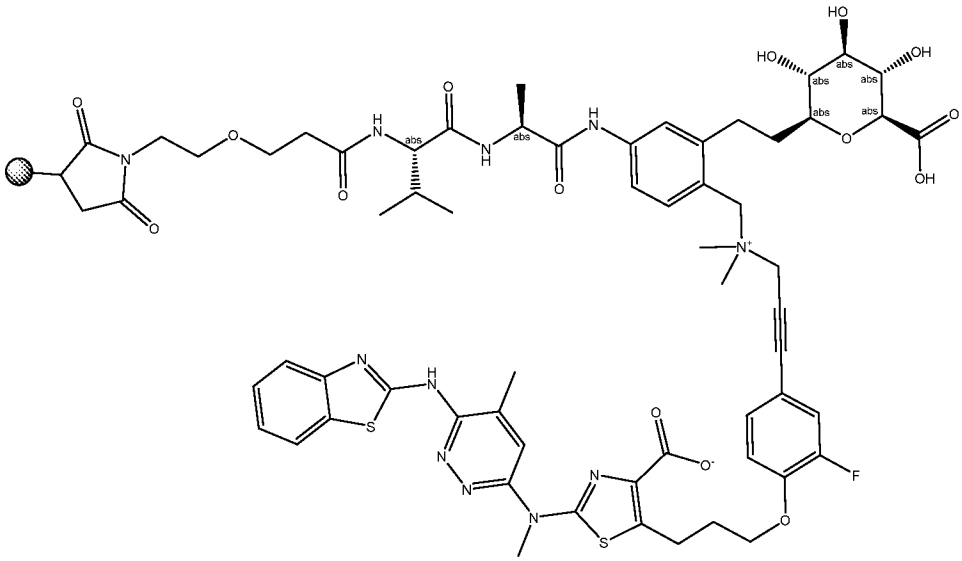
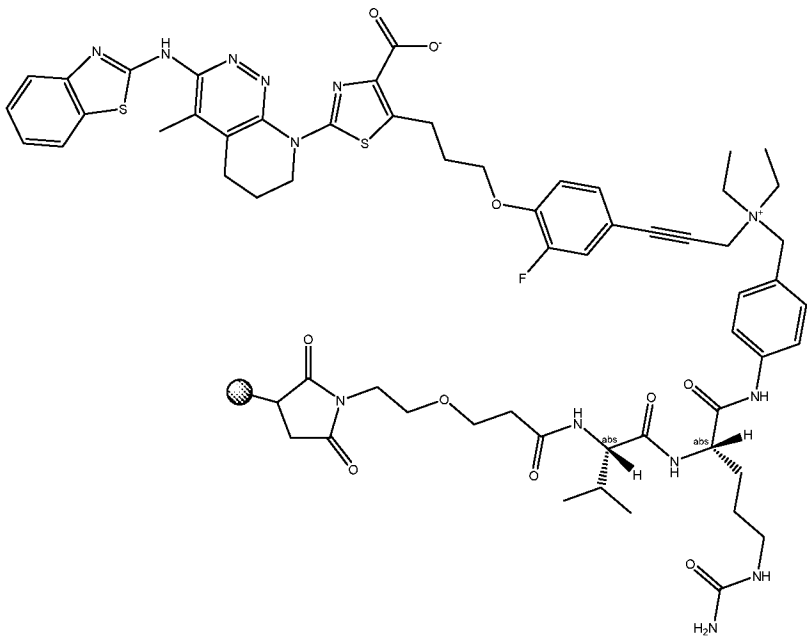
L111A-P27	
L112A-P1	

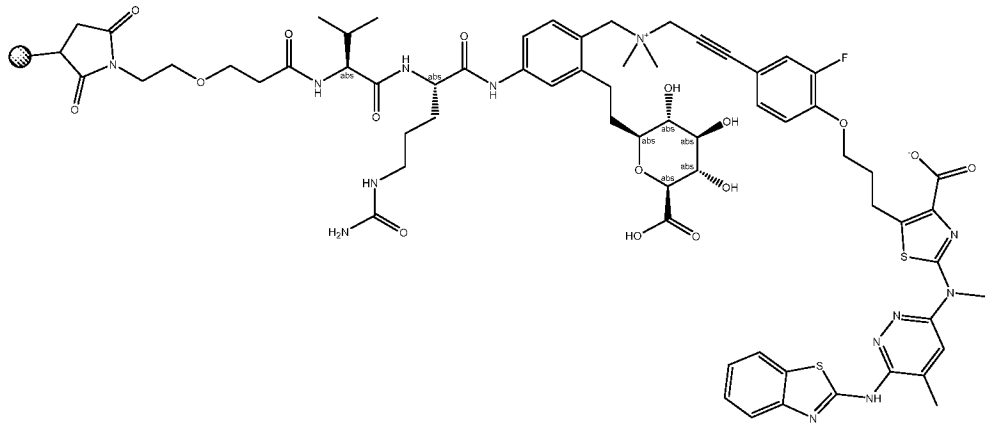
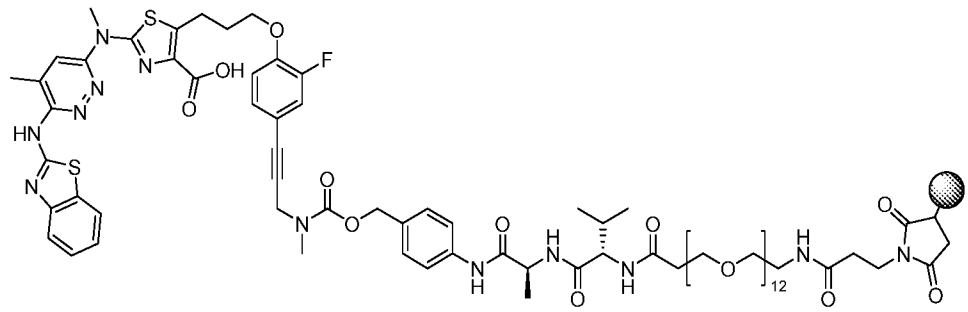
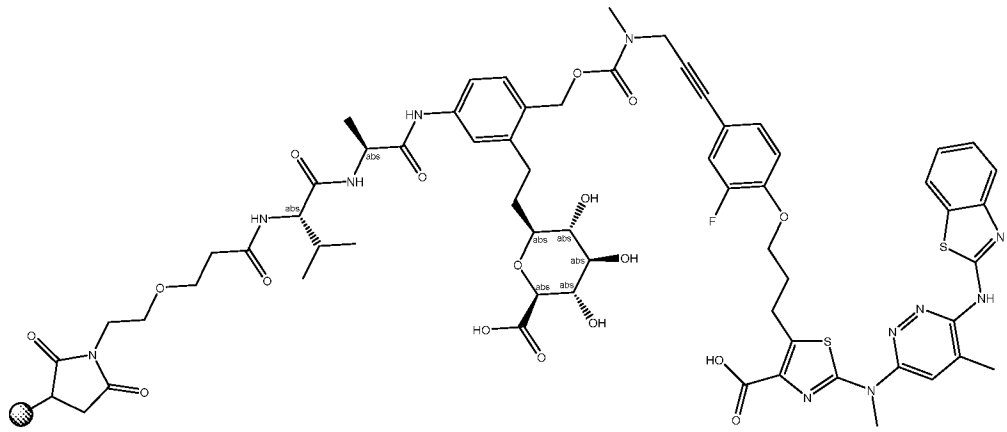
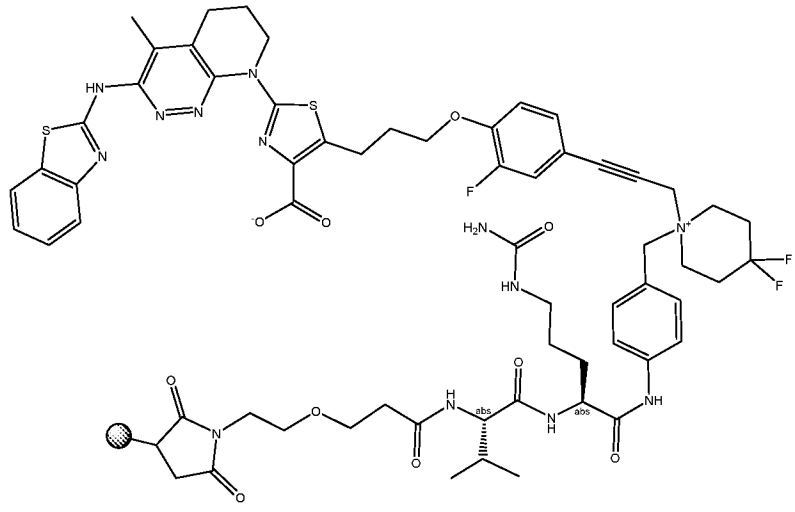
[96] In some embodiments, the antibody-drug conjugate has a formula according to any one of the structures shown in Table 1.

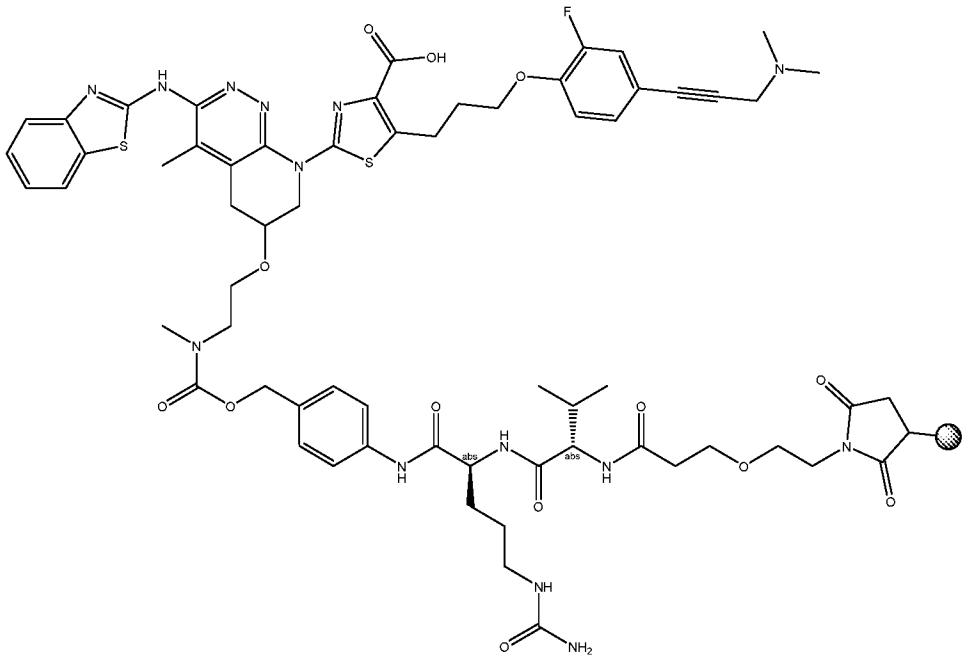
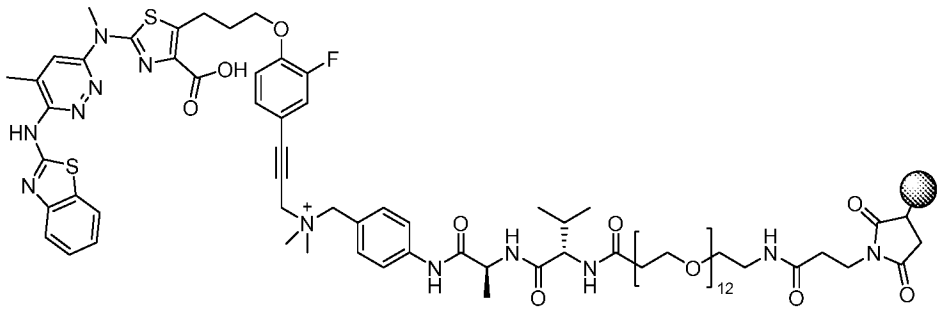
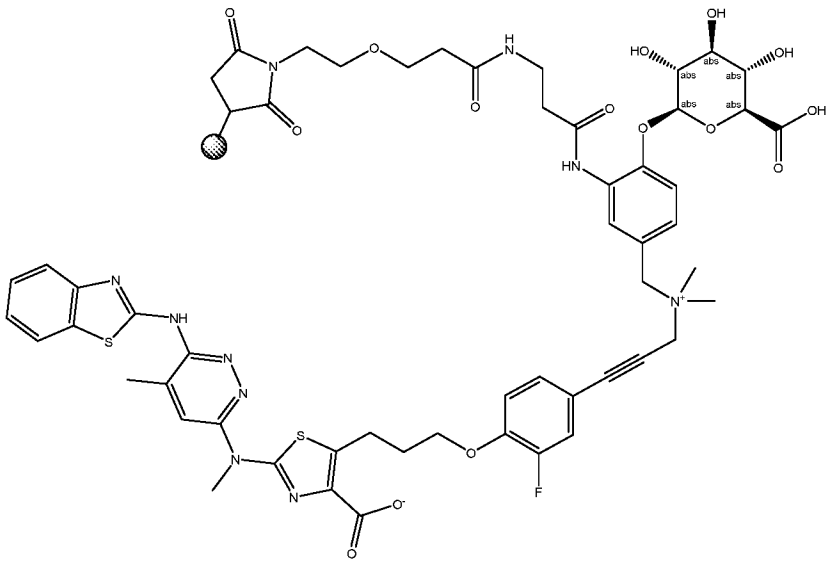
Table 1. ADC Structures

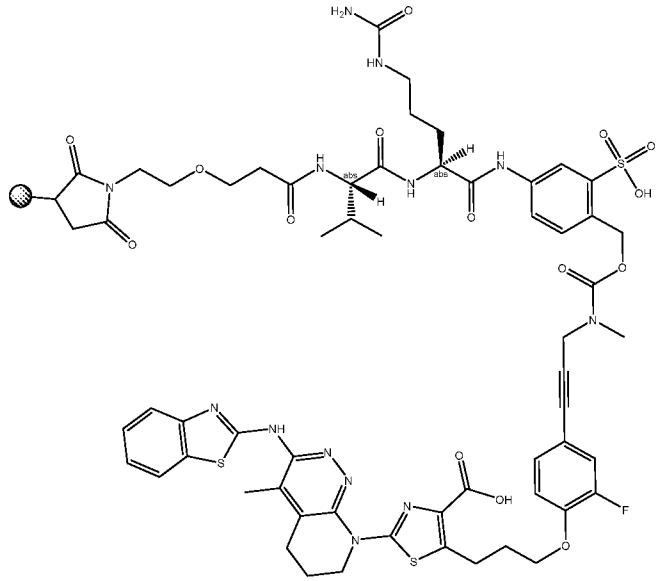
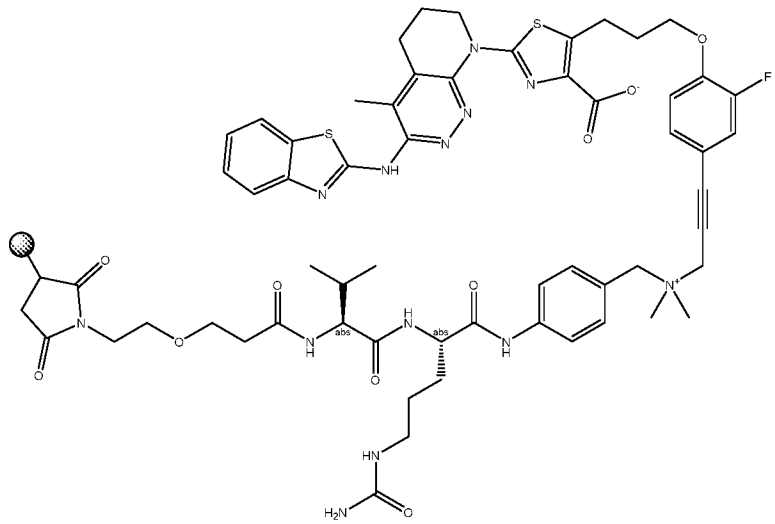
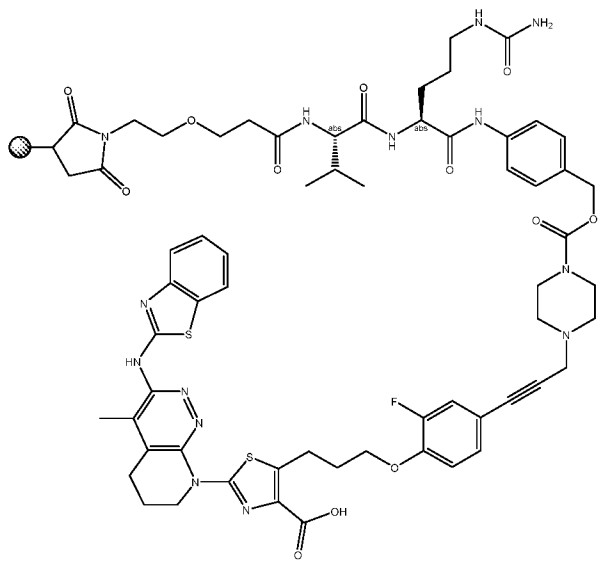
ADC	Structure
Ab C – L9A-P8	
Ab C – L9A-P9	

ADC	Structure
Ab C – L9A-P10	
Ab C – L9A-P11	
Ab C - L9C-P12	

ADC	Structure
Ab C – L9A-P13	
Ab C – L106A-P2	
Ab C – L9A-P14	

ADC	Structure
Ab C – L21A-P2	
Ab C – L107C-P7	
Ab C – L106C-P7	
Ab C – L9A-P15	

ADC	Structure
Ab C – L9C-P16	
Ab C – L107A-P2	
Ab C – L108A-P2	

ADC	Structure
Ab C – L27C-P3	
Ab C – L9A-P1	
Ab C – L9C-P17	

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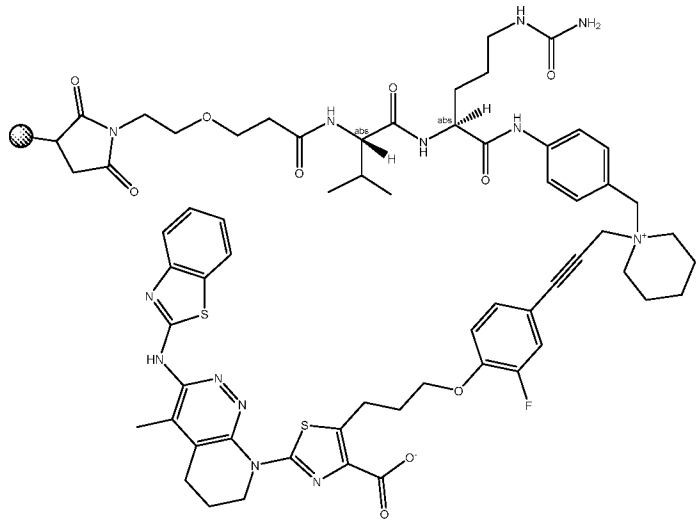
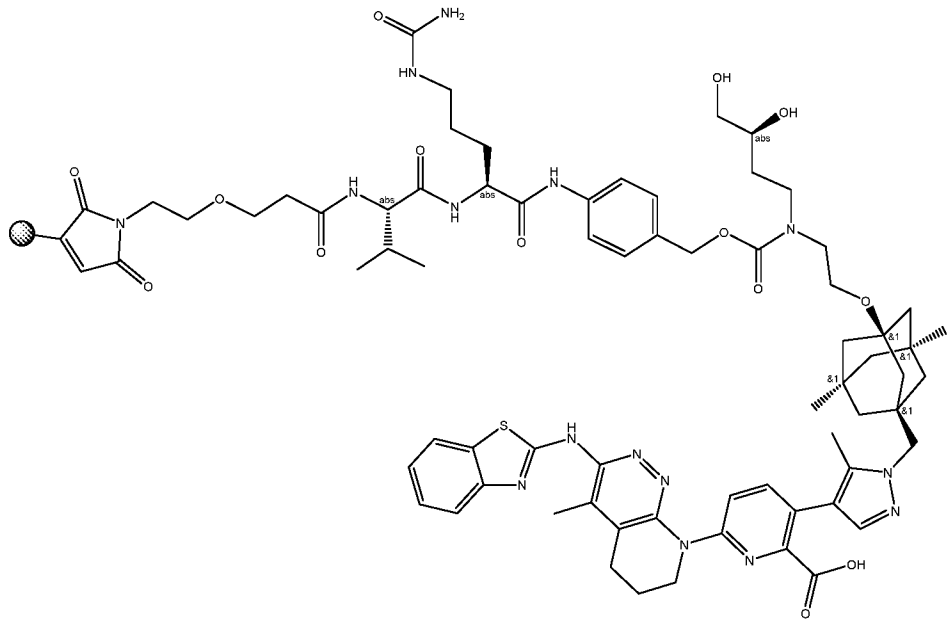
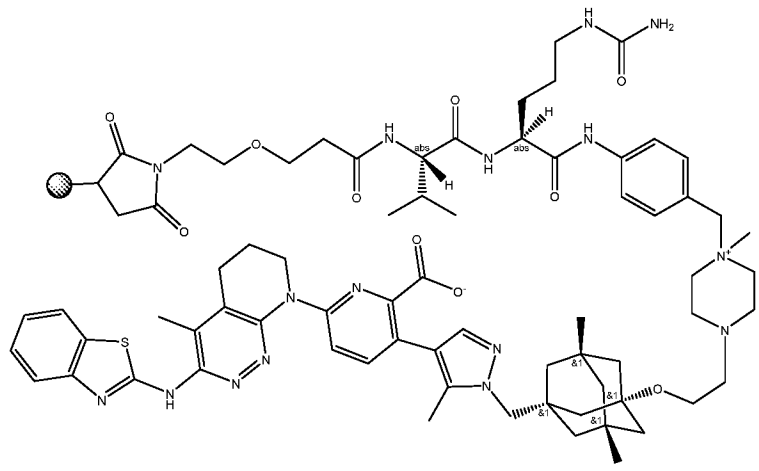
Ab C – L9A-P18	
Ab C – L9C-P19	
Ab C – L9A-P20	

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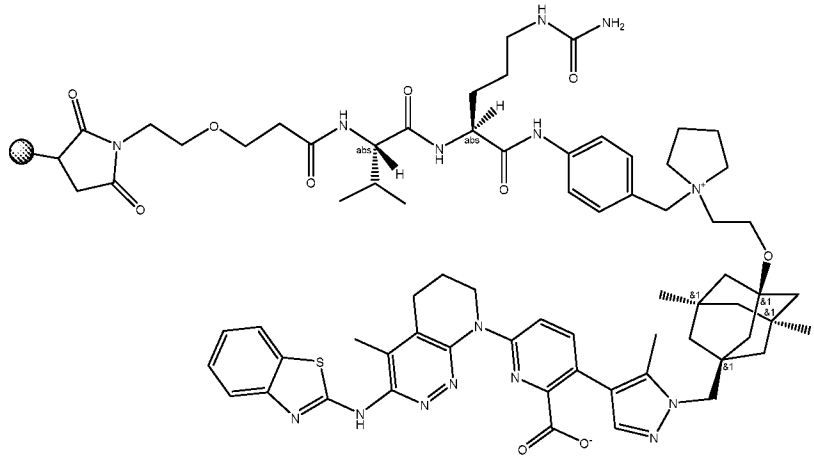
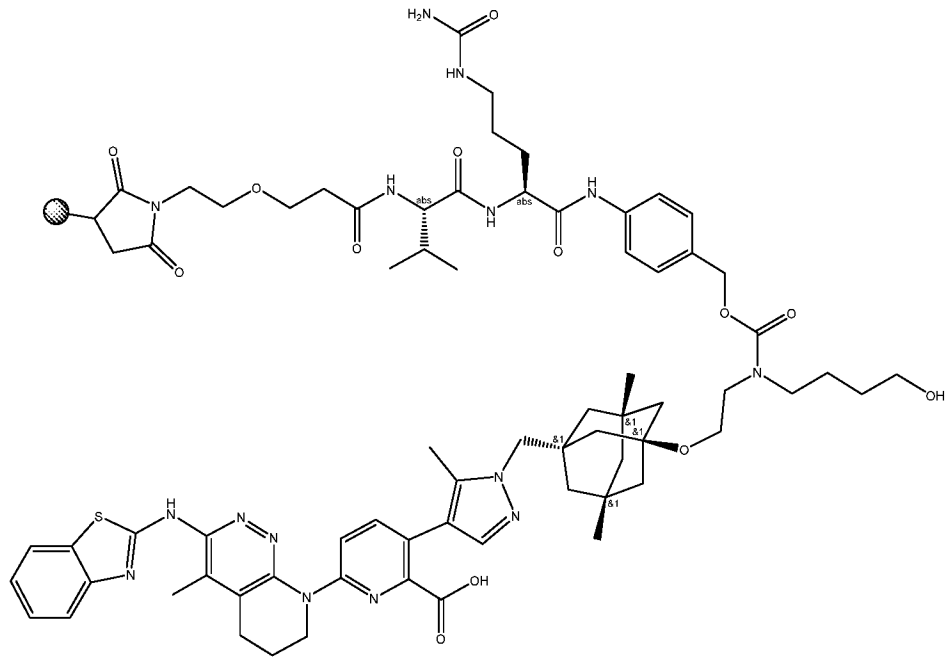
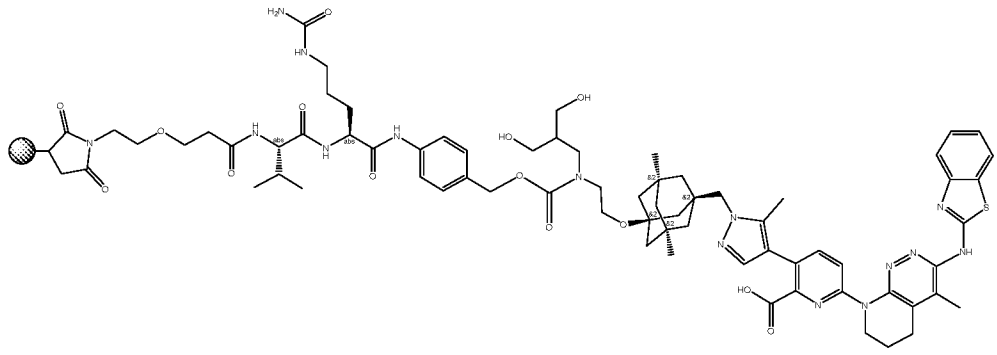
Ab C - L9A-P21	
Ab C – L9C-P22	
Ab C – L9C-P23	

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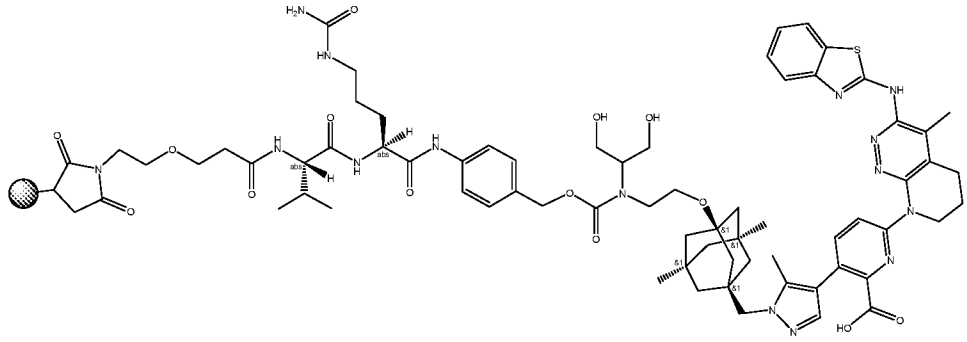
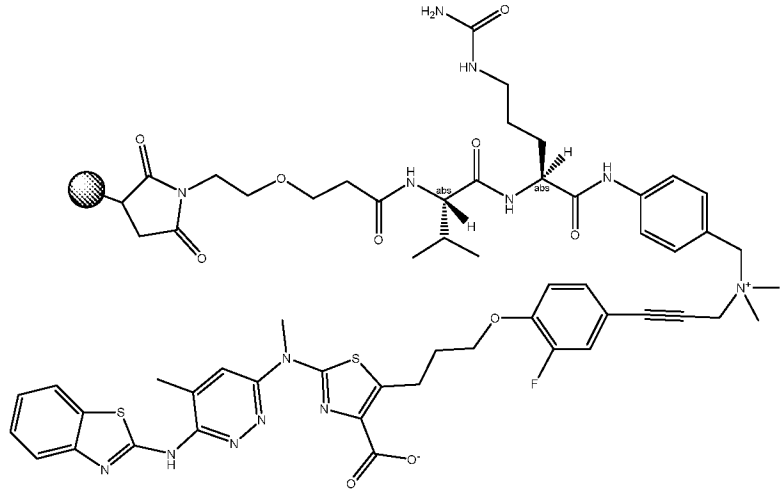
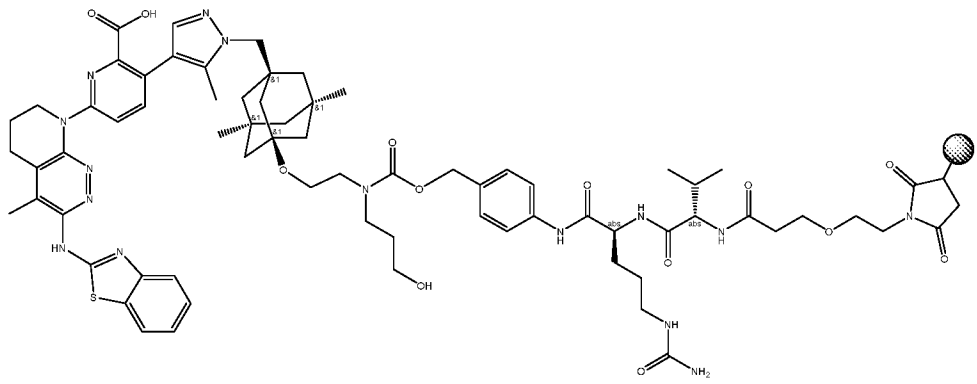
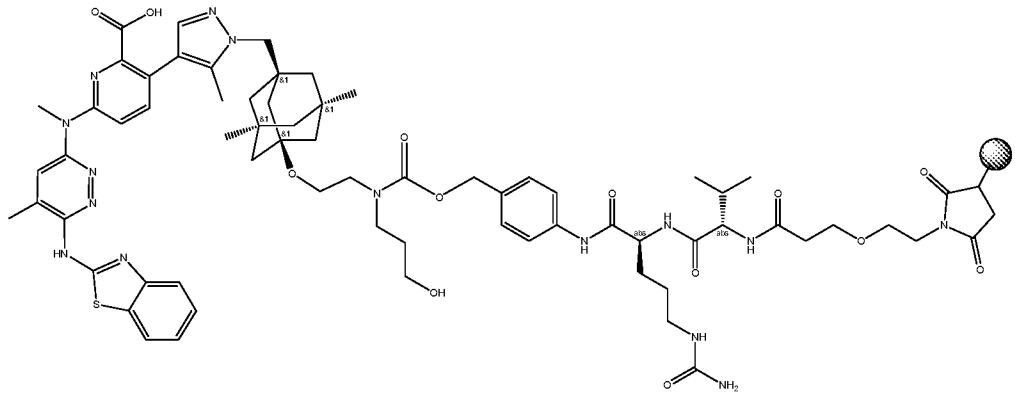
Ab C – L9C-P24	
Ab C – L9A-P2	
Ab C - L9C-P25	
Ab C – L9C-P26	

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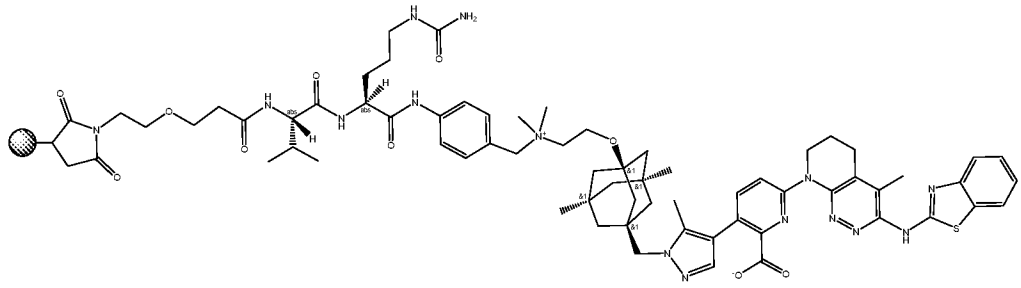
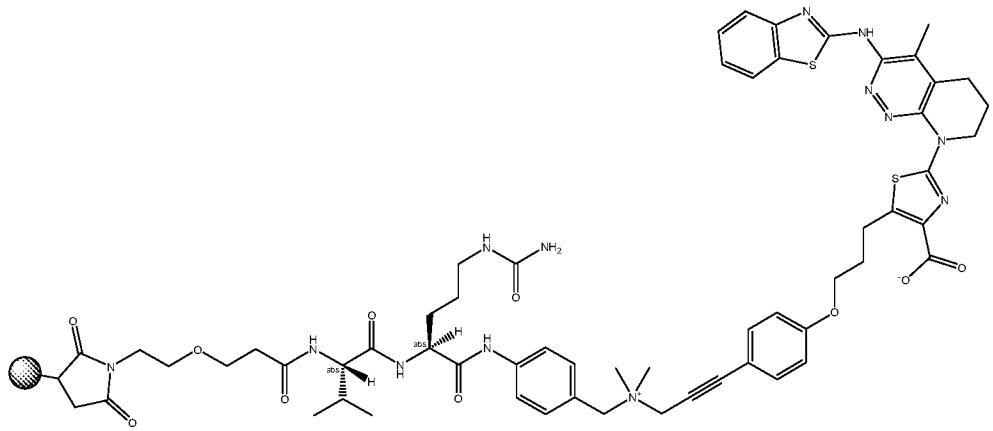
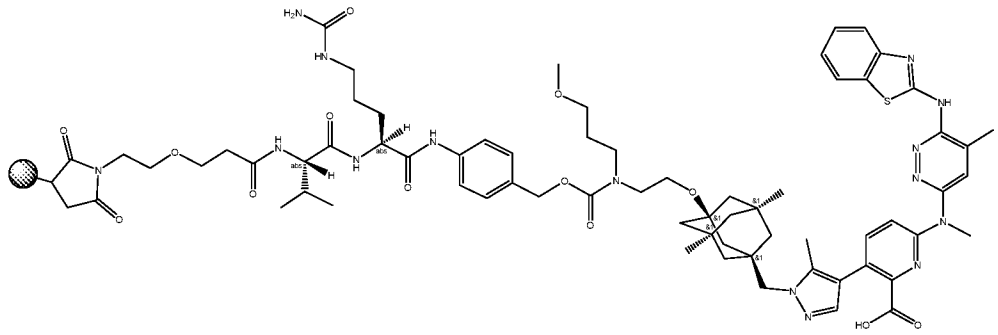
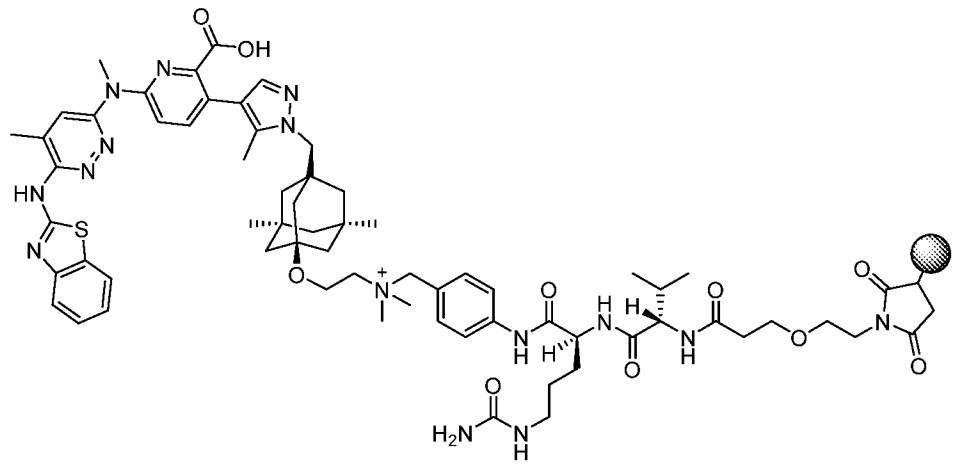
Ab C – L9A-P27	
Ab C – L9A-P28	
Ab C – L9C-P29	
Ab C - L9A-P30	

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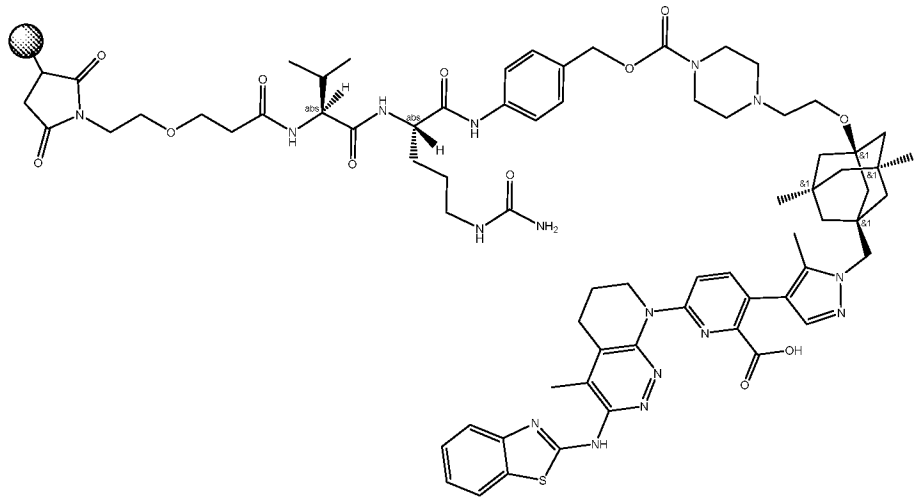
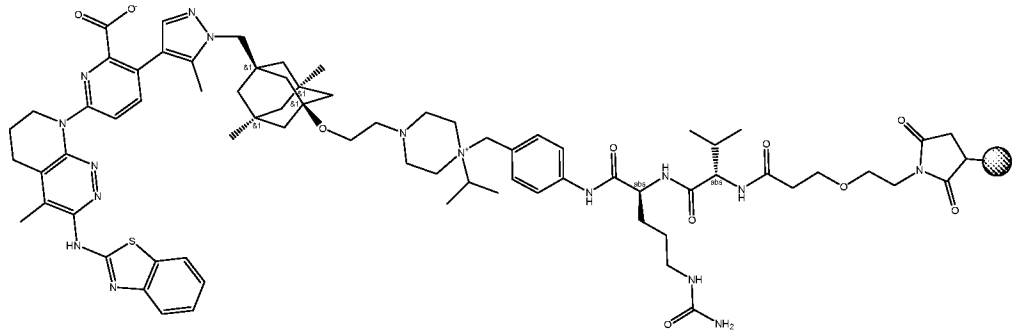
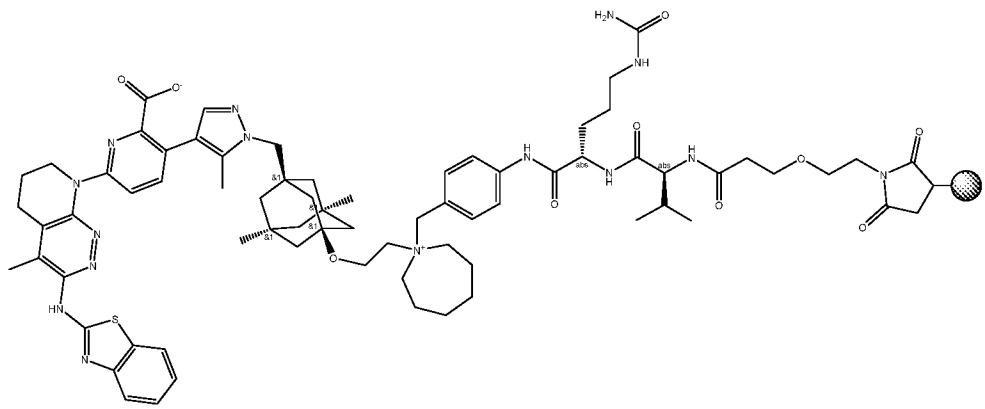
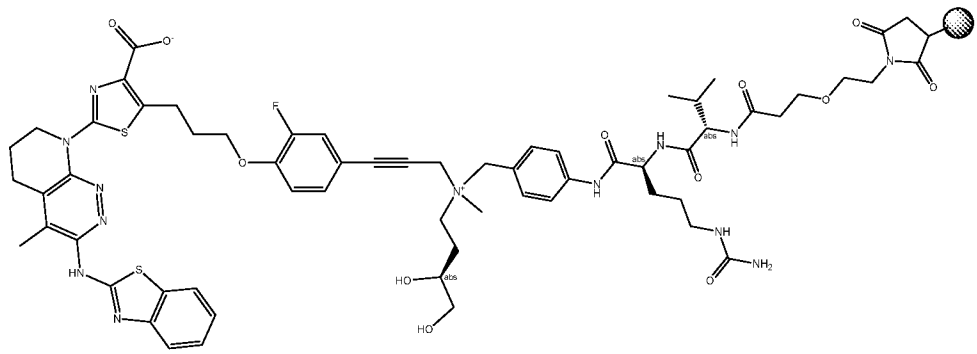
Ab C – L9C-P31	
Ab C – L9A-P32	
Ab C – L9A-P33	
Ab C – L9A-P34	

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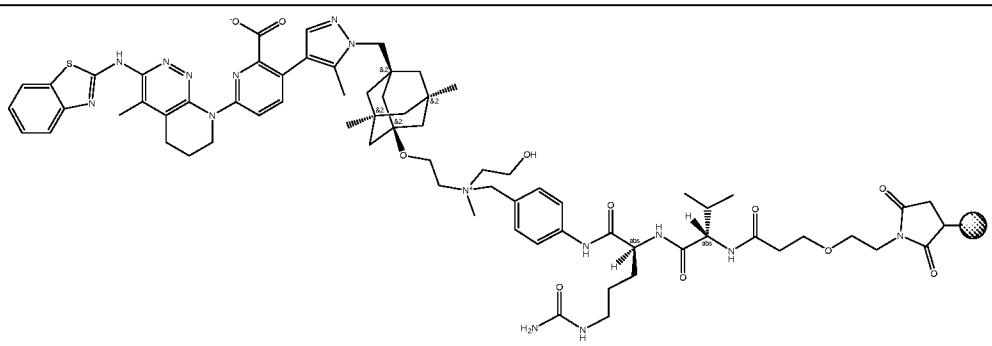
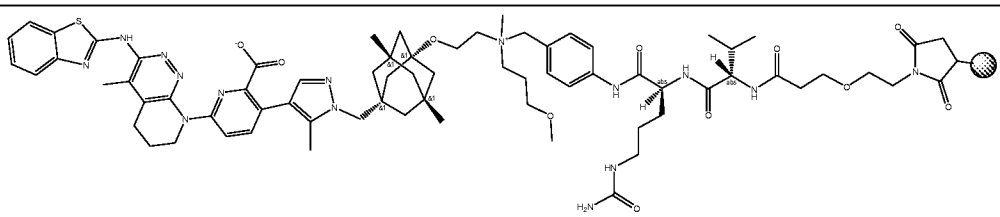
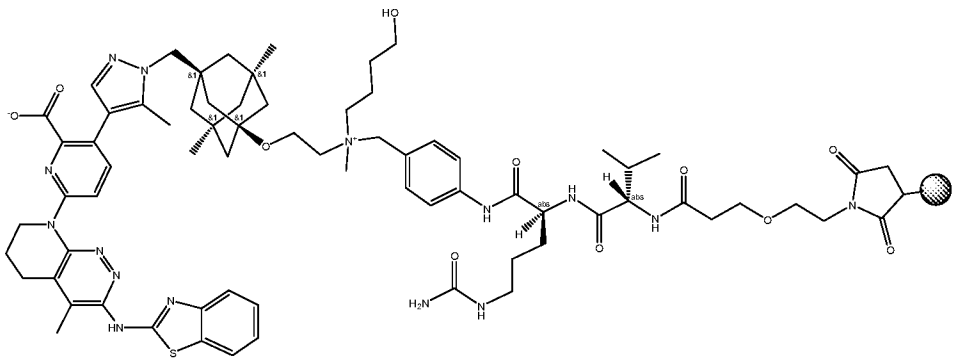
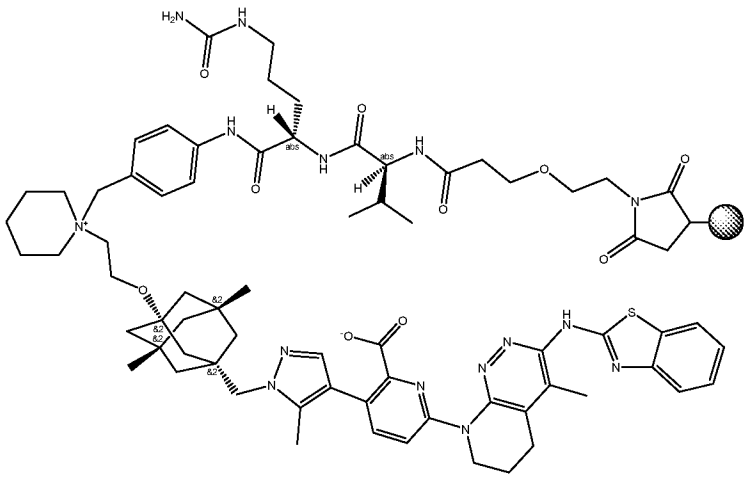
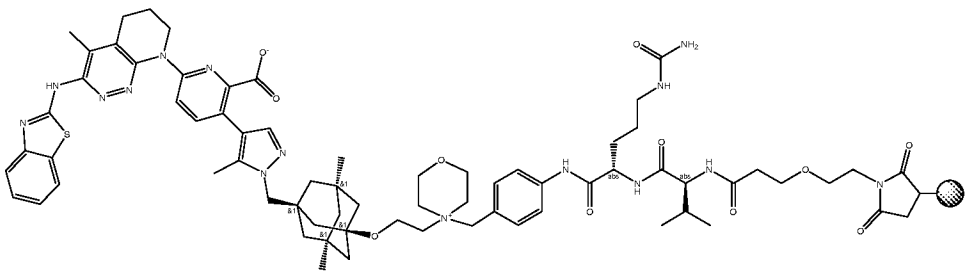
Ab C - L9A-P35	
Ab C - L9A-P36	
Ab C - L9A-P37	
Ab C - L9A-P38	
Ab C - L9A-P39	

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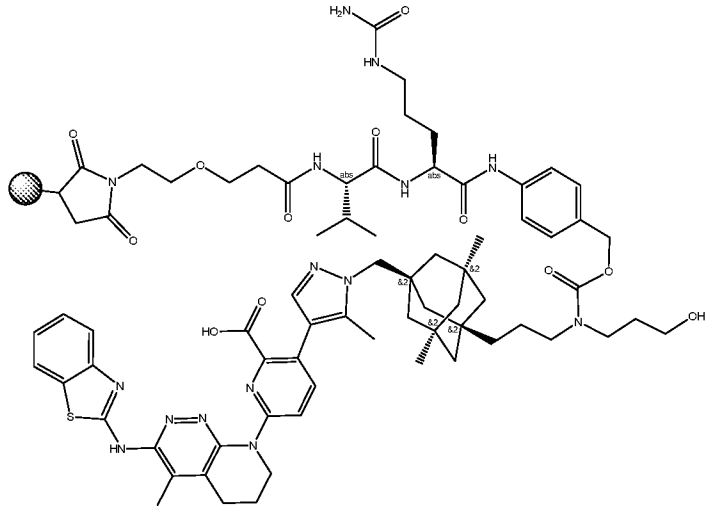
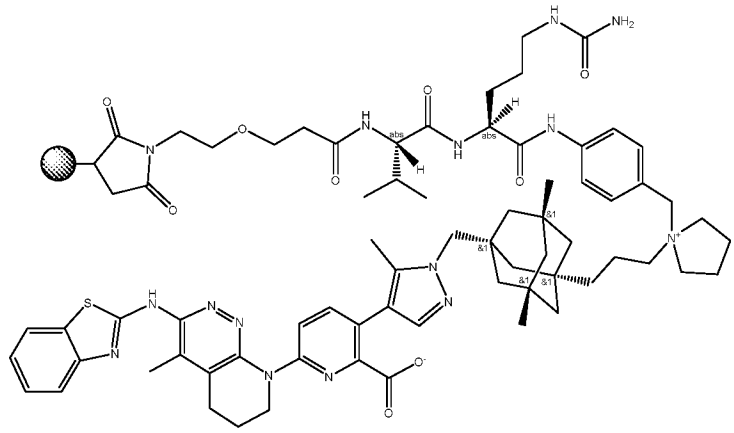
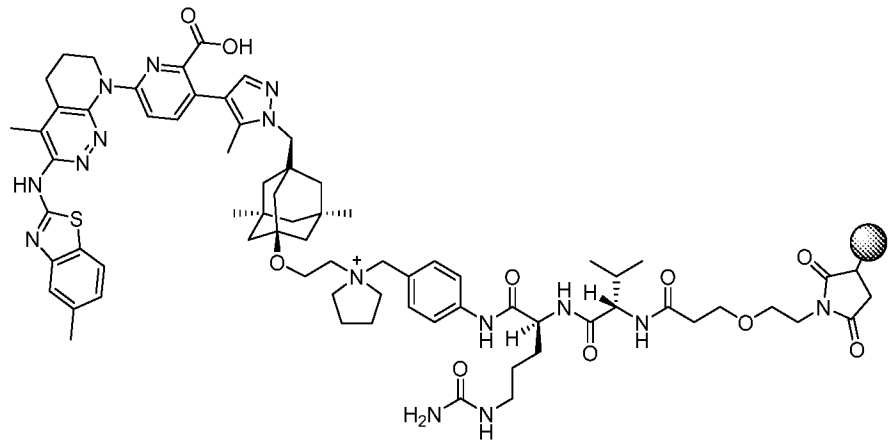
Ab C - L9C-P40	
Ab C - L9A-P41	
Ab C - L9A-P42	

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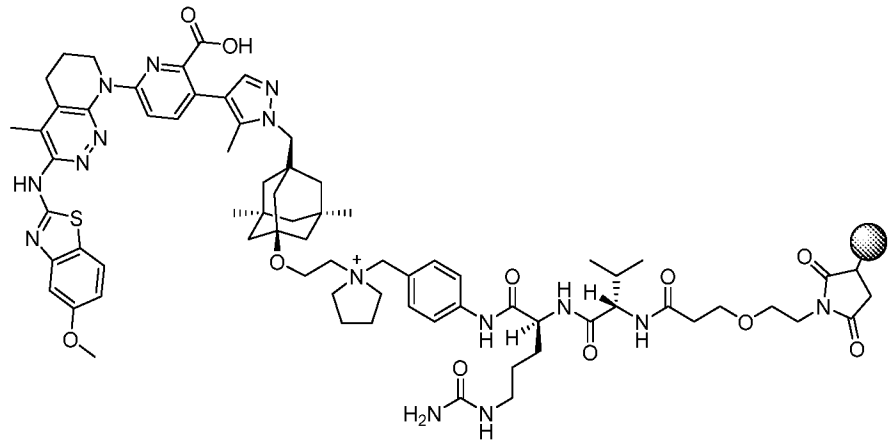
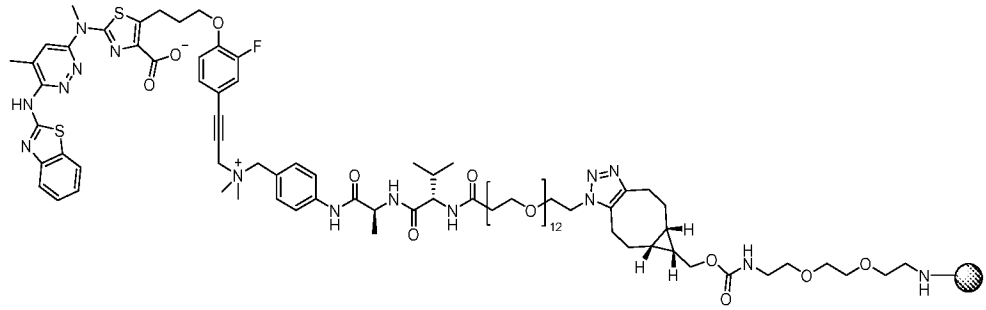
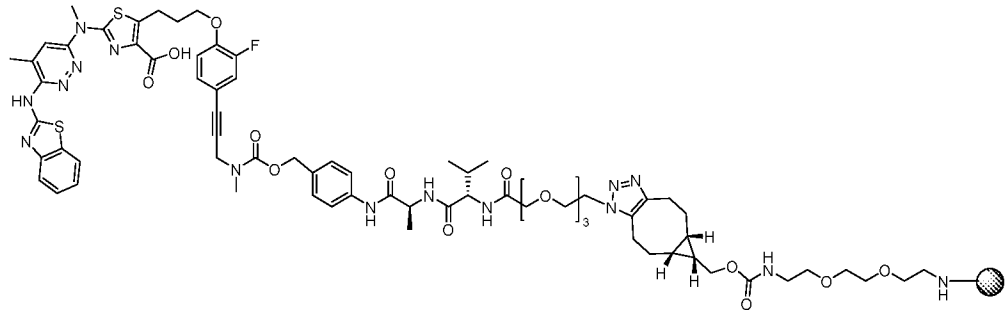
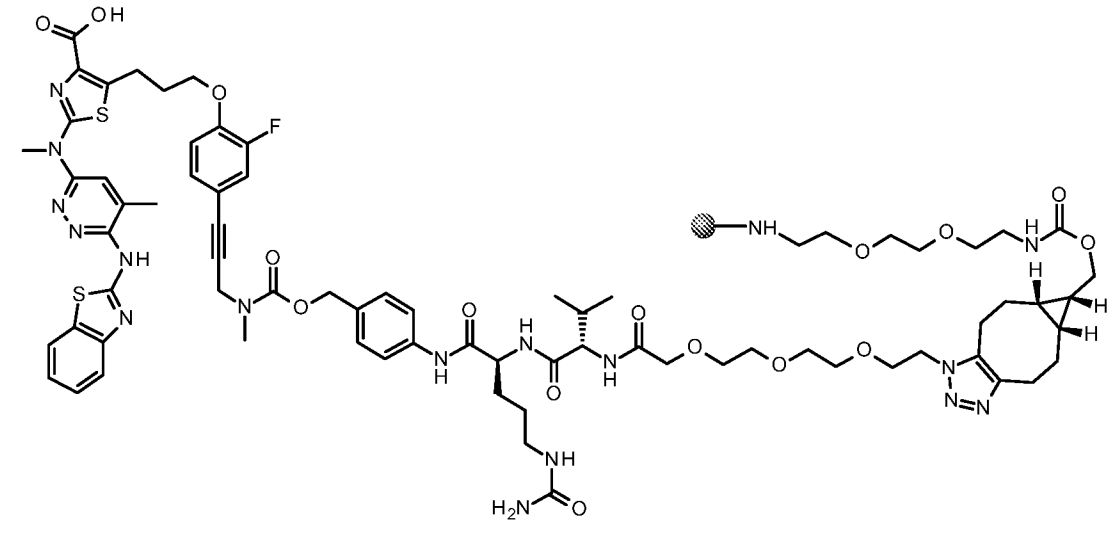
Ab C - L9A-P43	
Ab T – L13A-P2	
Ab T – L19C-P7	
Ab T – L23C-P7	

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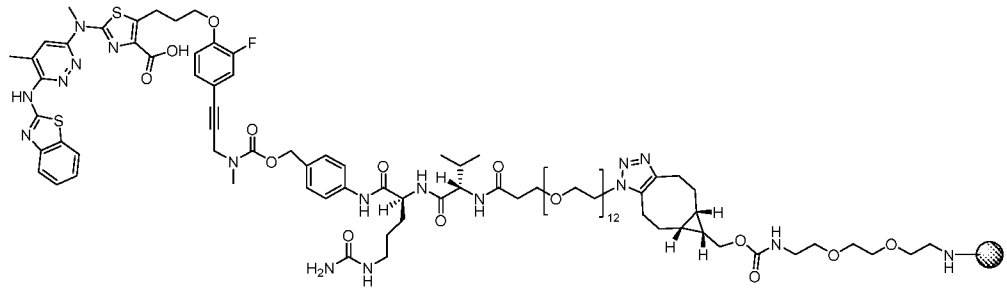
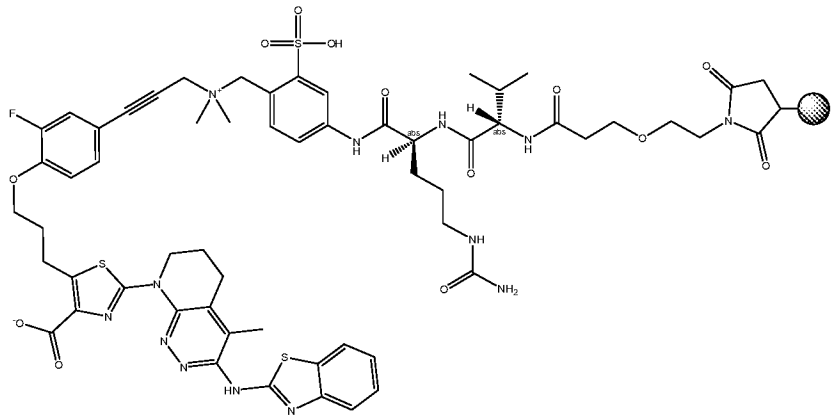
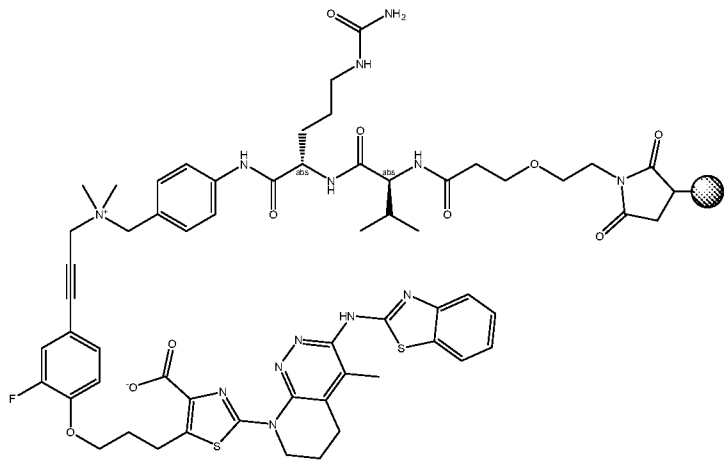
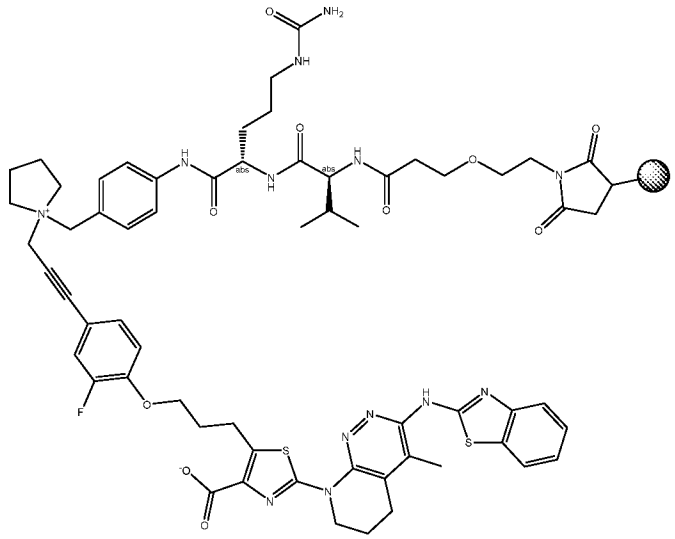
Ab T – L110C-P7	
Ab D – L27A -P1	
Ab D – L9A-P1	
Ab D – L9A-P9	

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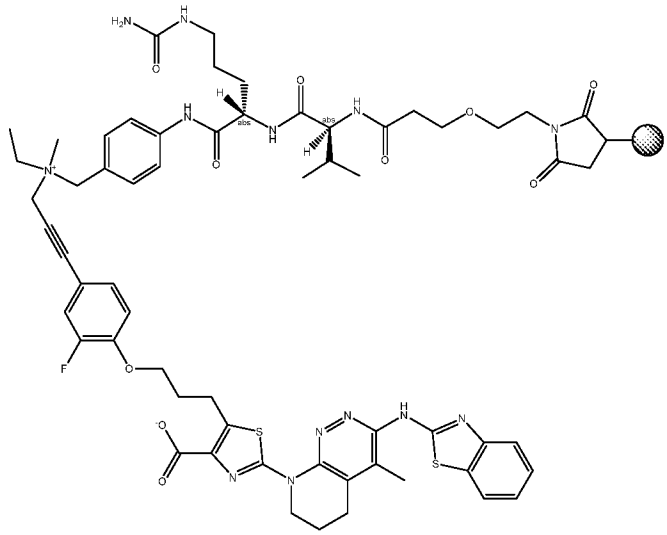
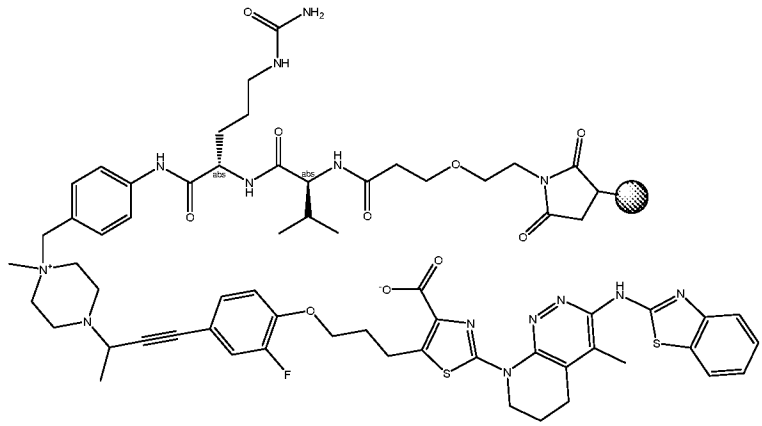
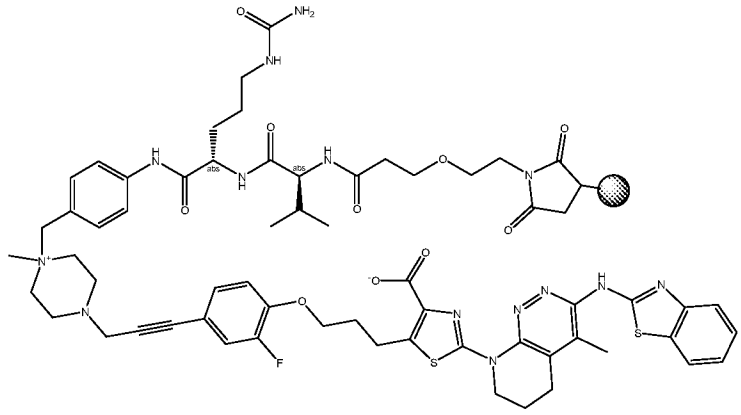
Ab D – L9A-P13	
Ab D – L9A-P8	
Ab D – L9A-P10	

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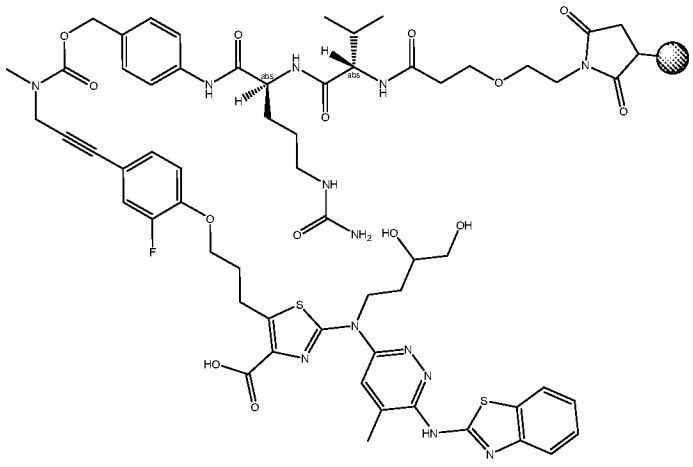
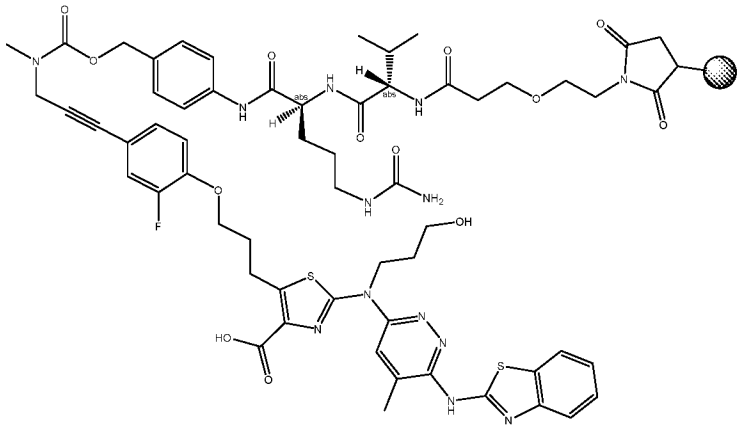
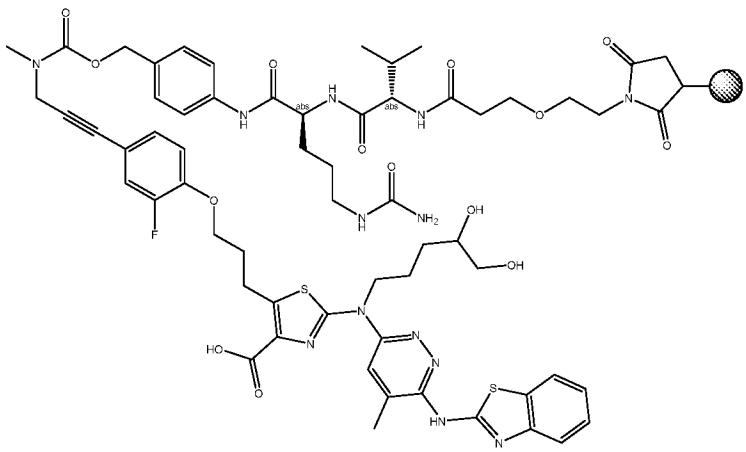
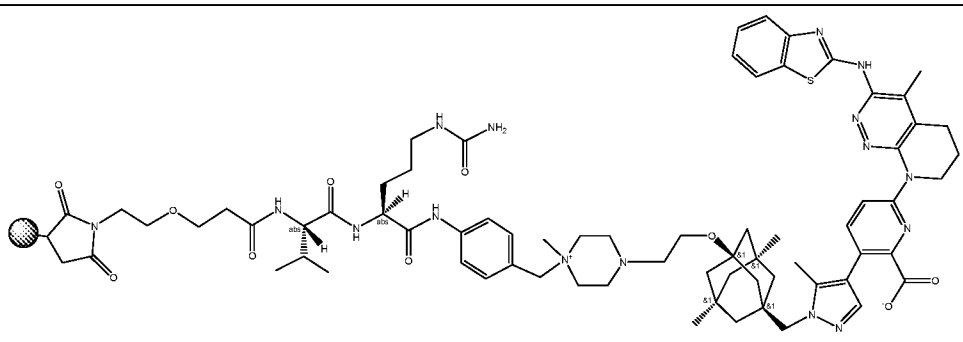
Ab D – L9C-P44	
Ab D - L9C-P45	
Ab D – L9C-P46	
Ab D – L9A-P20	

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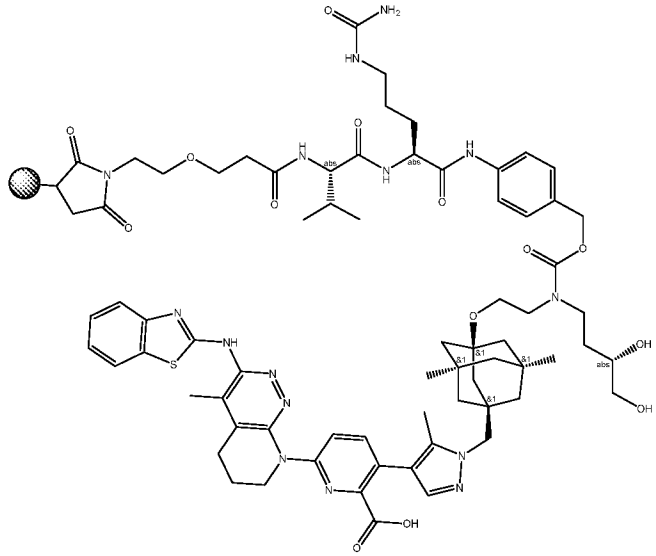
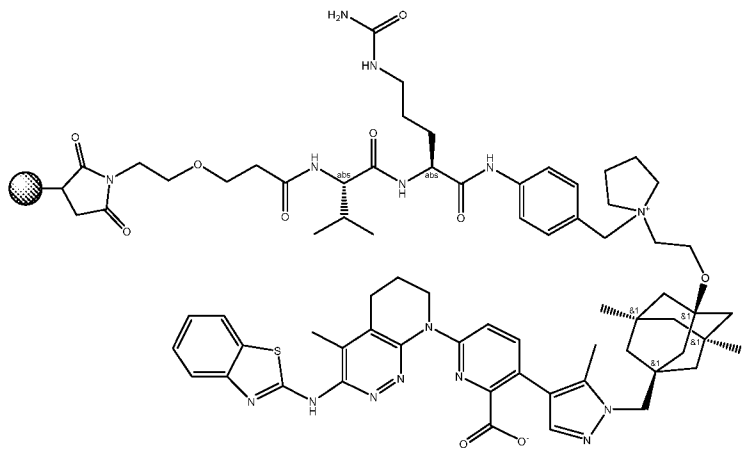
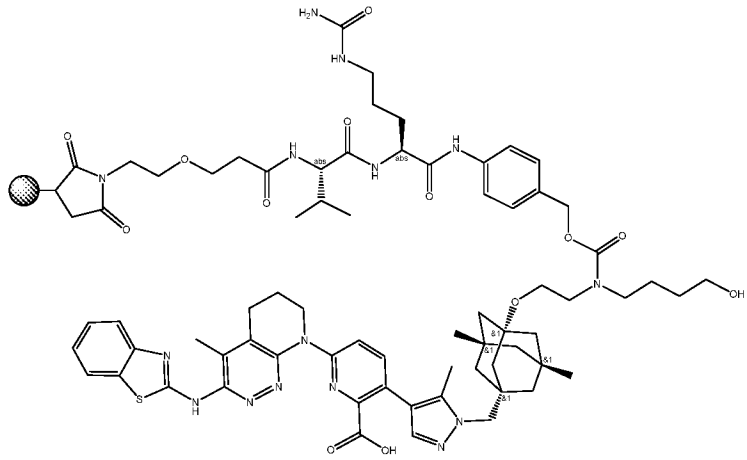
Ab D – L9C-P19	
Ab D - L9A-P21	
Ab D – L9C-P22	

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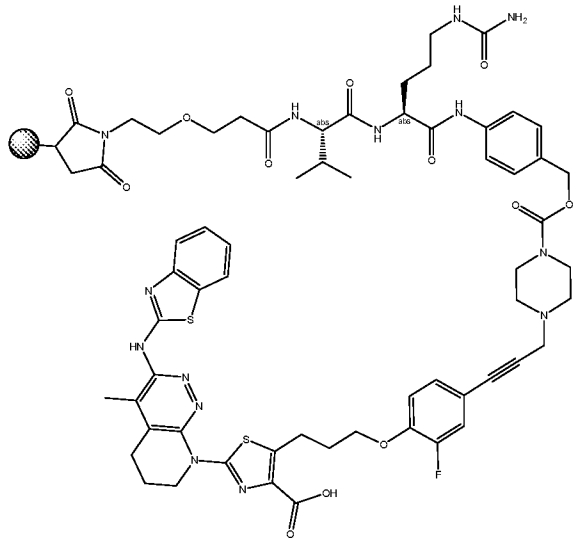
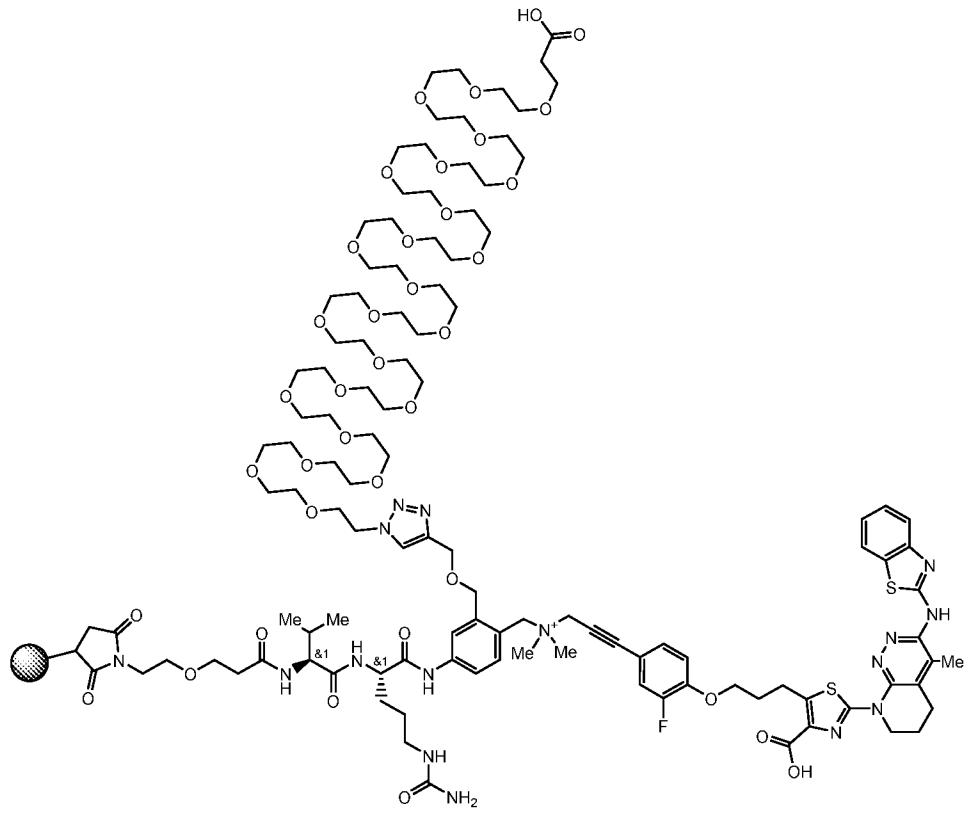
Ab D – L9C-P17	
EGFR1 CysMab-L109A-P1	

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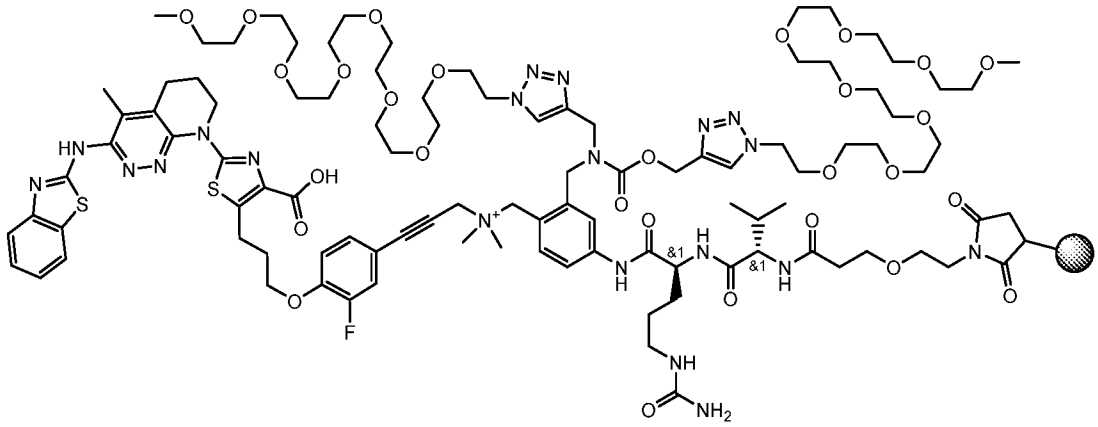
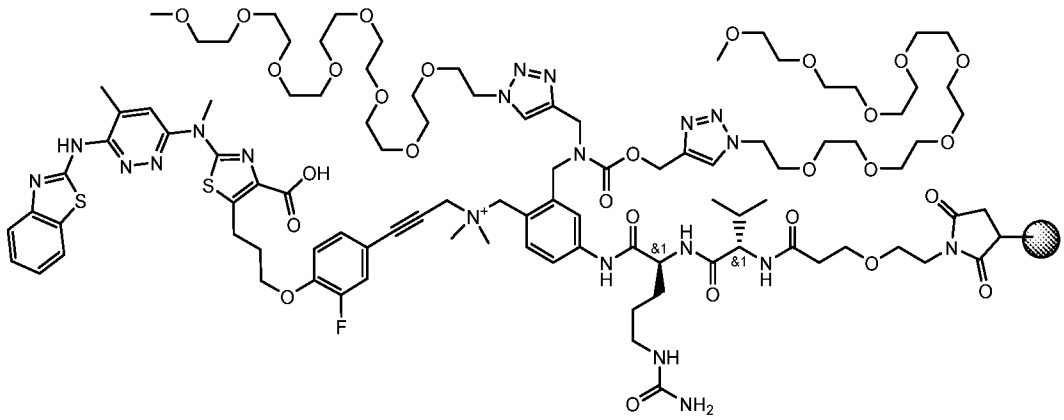
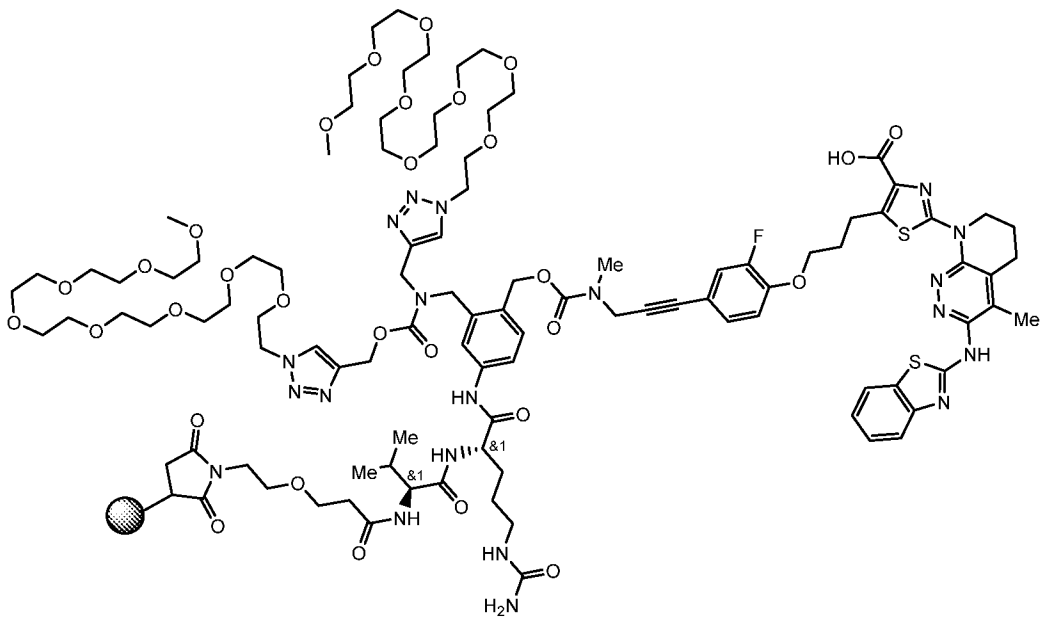
EGFR1 CysMab-L1A-P1	
EGFR1 CysMab-L1A-P2	
EGFR1 CysMab-L1C-P3	

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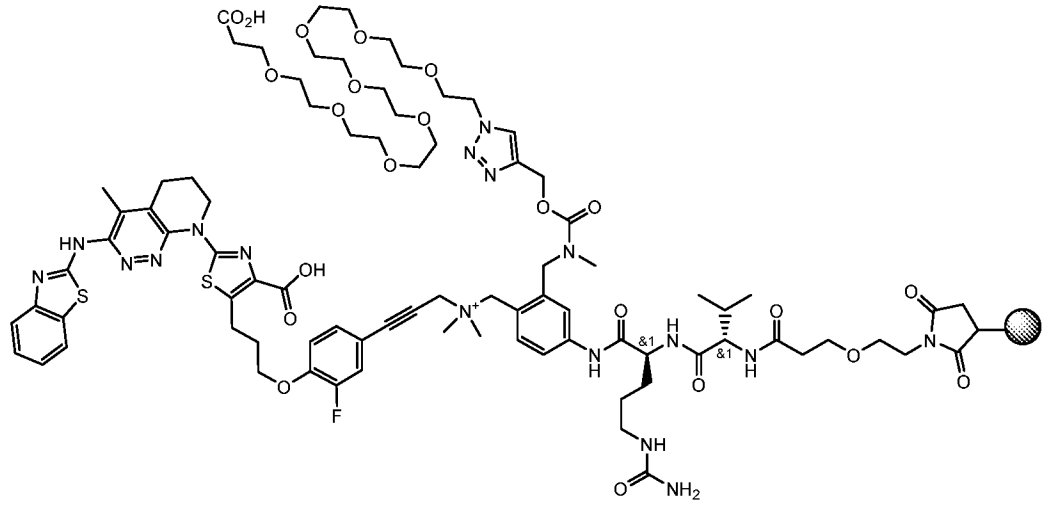
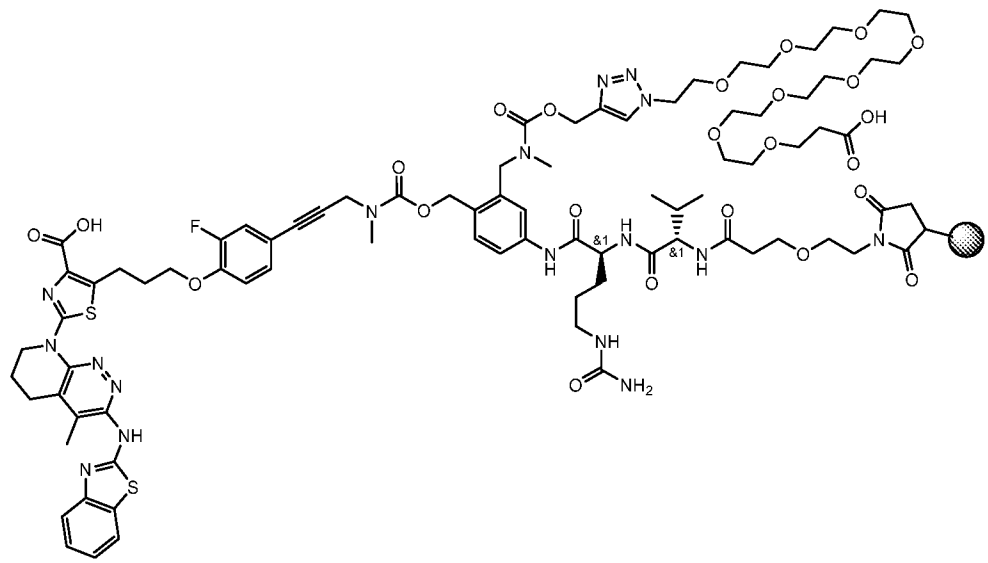
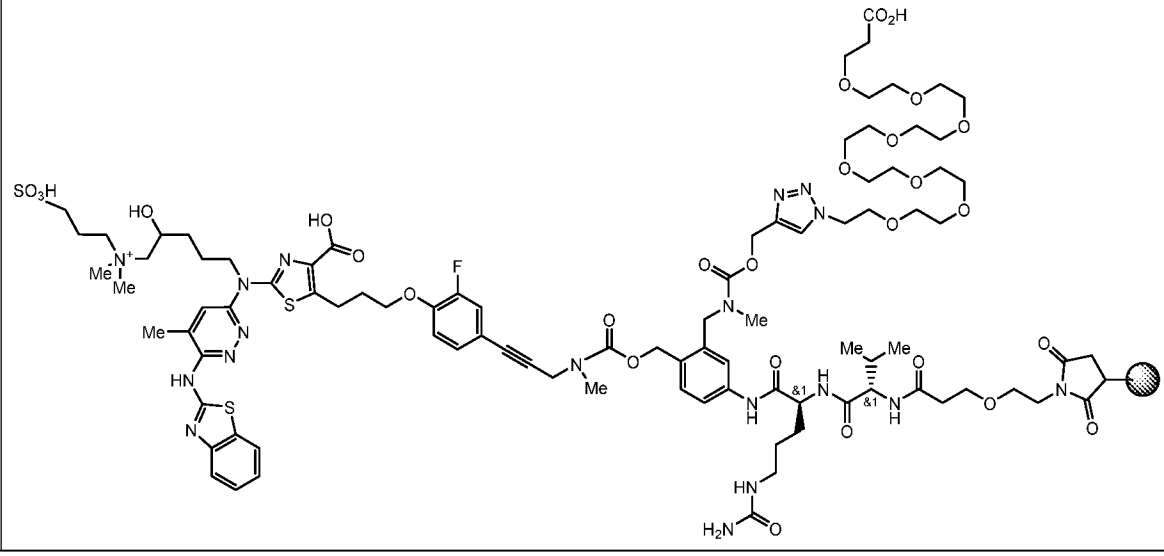
EGFR1 CysMab-L3A-P1	
EGFR1 CysMab-L3C-P3	
EGFR1 CysMab-L3C-P4	

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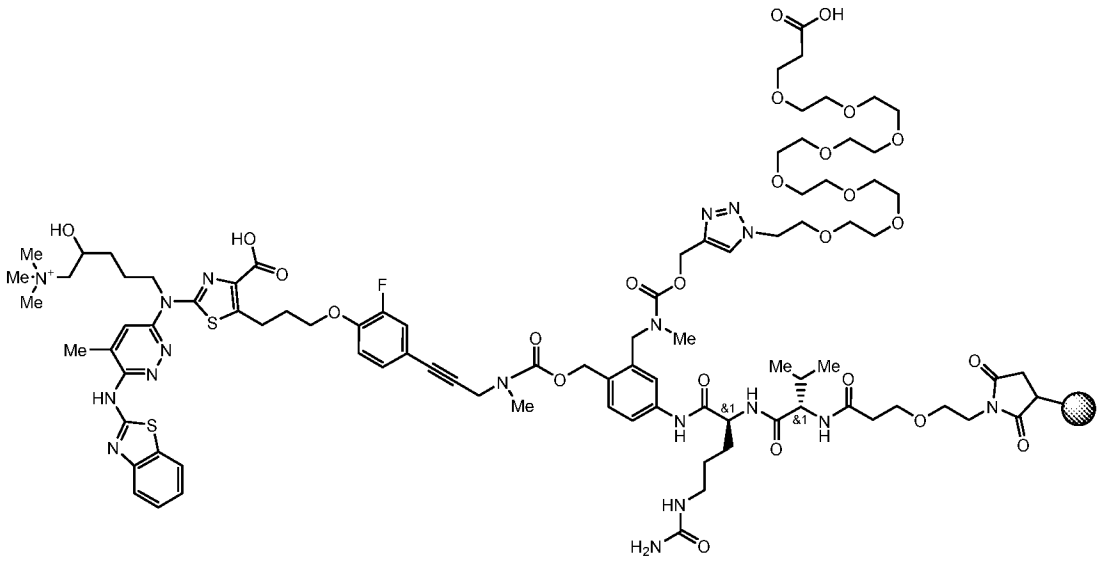
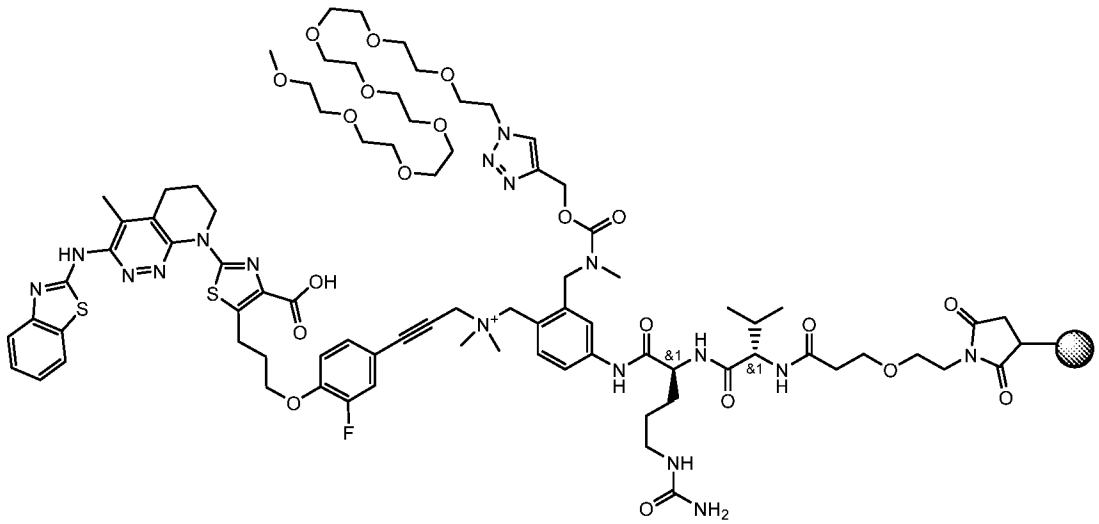
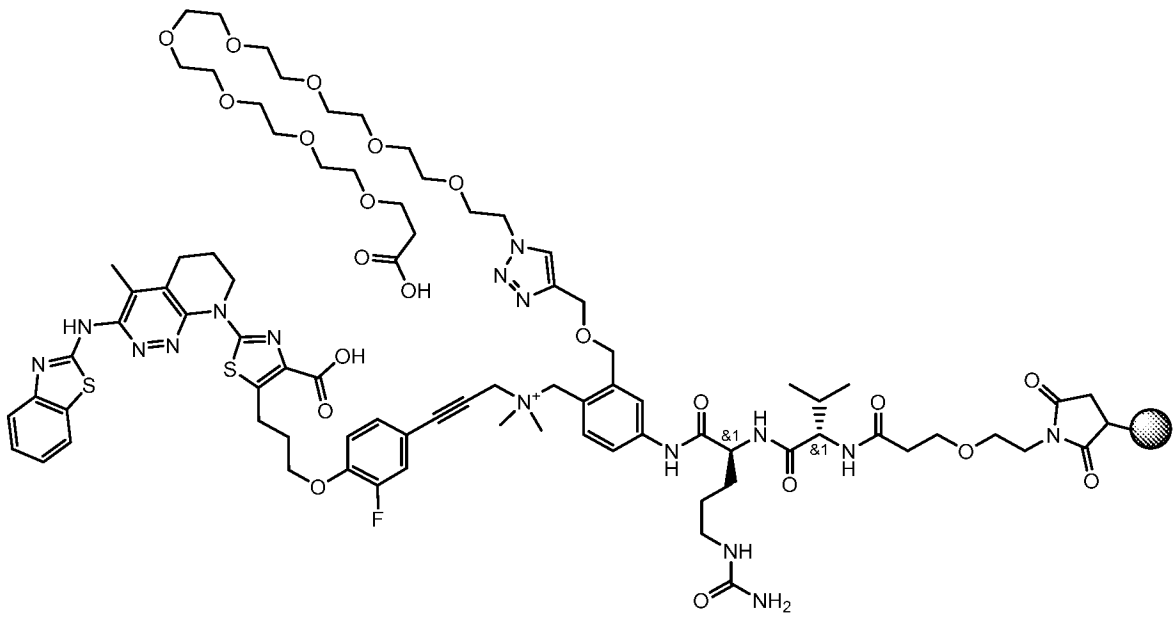
EGFR1 CysMab-L3C-P5	
EGFR1 CysMab-L4A-P1	
EGFR1 CysMab-L7A-P1	

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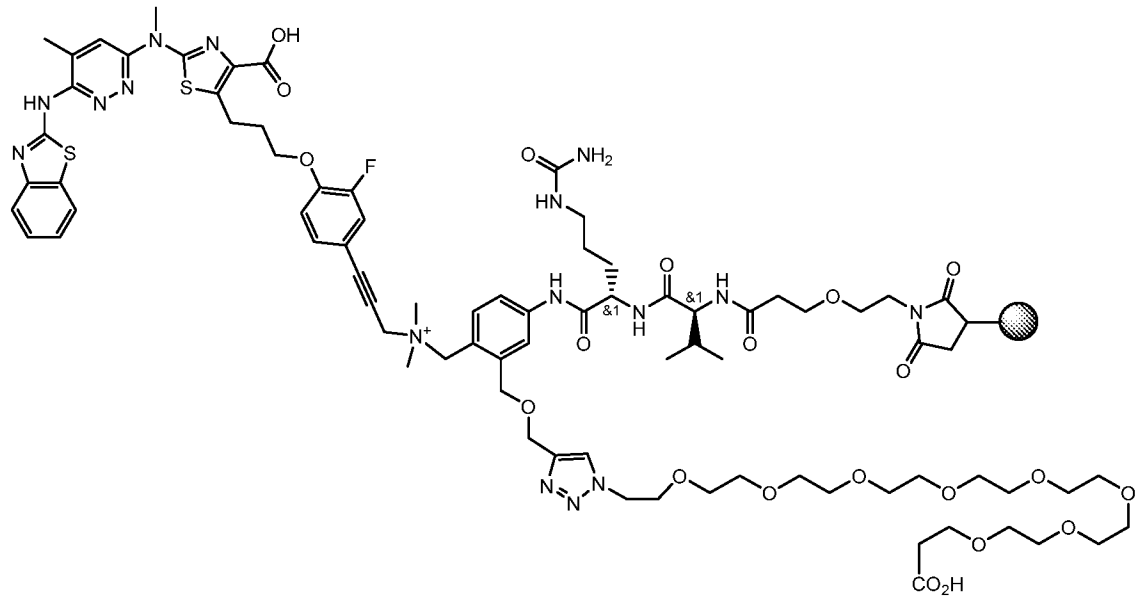
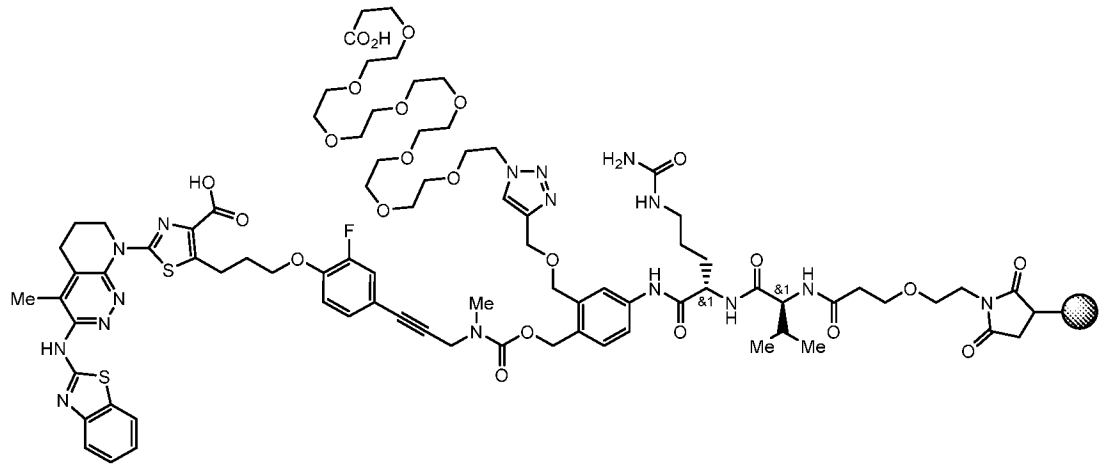
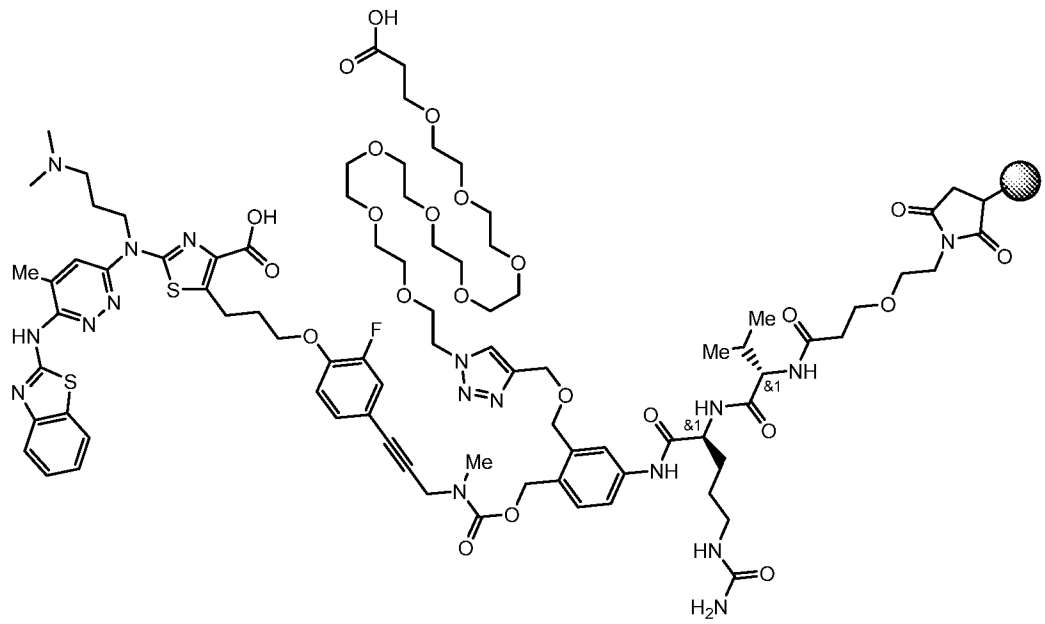
EGFR1 CysMab-L7A-P2	
EGFR1 CysMab-L7C-P3	
EGFR1 CysMab-L7C-P6	

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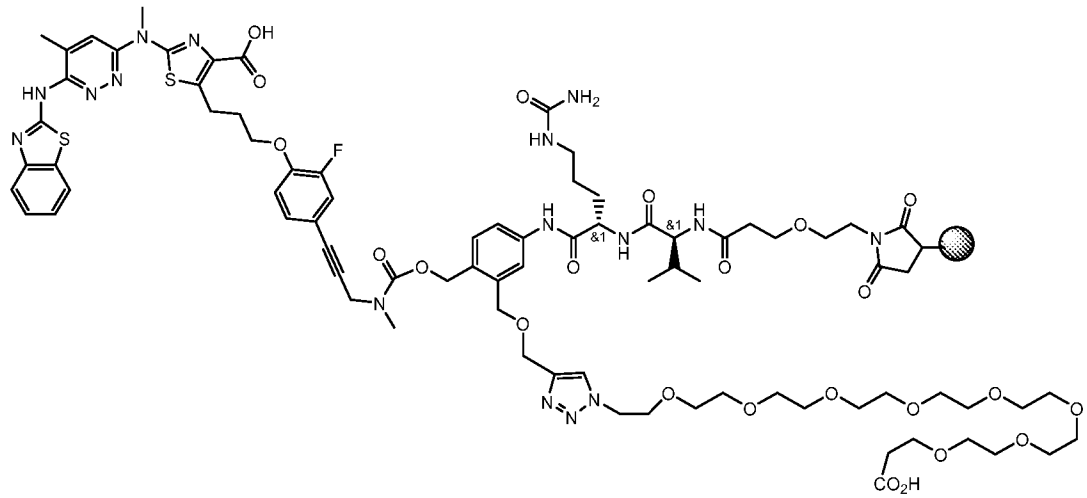
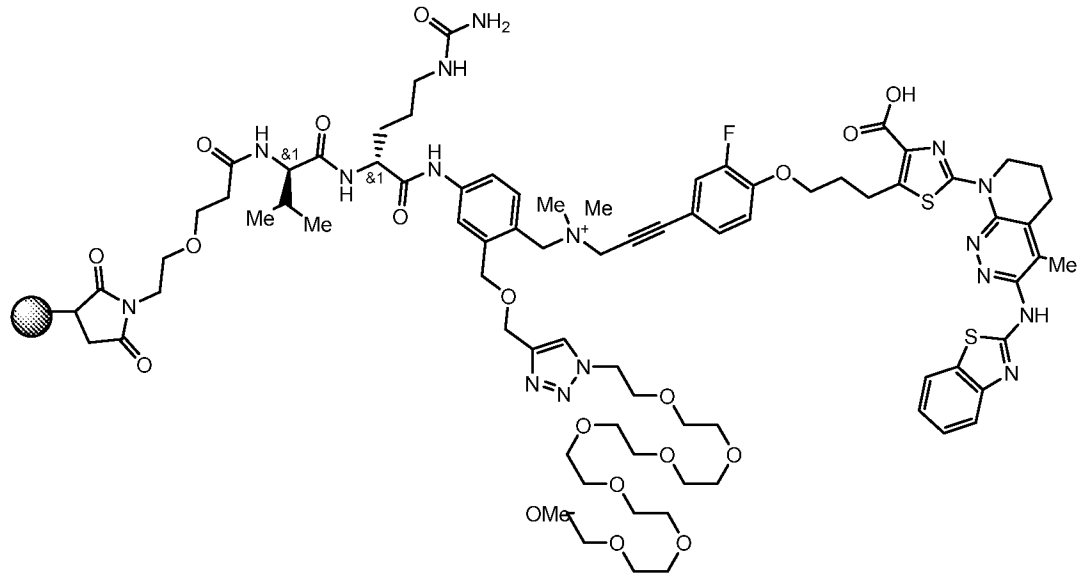
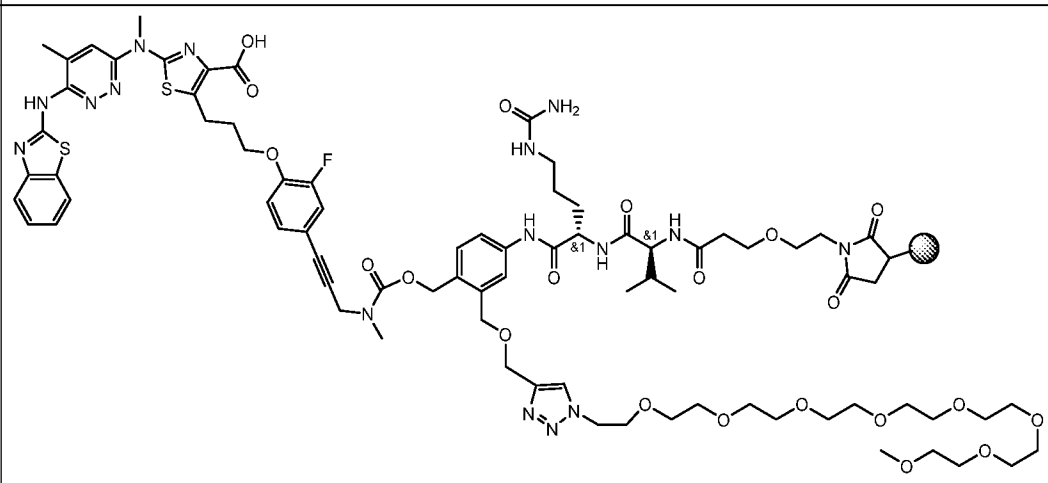
EGFR1 CysMab-L7C-P7	
EGFR1 CysMab-L8A-P1	
EGFR1 CysMab-L8C-P7	

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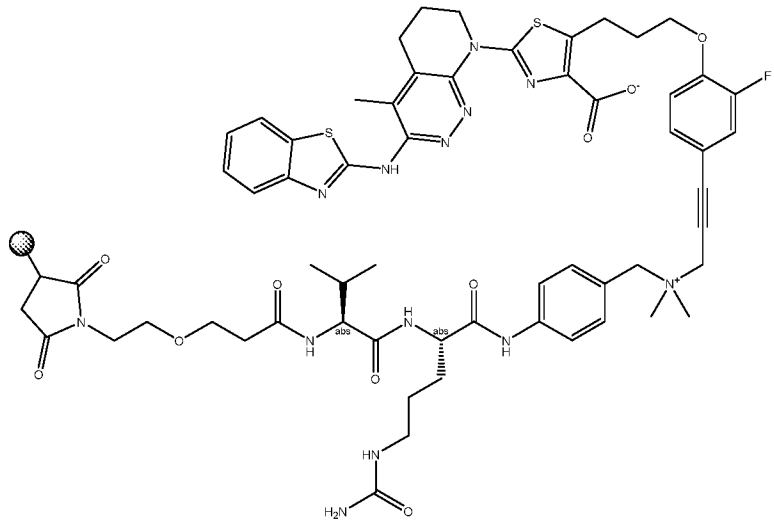
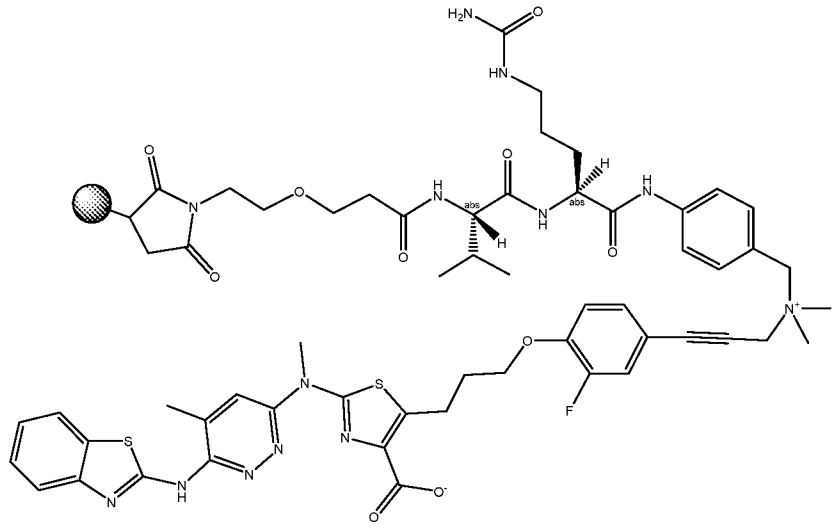
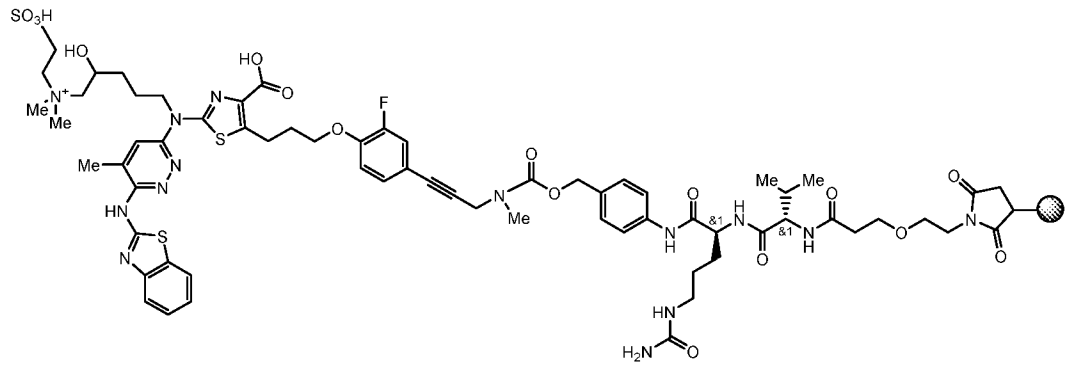
EGFR1 CysMab-L9A-P1	
EGFR1 CysMab-L9A-P2	
EGFR1 CysMab-L9C-P4	

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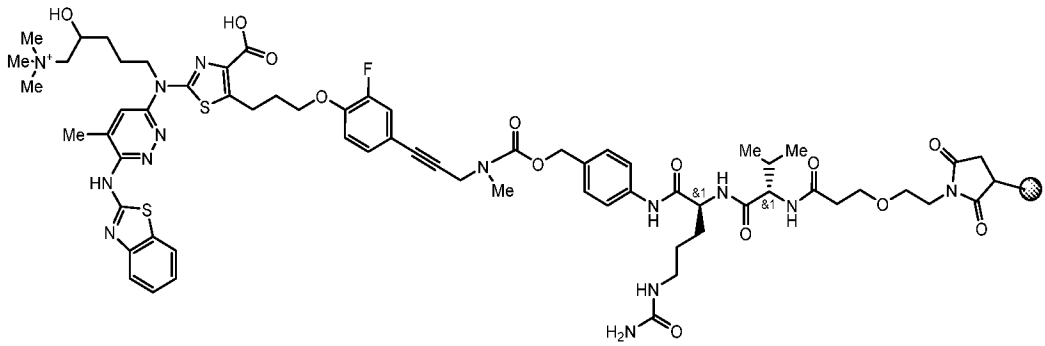
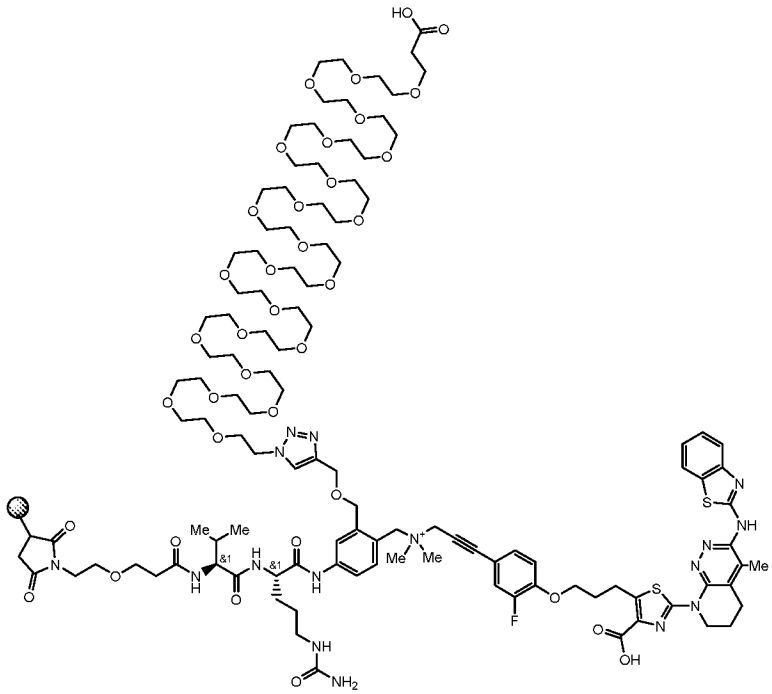
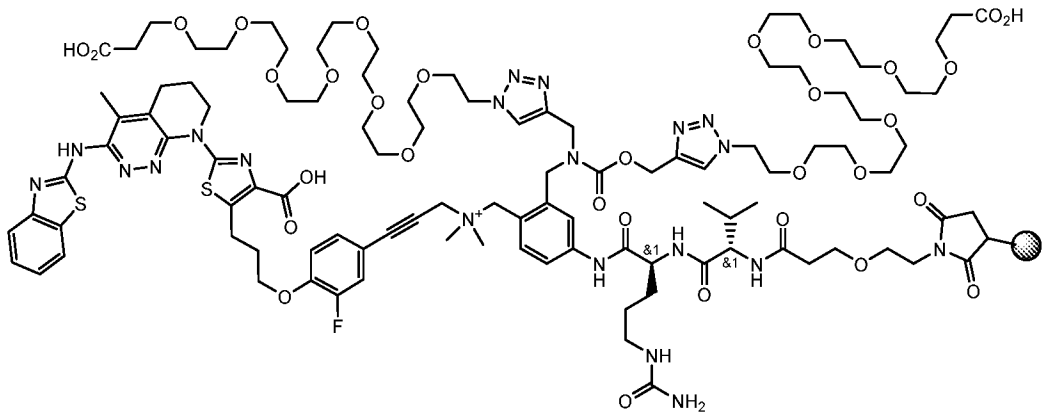
EGFR1 CysMab-L9C-P5	
EGFR1 CysMab-L109A-P1	
EGFR1 CysMab-L10A-P1	

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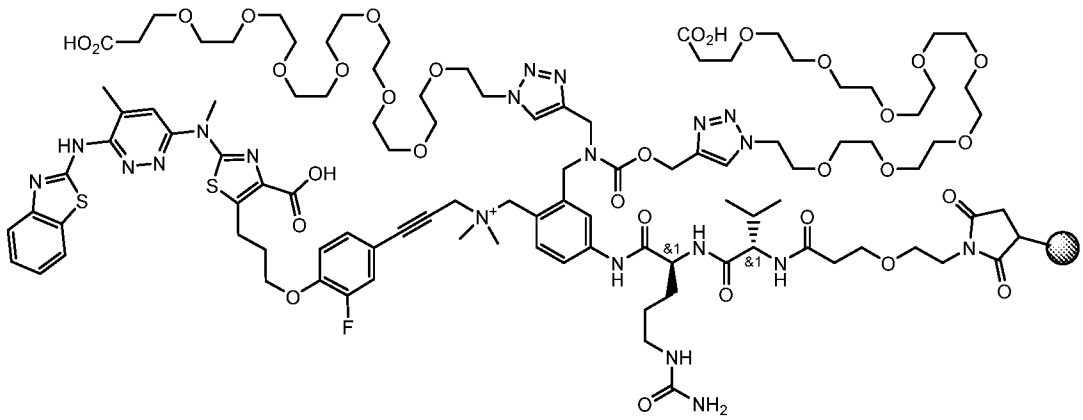
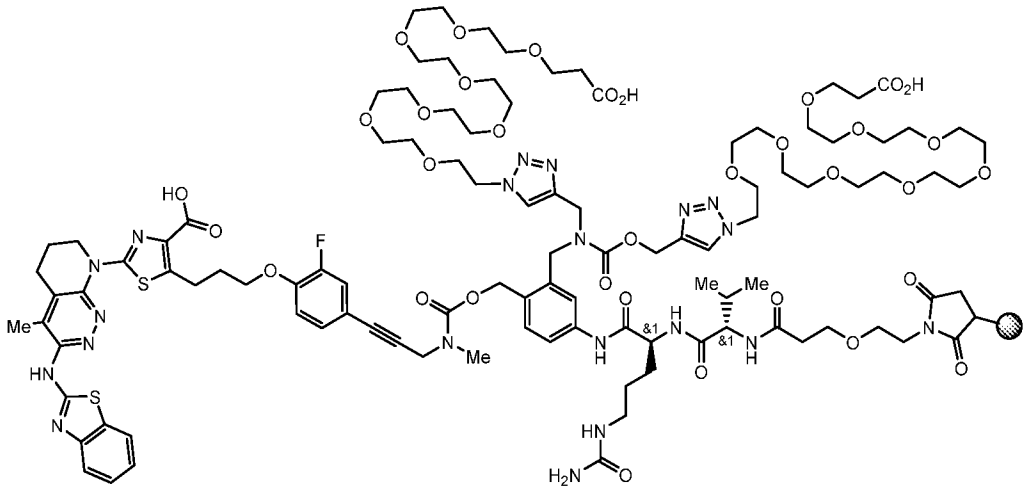
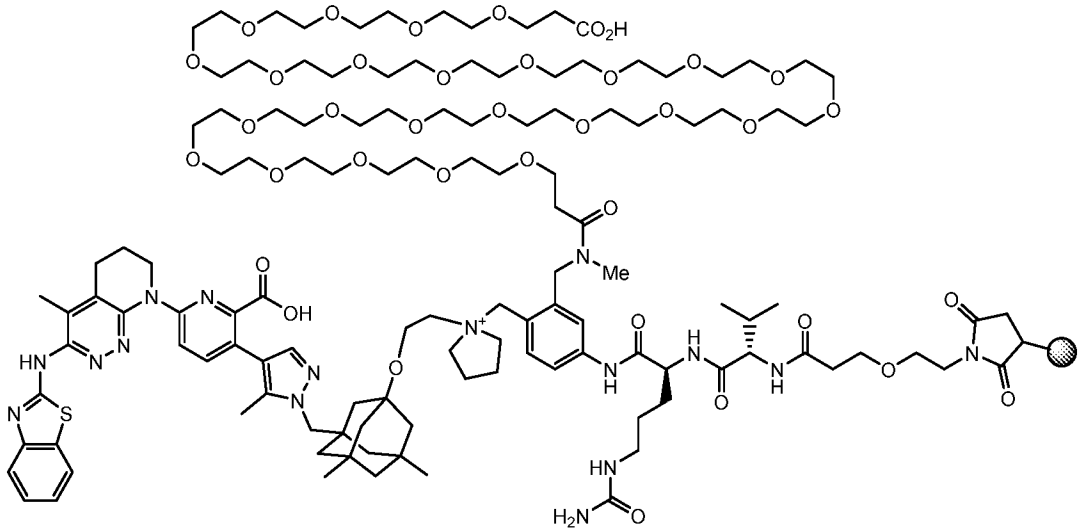
EGFR1 CysMab-L10A-P2	
EGFR1 CysMab-L10C-P3	
EGFR1 CysMab-L11A-P21	

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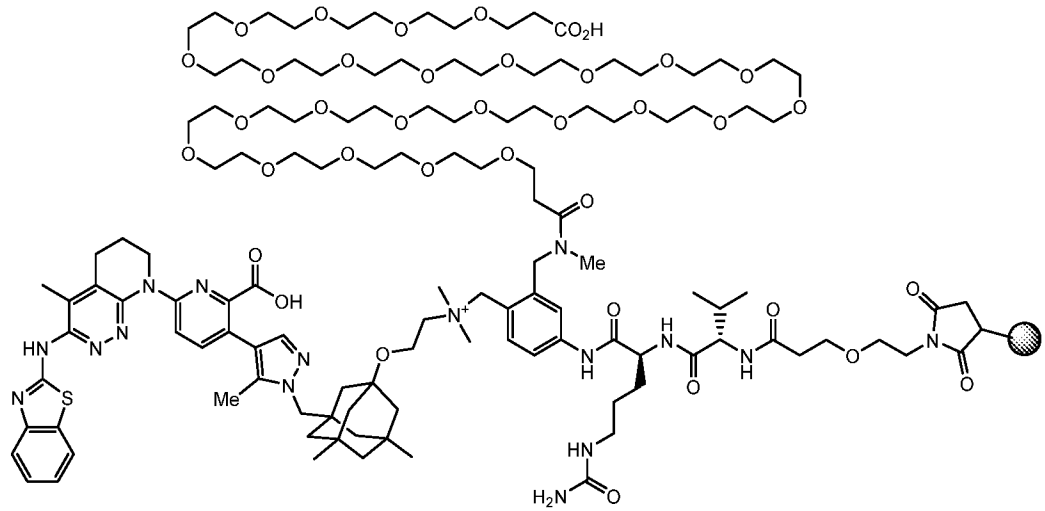
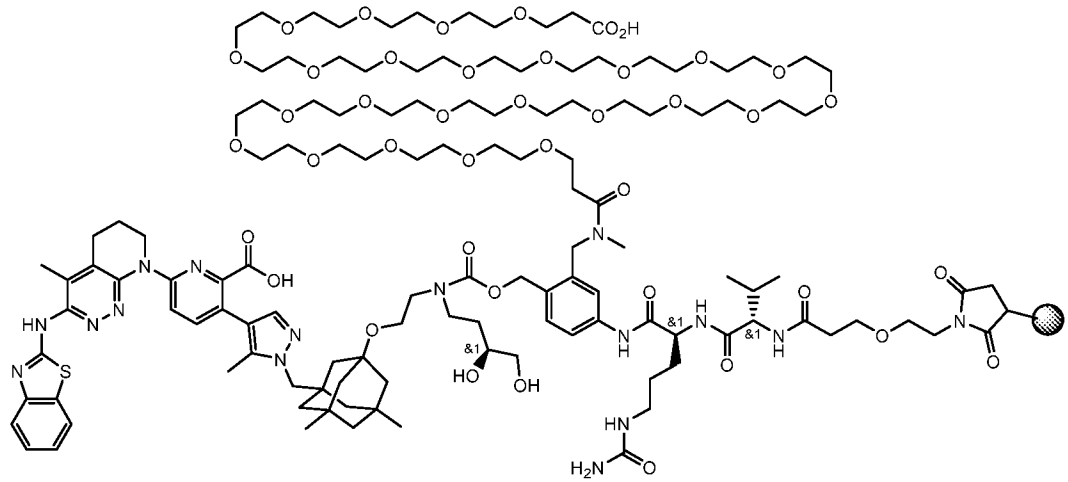
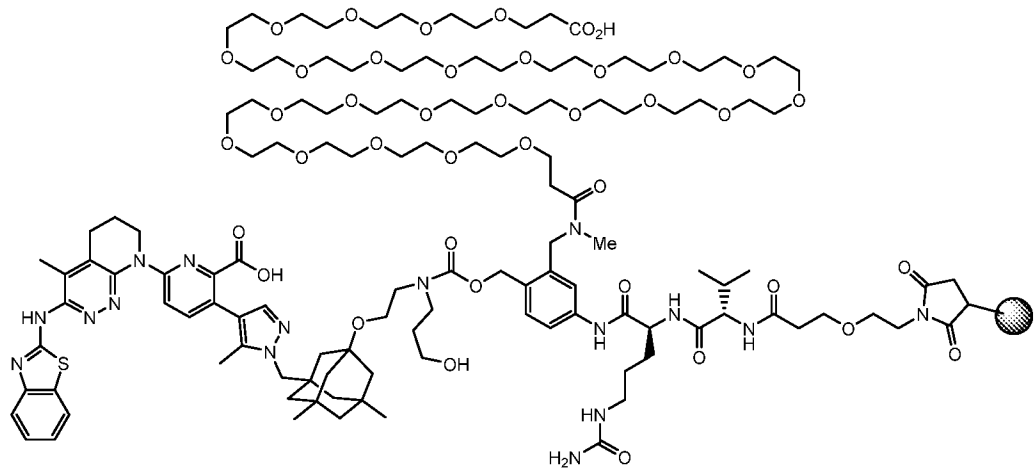
EGFR1 CysMab-L11A-P27	
EGFR1 CysMab-L11C-P19	
EGFR1 CysMab-L11C-P25	

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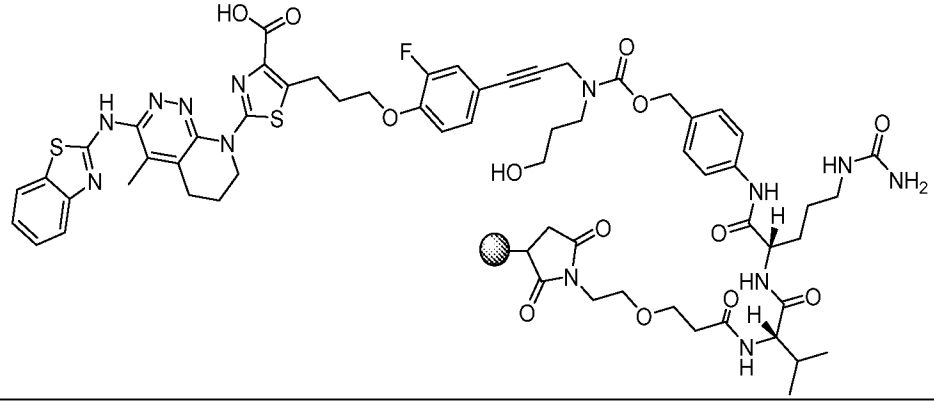
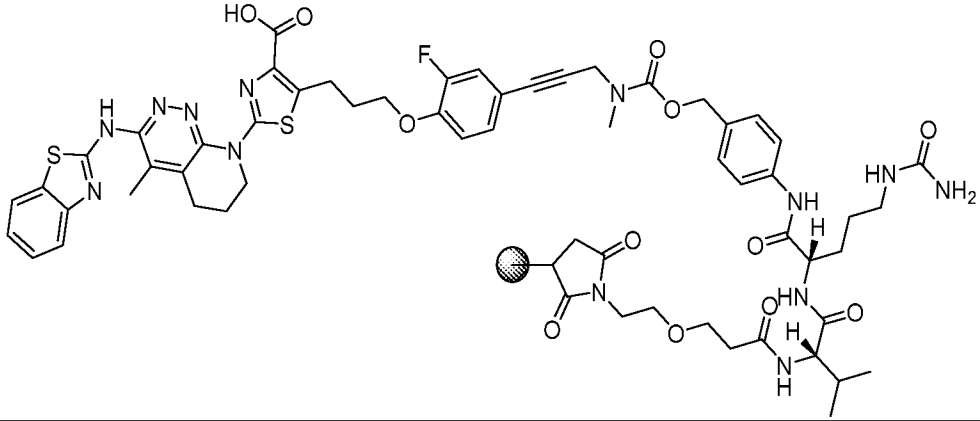
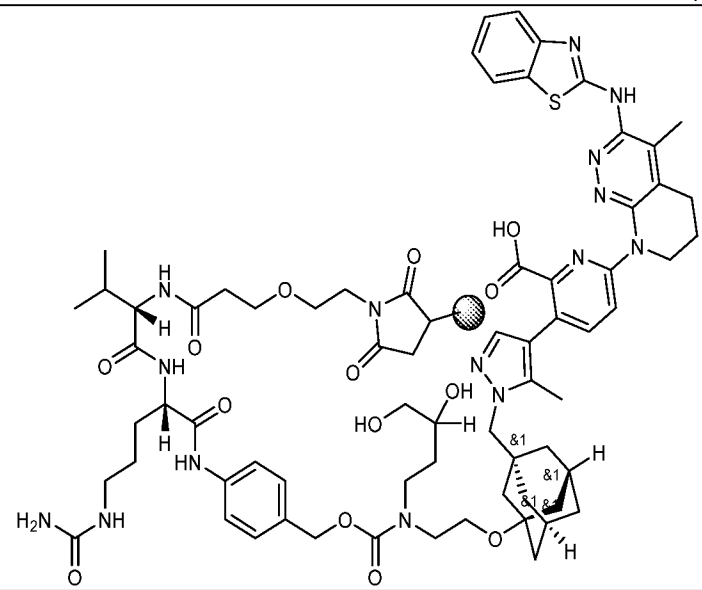
Ab-L9C-P59	
Ab-L9C-P3	
Ab-L9C-P60	

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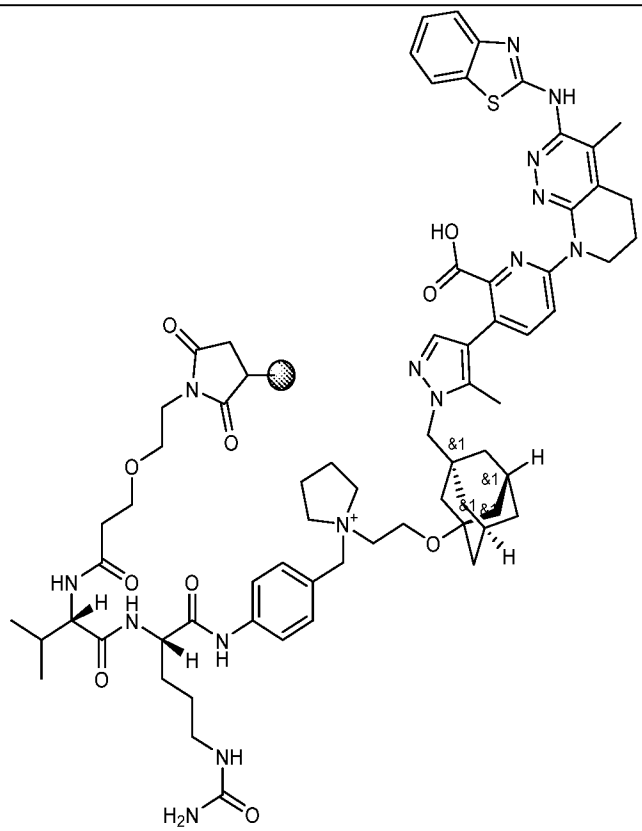
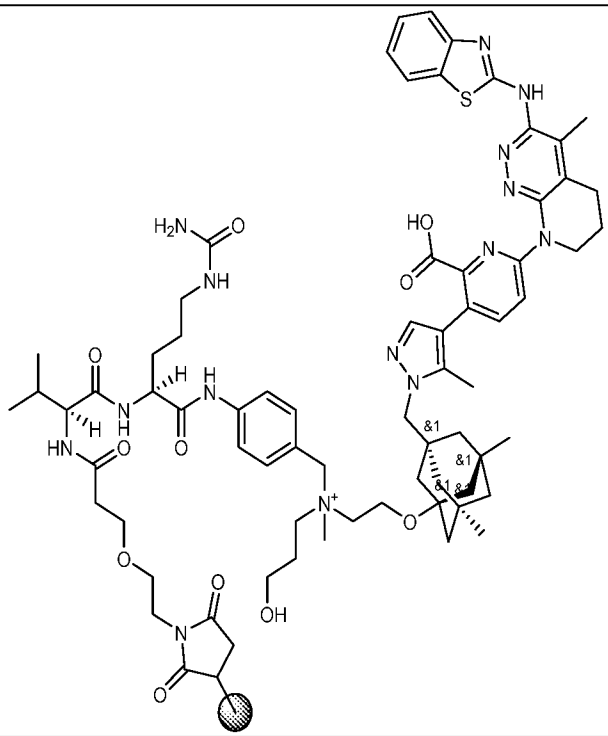
Ab-L9A-P61	
Ab-L9A-P62	

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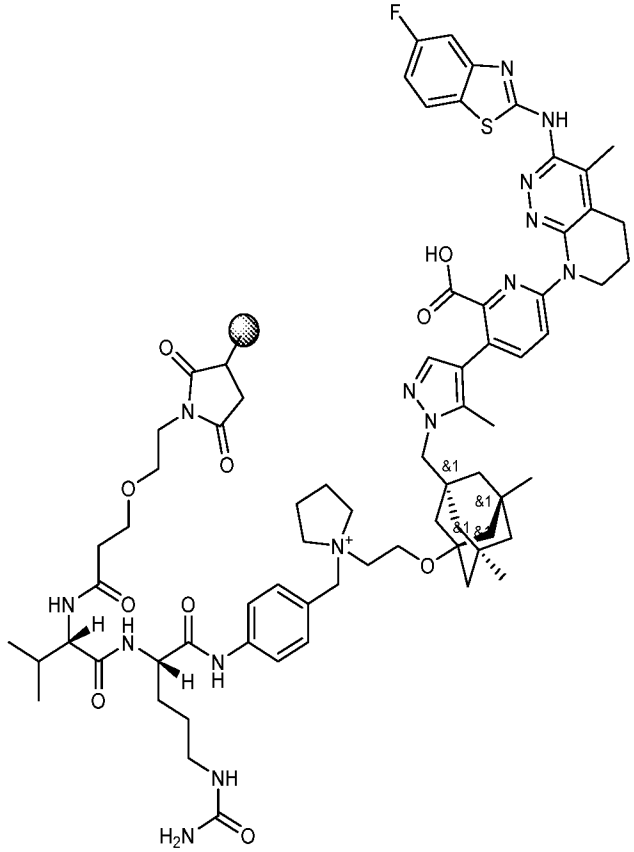
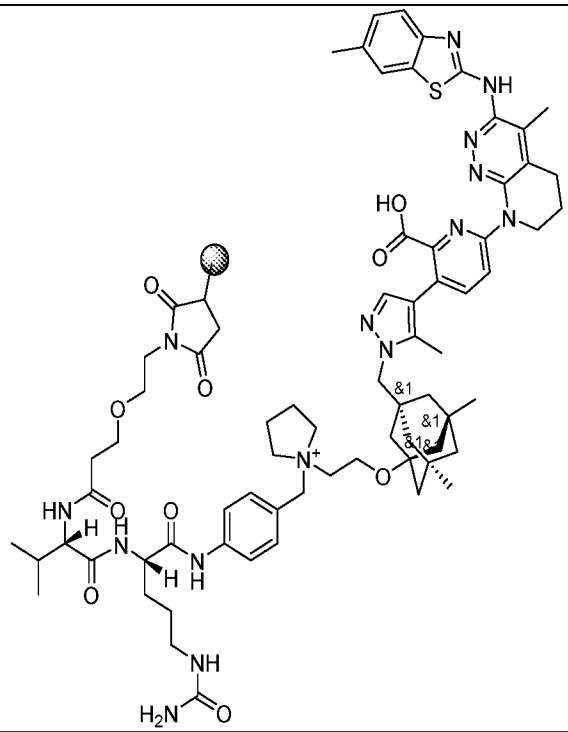
Ab-L9A-P63	
Ab-L9A-P64	

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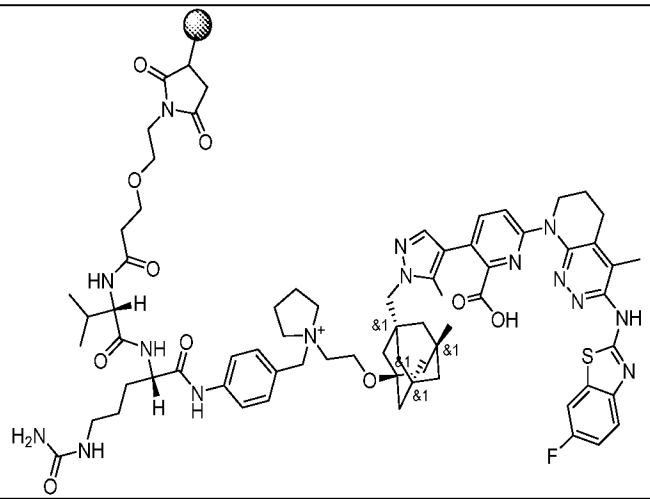
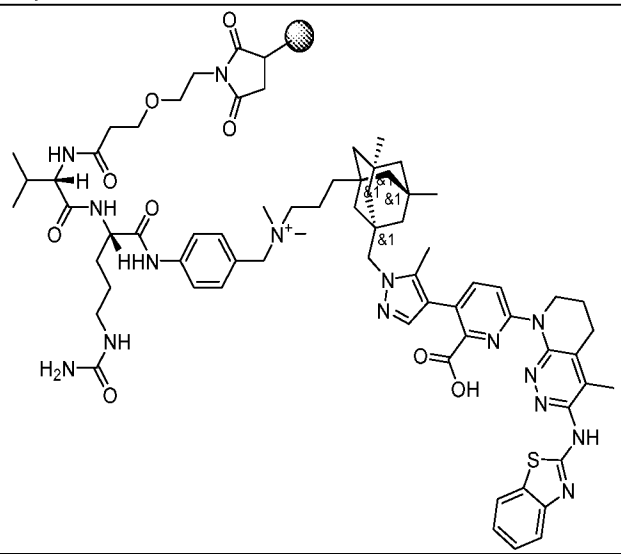
Ab-L9A-P65	
Ab-L9A-P66	

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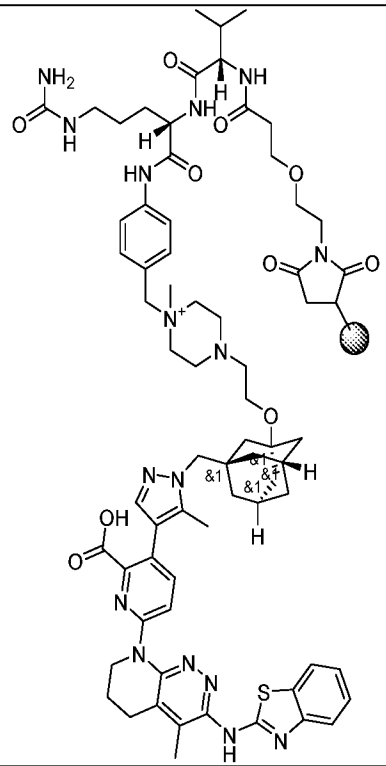
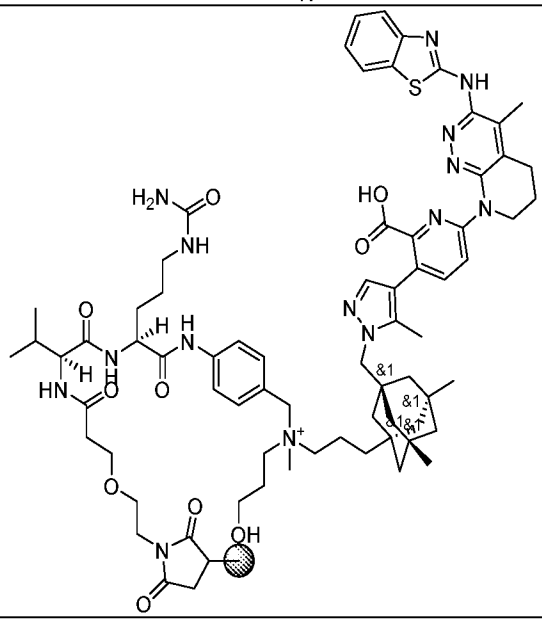
Ab-L9A-P67	
Ab-L9A-P68	

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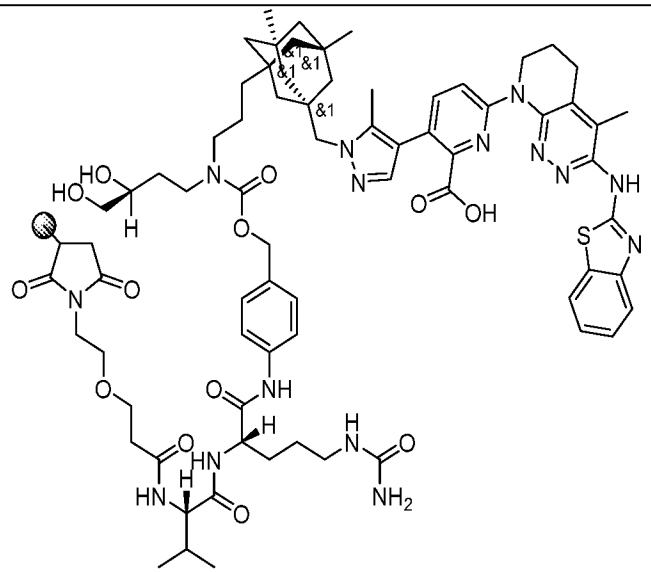
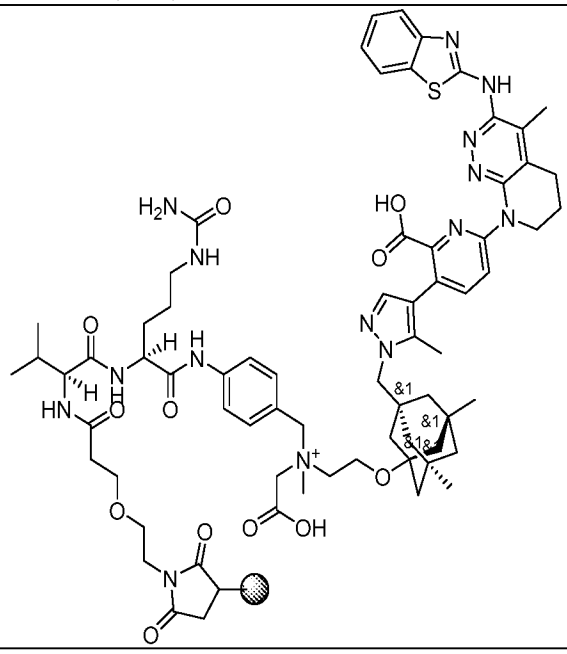
Ab-L9C-P69	
Ab-L9A-P48	

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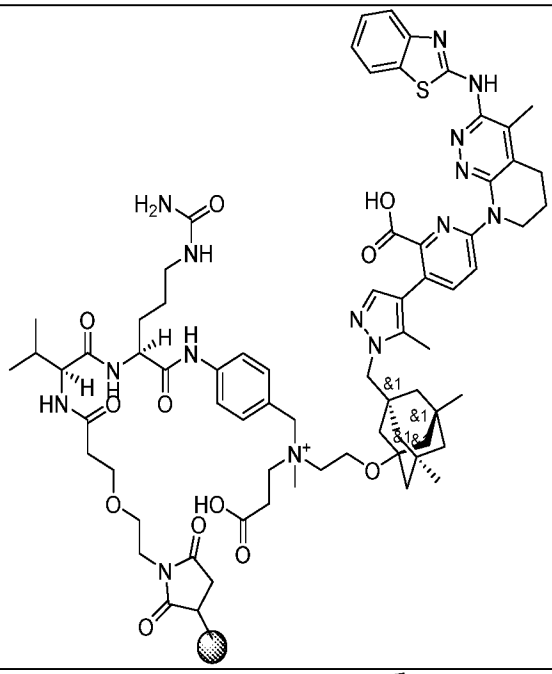
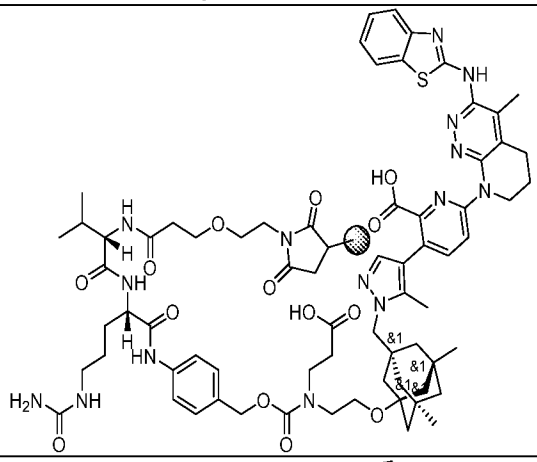
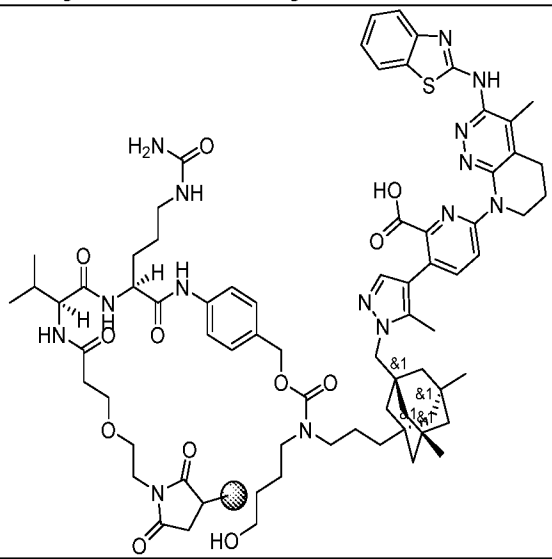
Ab-L9A-P70	
Ab- L9C-P71	
Ab-L9C-P72	

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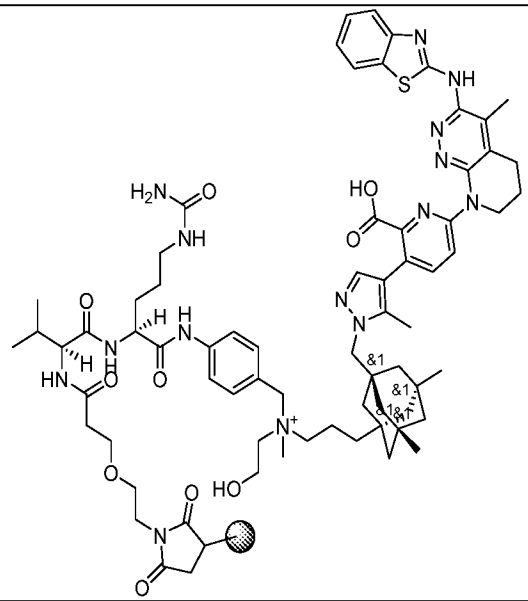
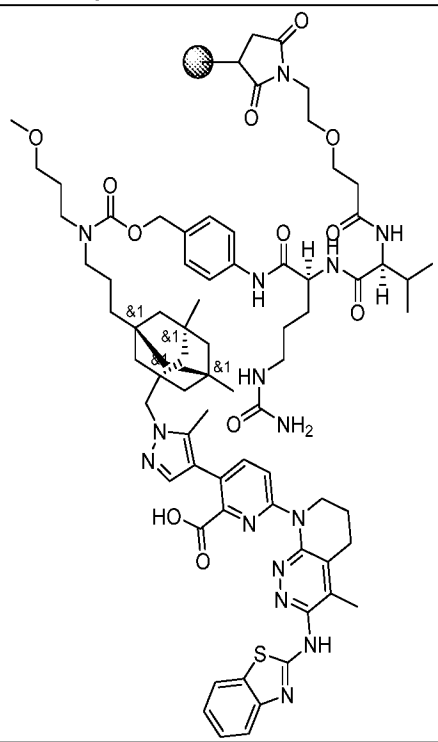
Ab-L9A-P49	
Ab-L9C-P51	

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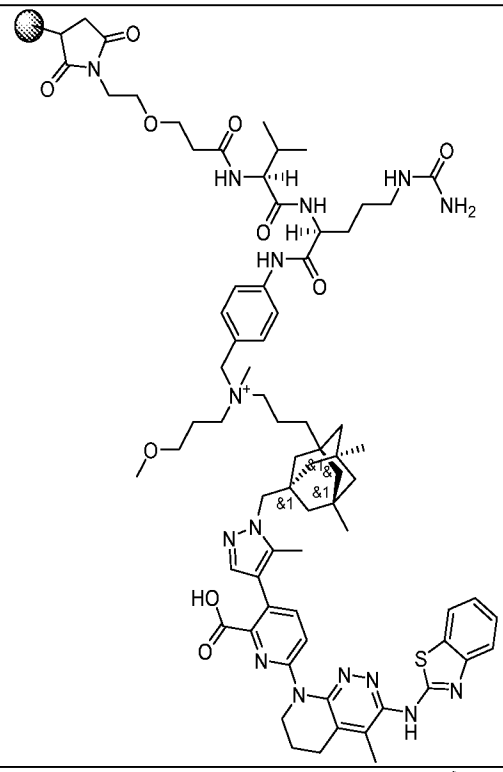
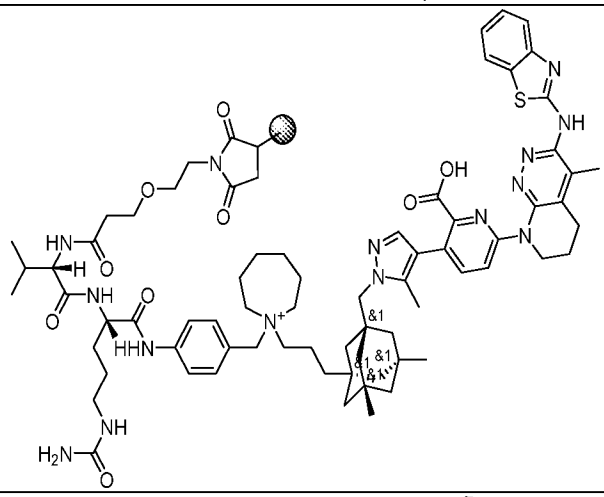
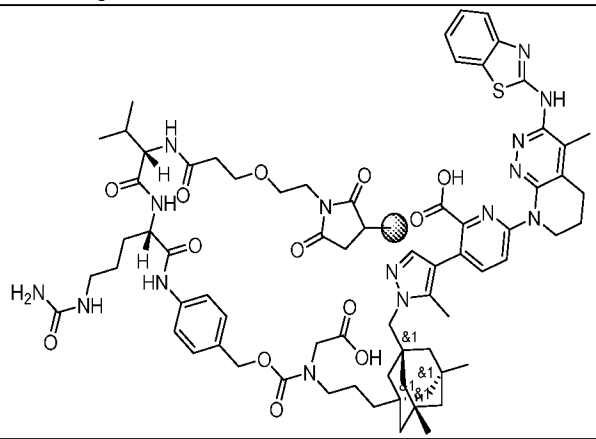
Ab-L9A-P50	
Ab-L9A-P52	
Ab-L9C-P53	

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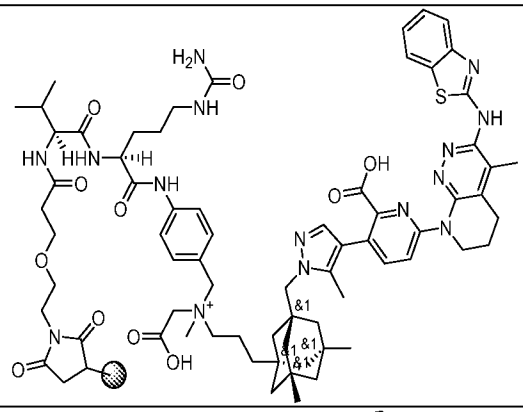
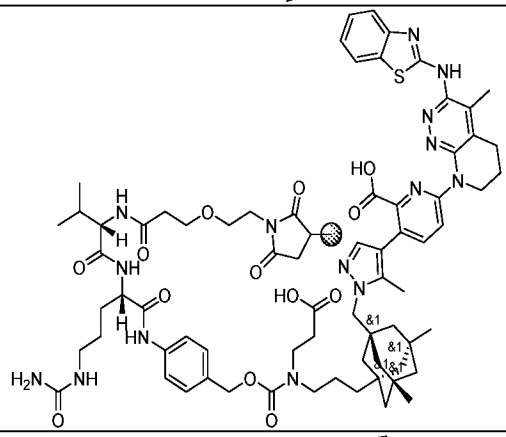
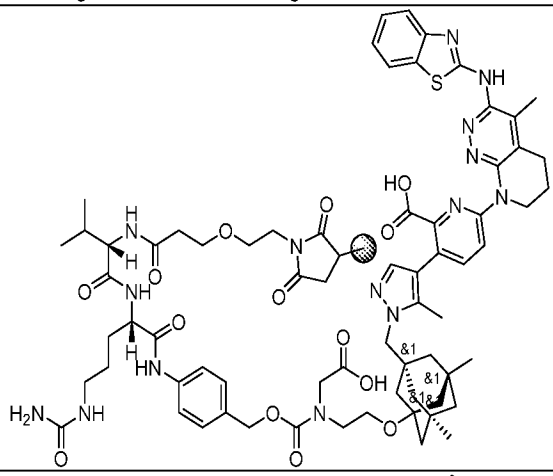
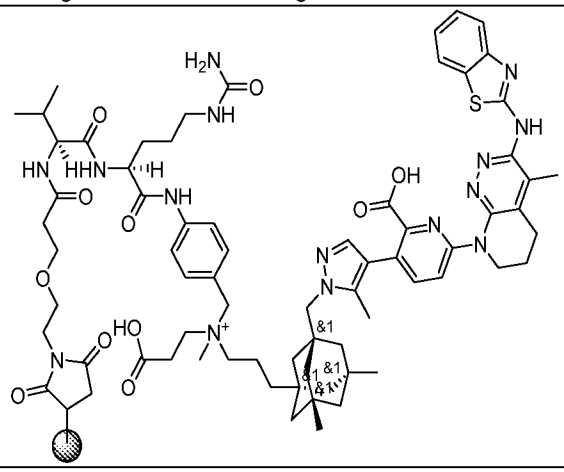
Ab-L9A-P55	
Ab-L9C-P54	
Ab-L9C-P47	
Ab-L9A-P56	

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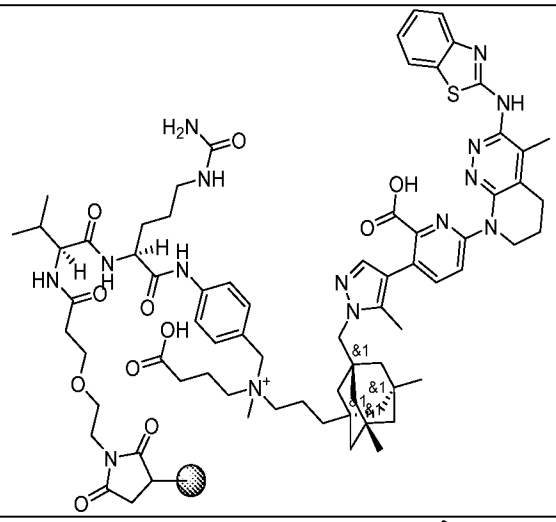
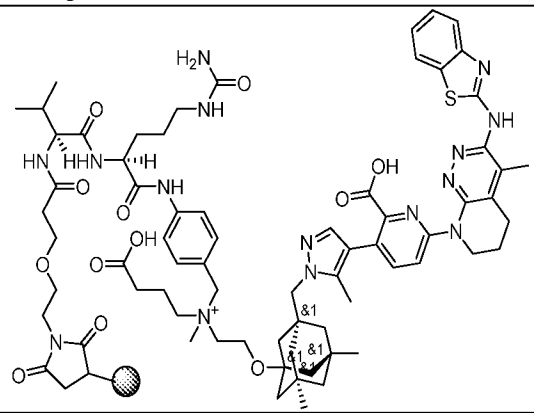
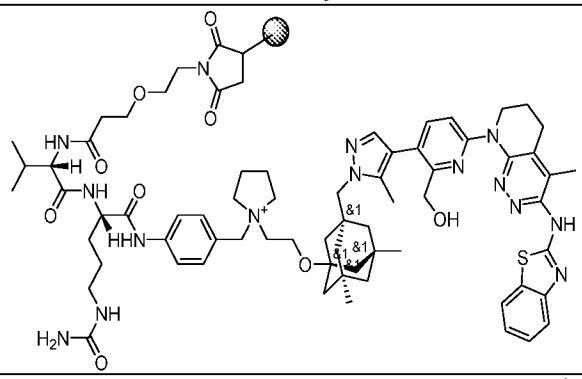
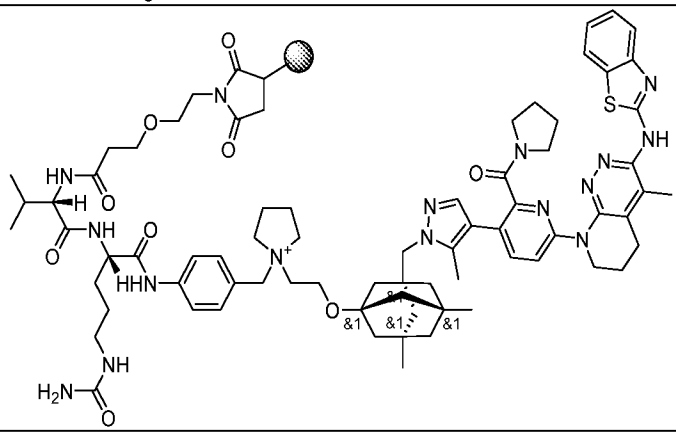
Ab-L9A-P58	
Ab-L9A-P57	
Ab-L9A-P73	
Ab-L9A-P74	

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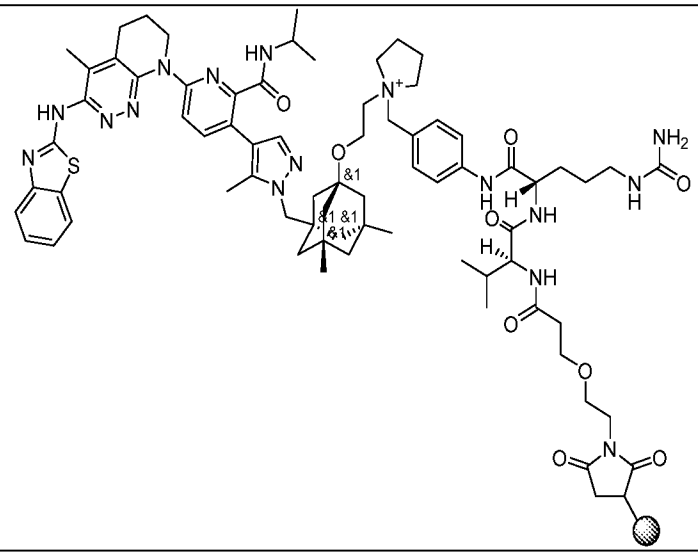
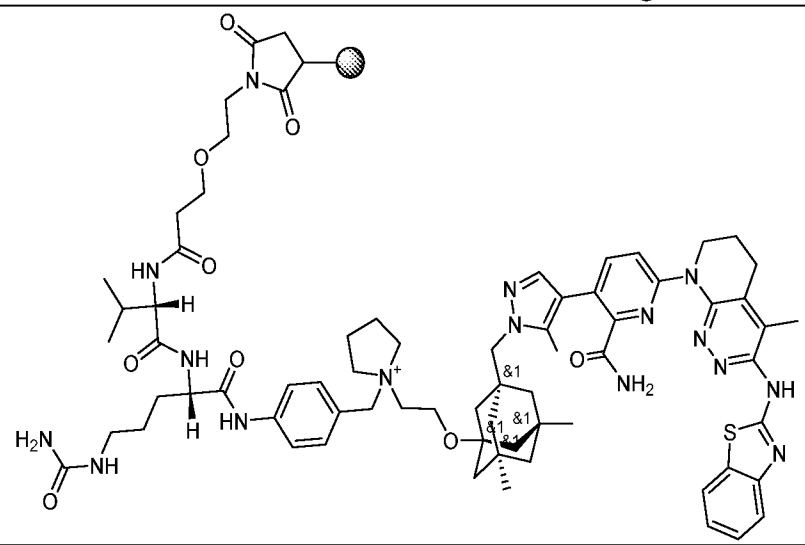
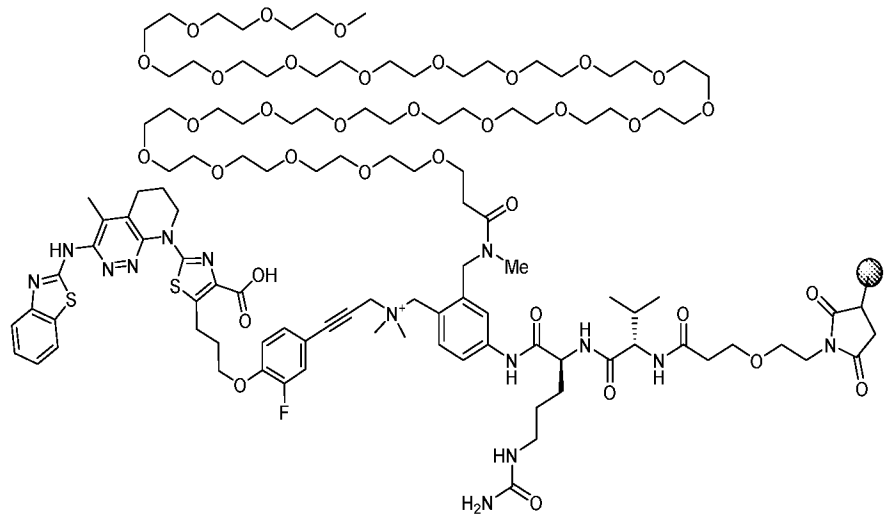
Ab-L9A-P75	
Ab-L9A-P76	
Ab-L30A-P1	

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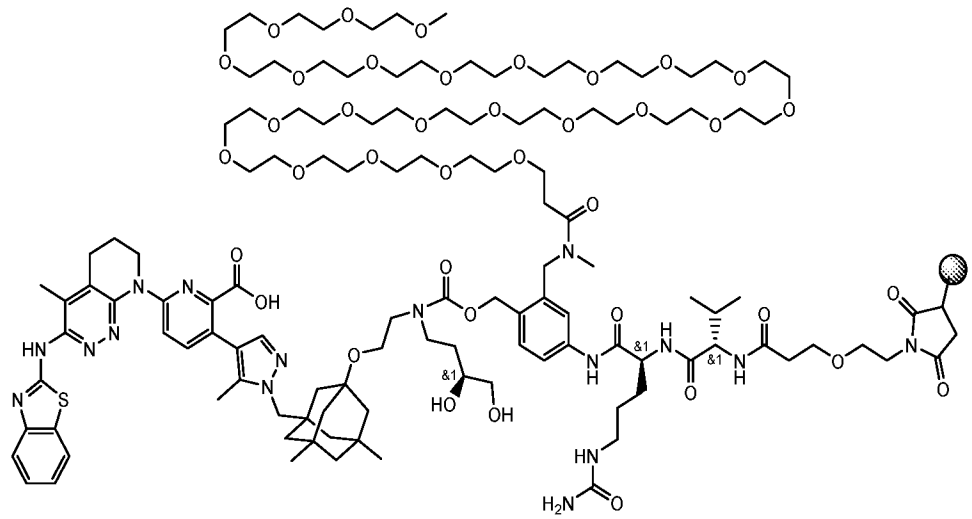
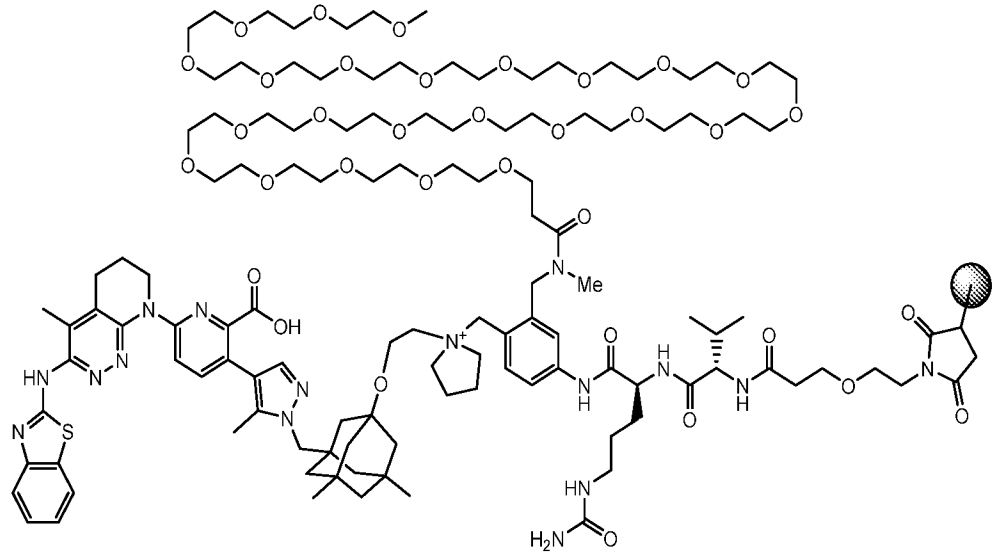
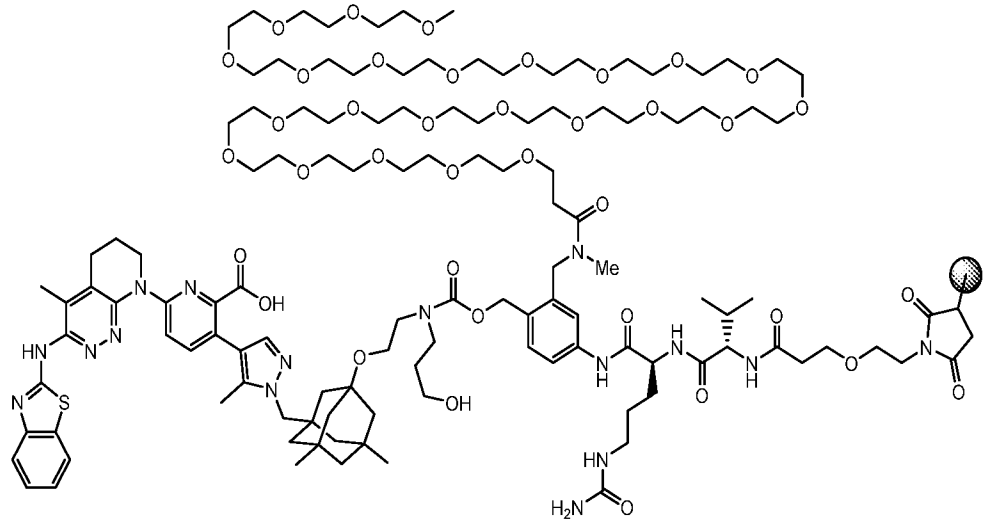
Ab-L30C-P19	
Ab-L30A-P21	
Ab-L30C-P25	

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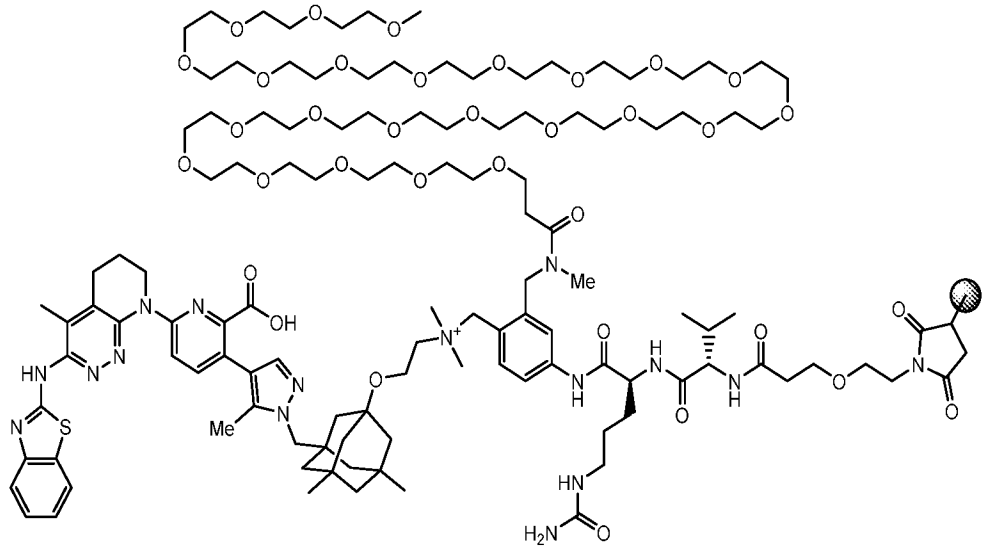
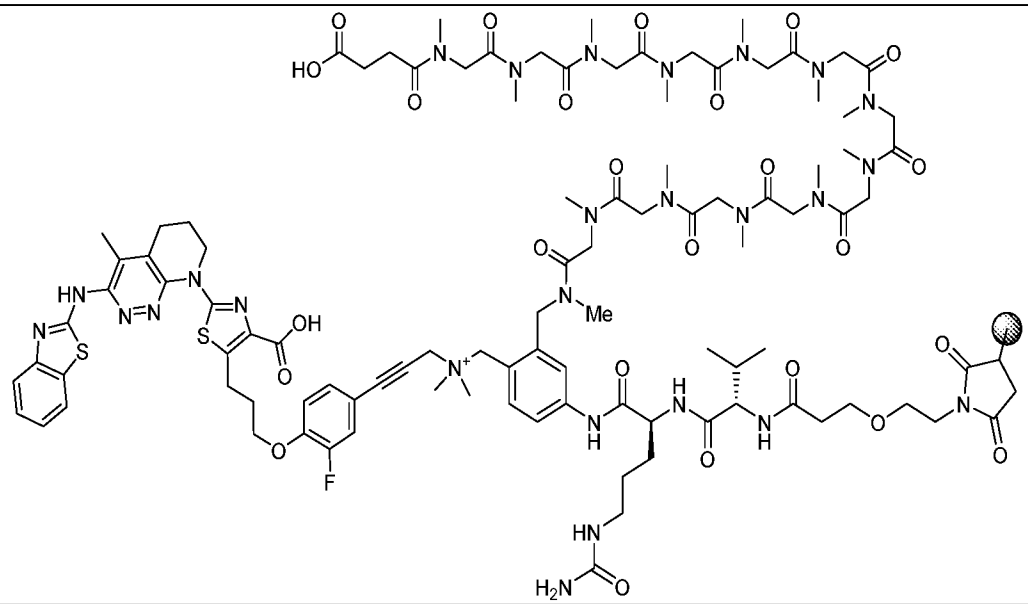
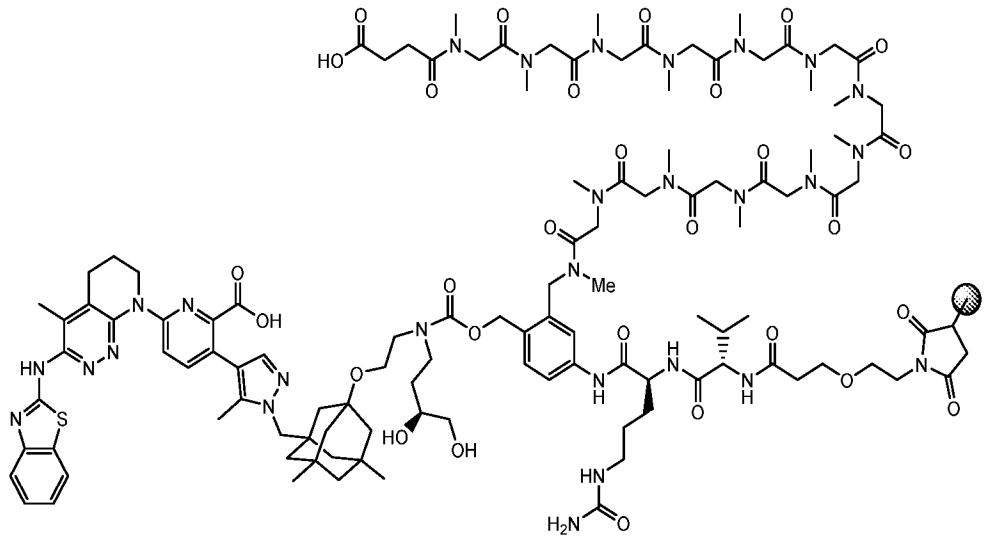
Ab-L30A-P27	
Ab-L35A-P1	
Ab-L35C-P19	

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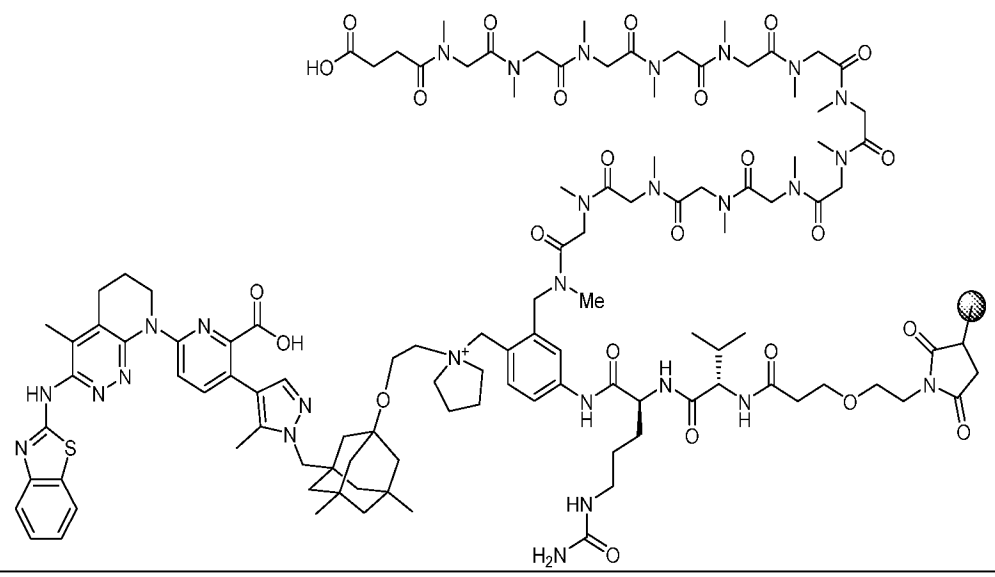
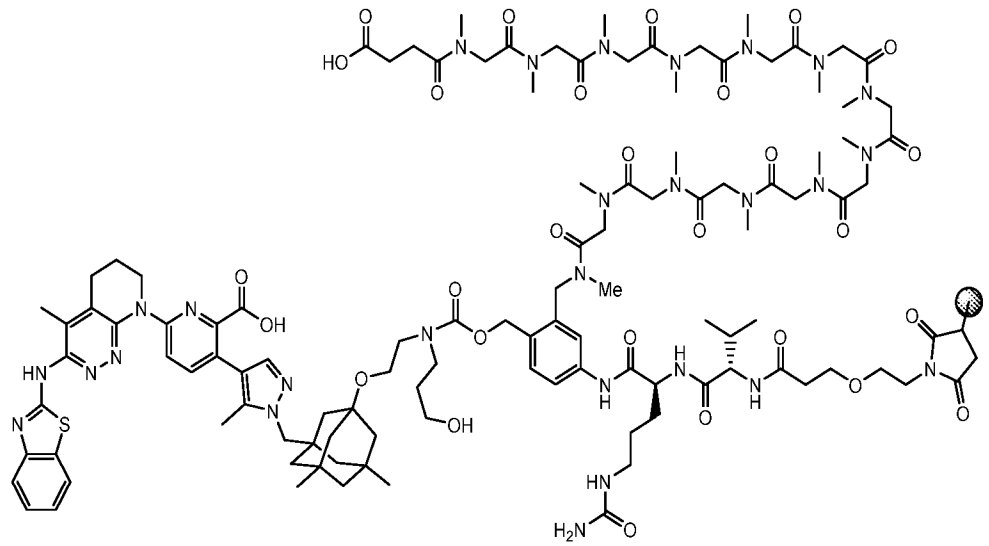
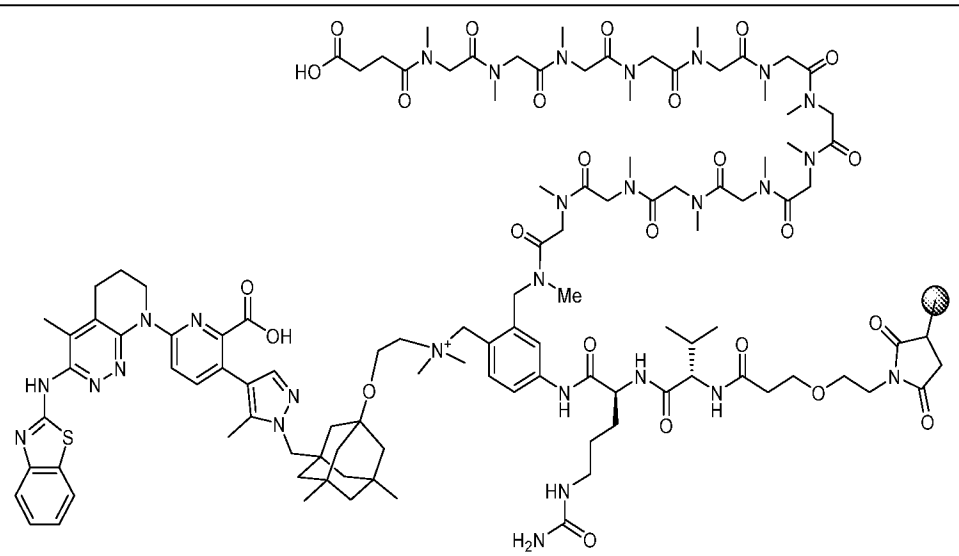
Ab-L35A-P21	
Ab-L35C-P25	
Ab-L35A-P27	

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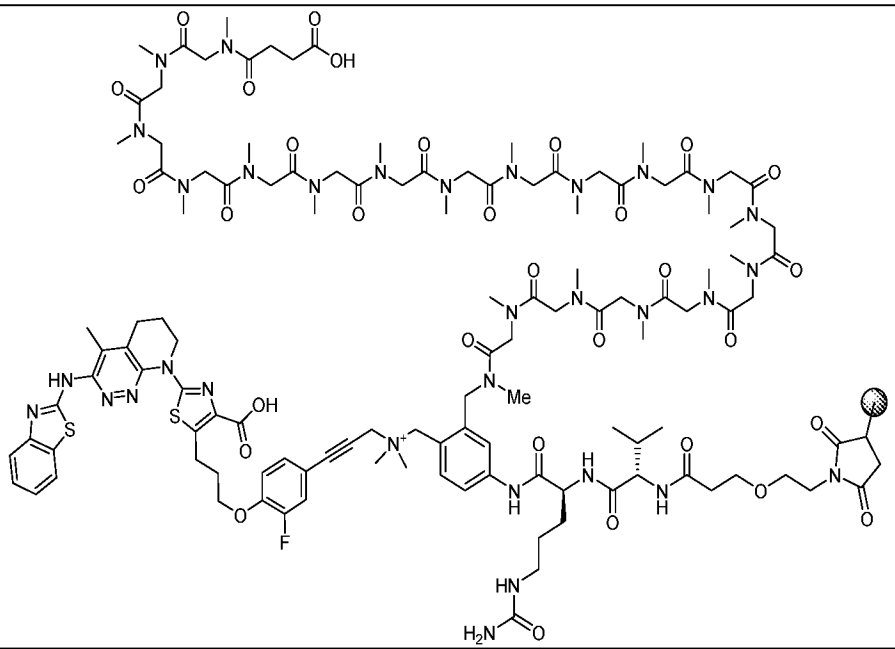
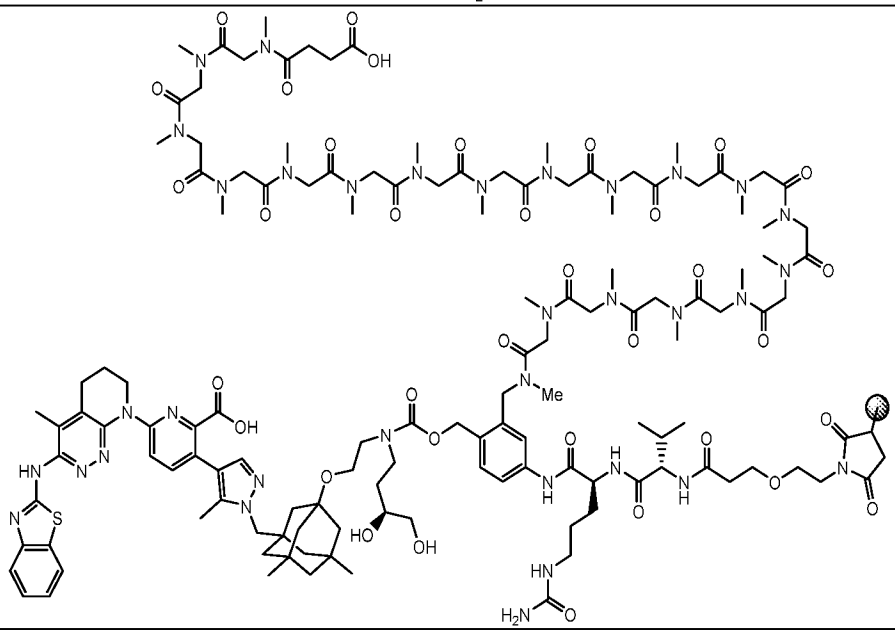
Ab-L36A-P1	
Ab-L36C-P19	

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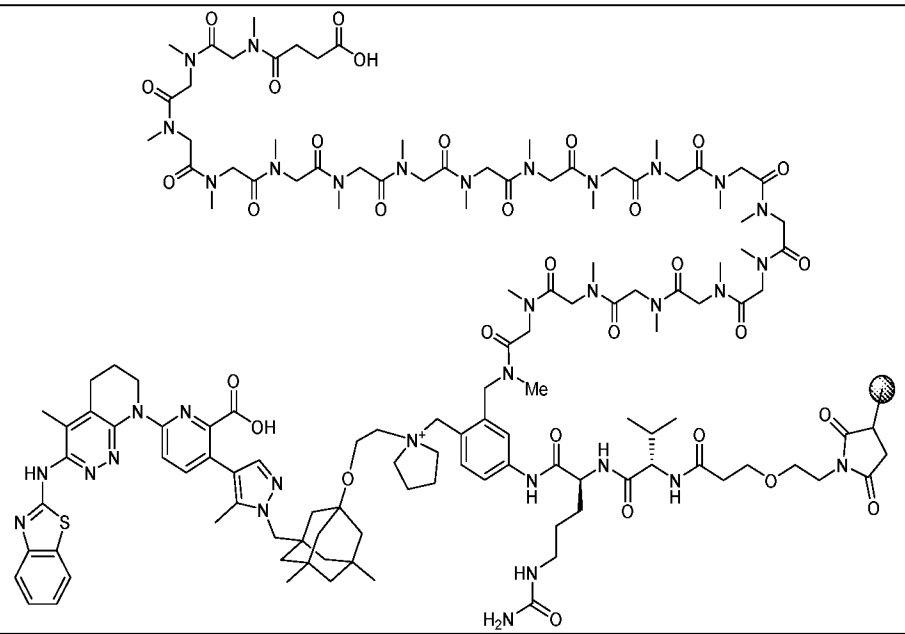
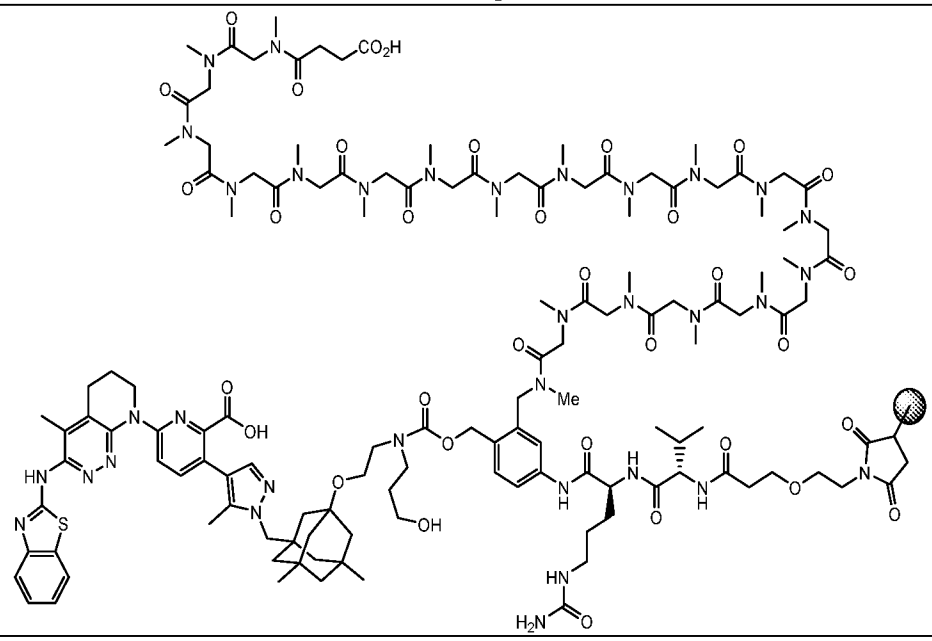
Ab-L36A-P21	
Ab-L36C-P25	

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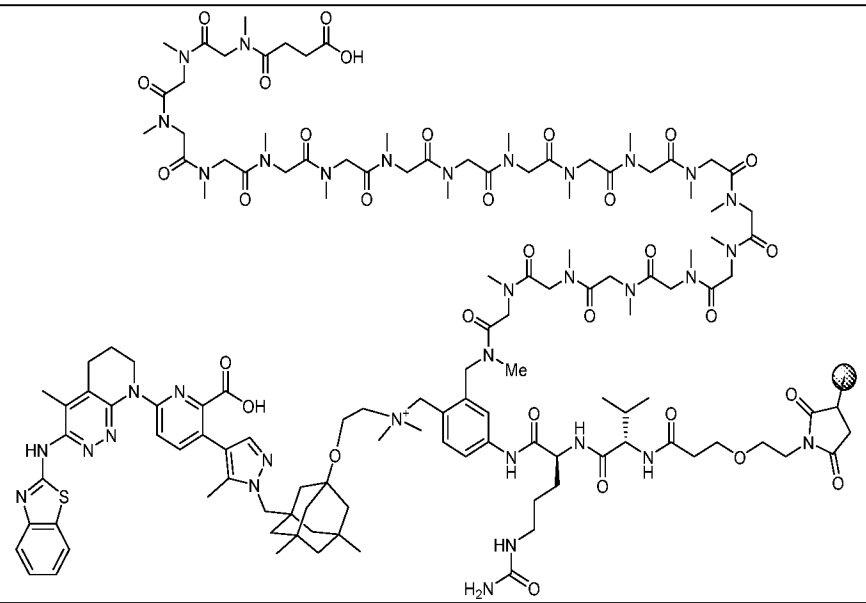
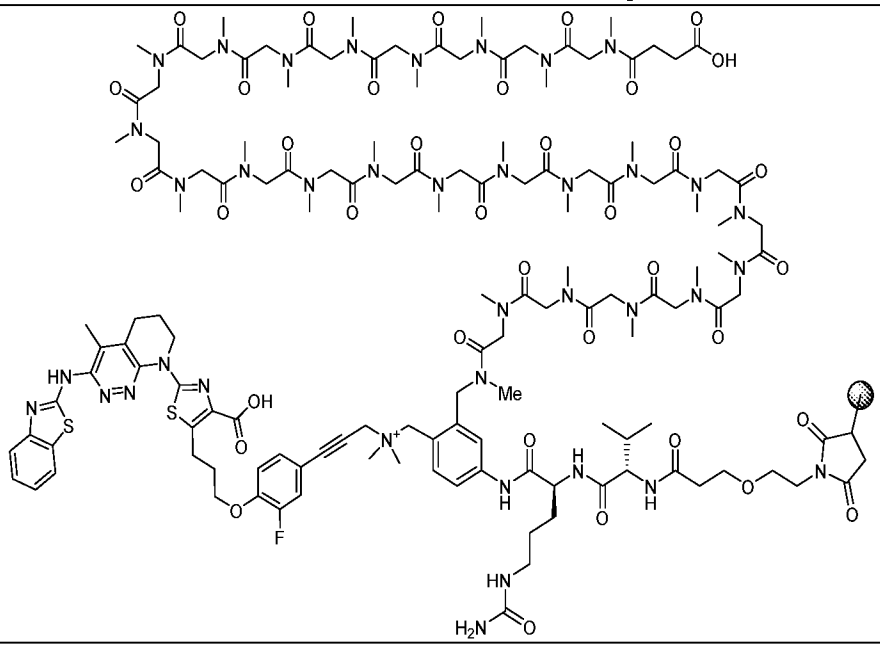
Ab-L36A-P27	
Ab-L37A-P1	

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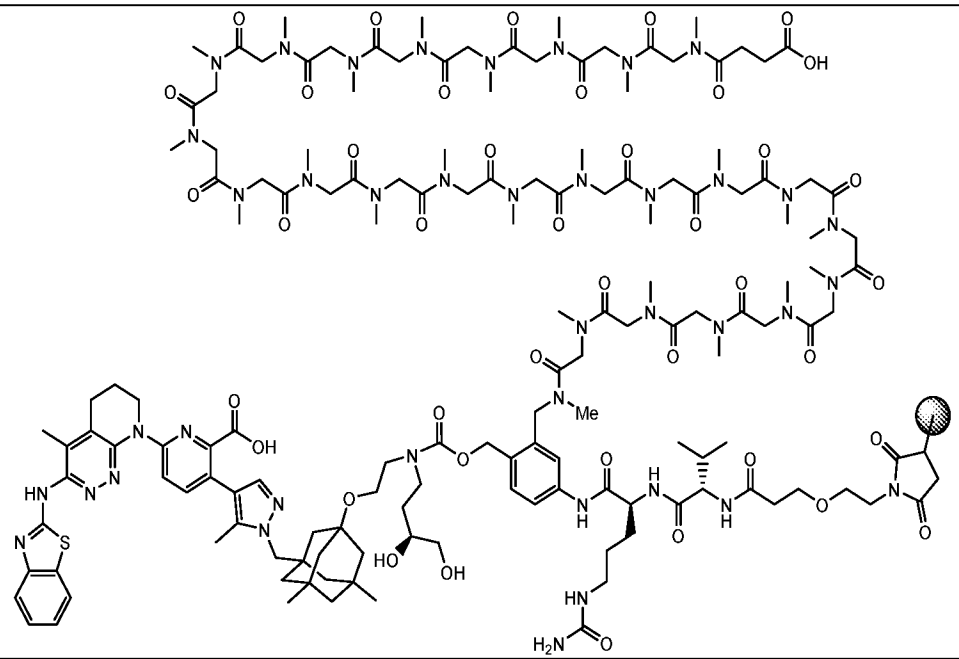
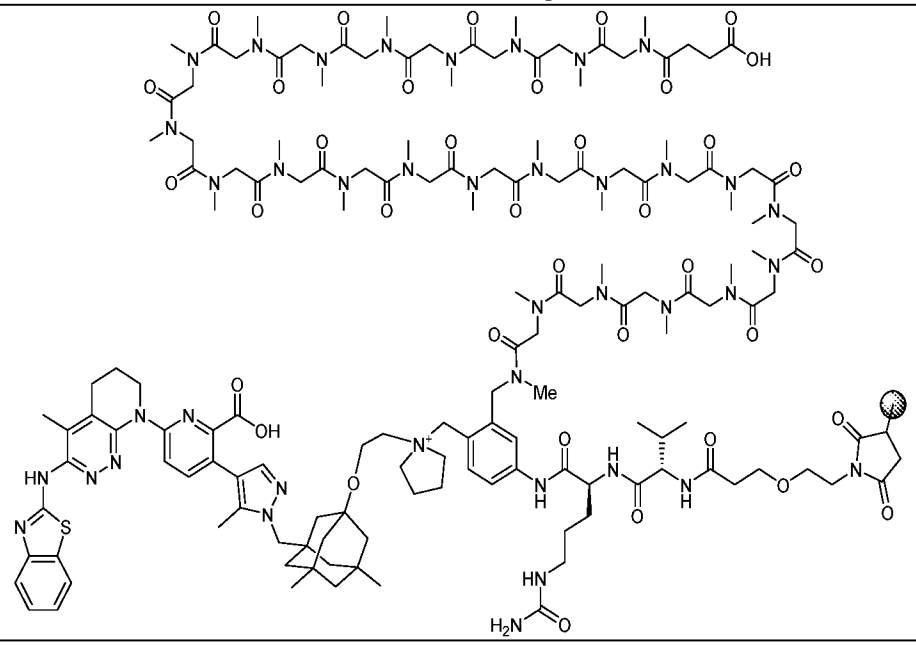
Ab- L37C-P19	
Ab-L37A-P21	

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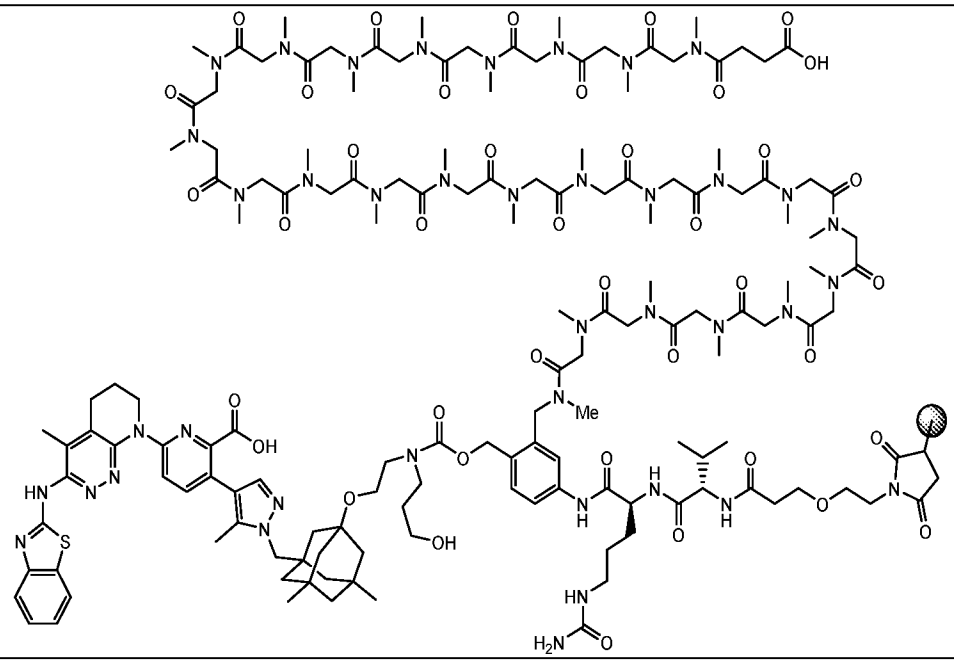
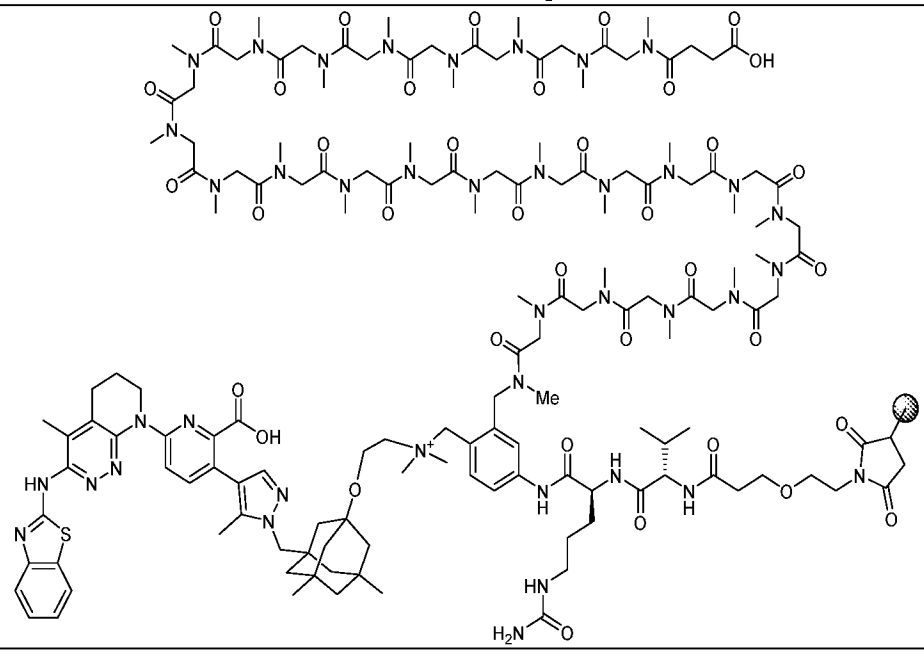
Ab-L37C-P25	
Ab-L37A-P27	

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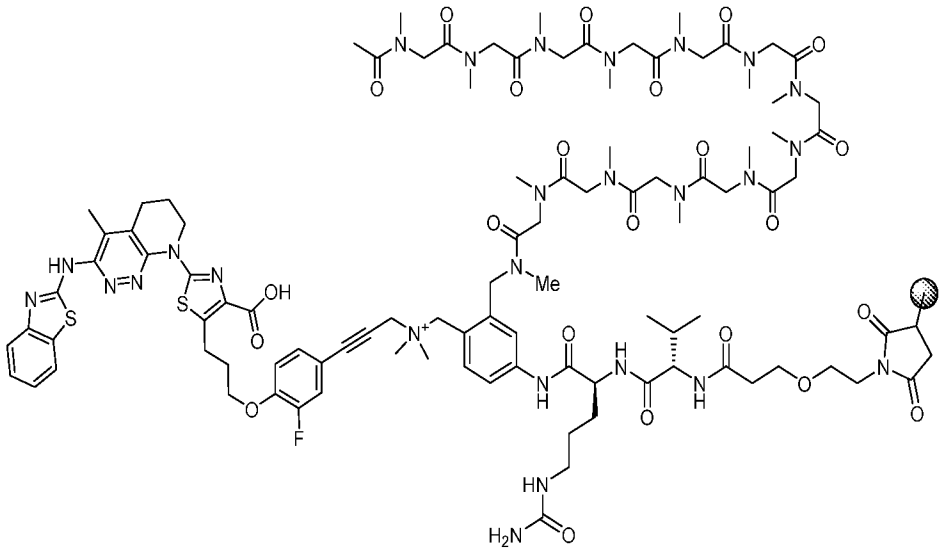
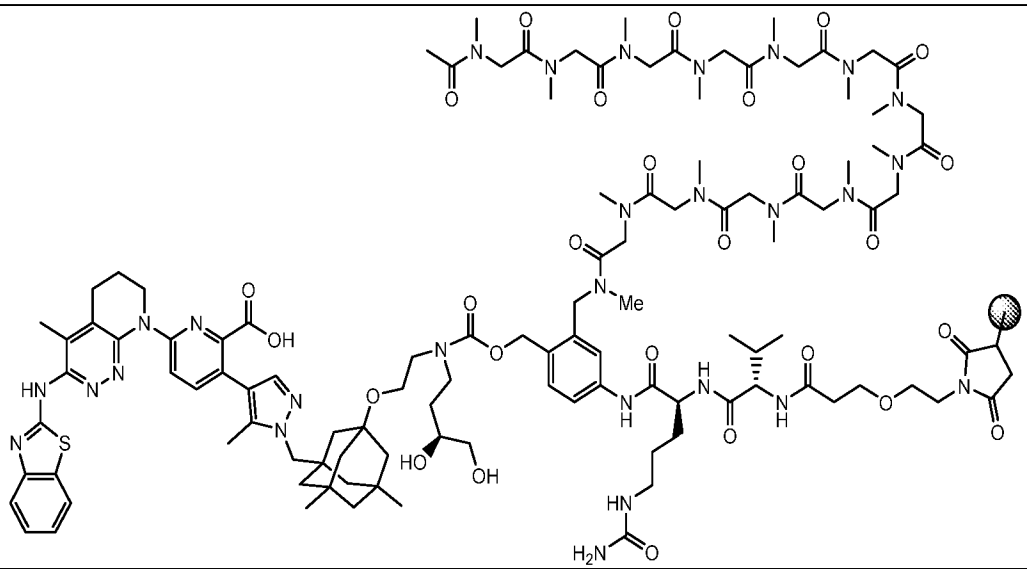
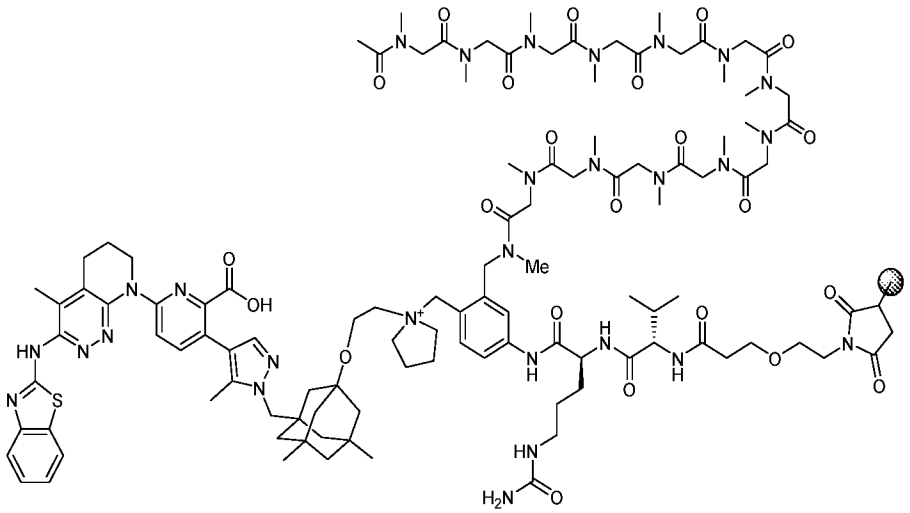
Ab-L38A-P1	
Ab-L38C-P19	
Ab-L38A-P21	

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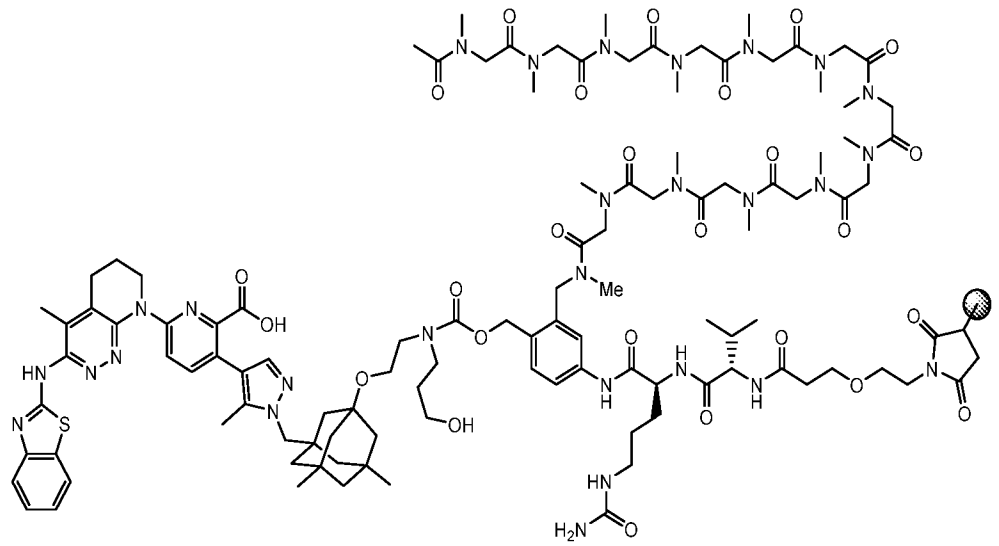
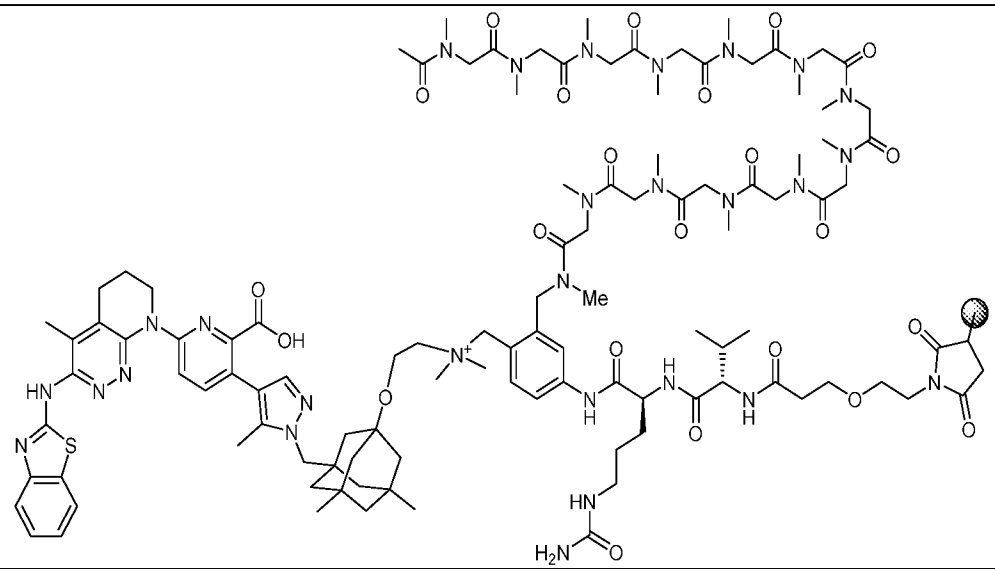
Ab-L38C-P25	
Ab-L38A-P27	

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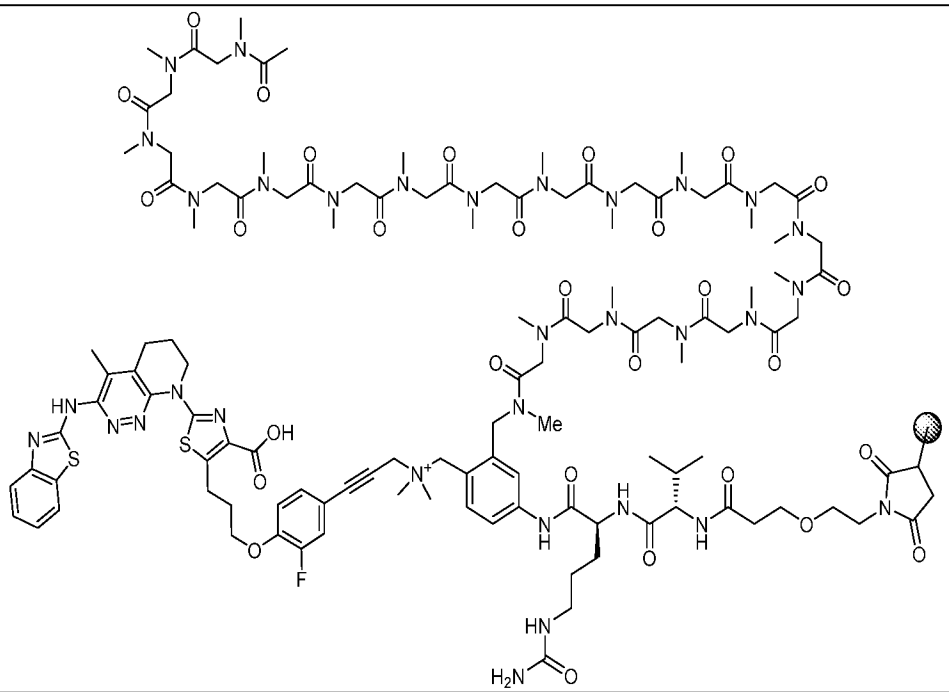
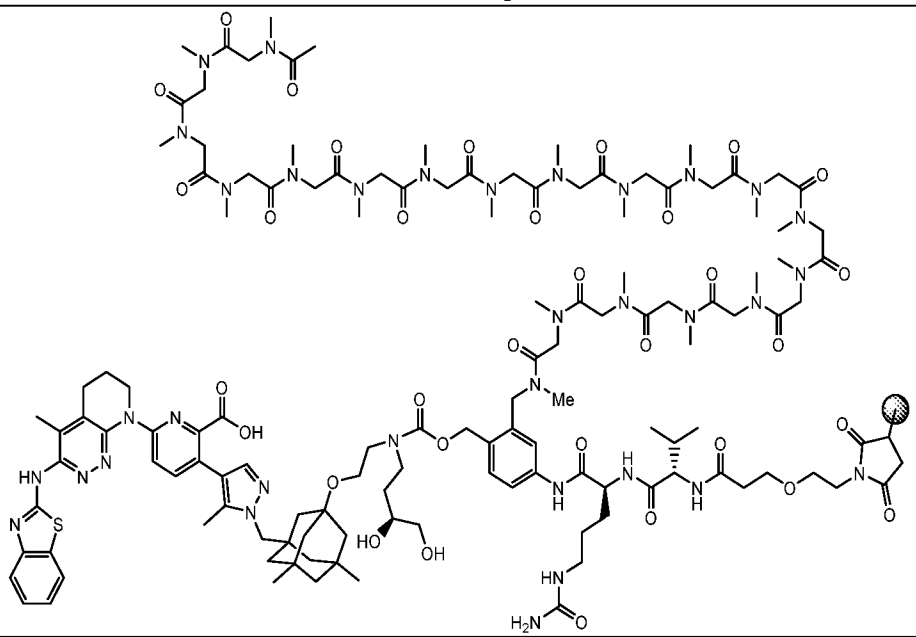
Ab-L39A-P1	
Ab-L39C-P19	

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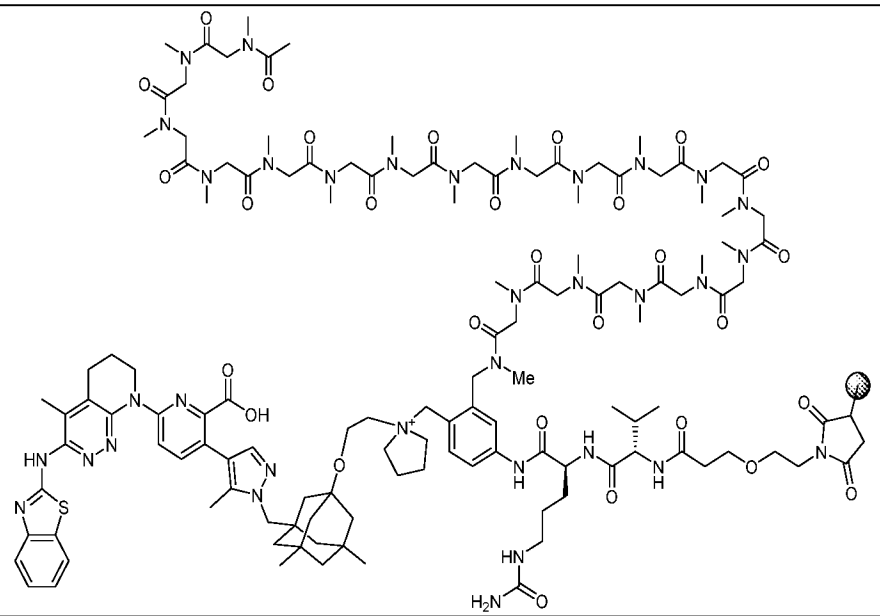
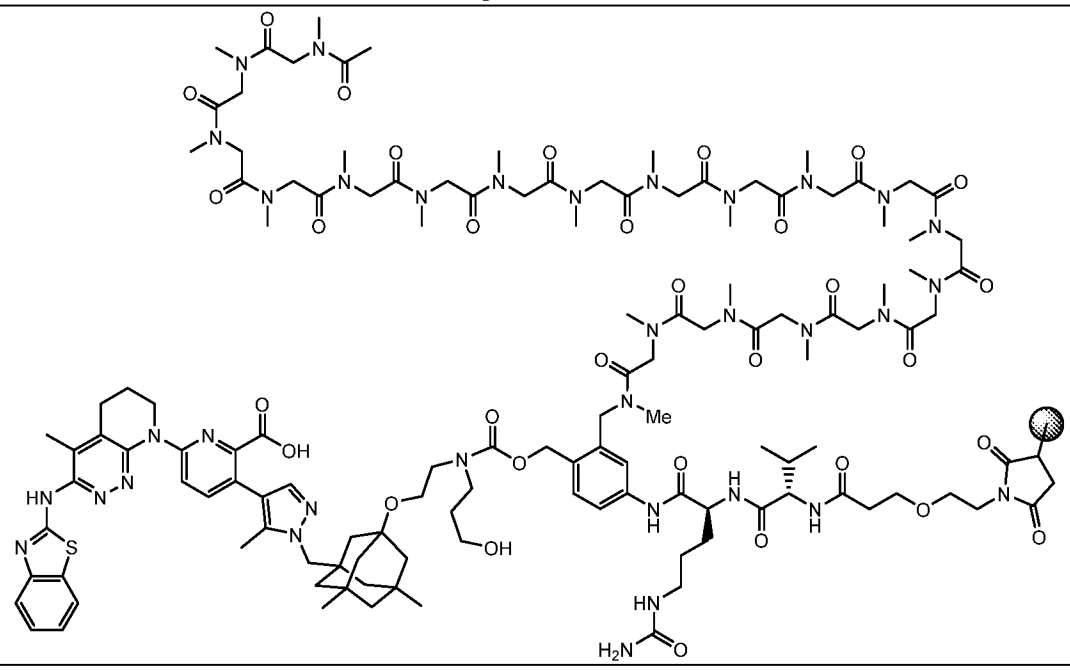
Ab-L39A-P21	
Ab-L39C-P25	

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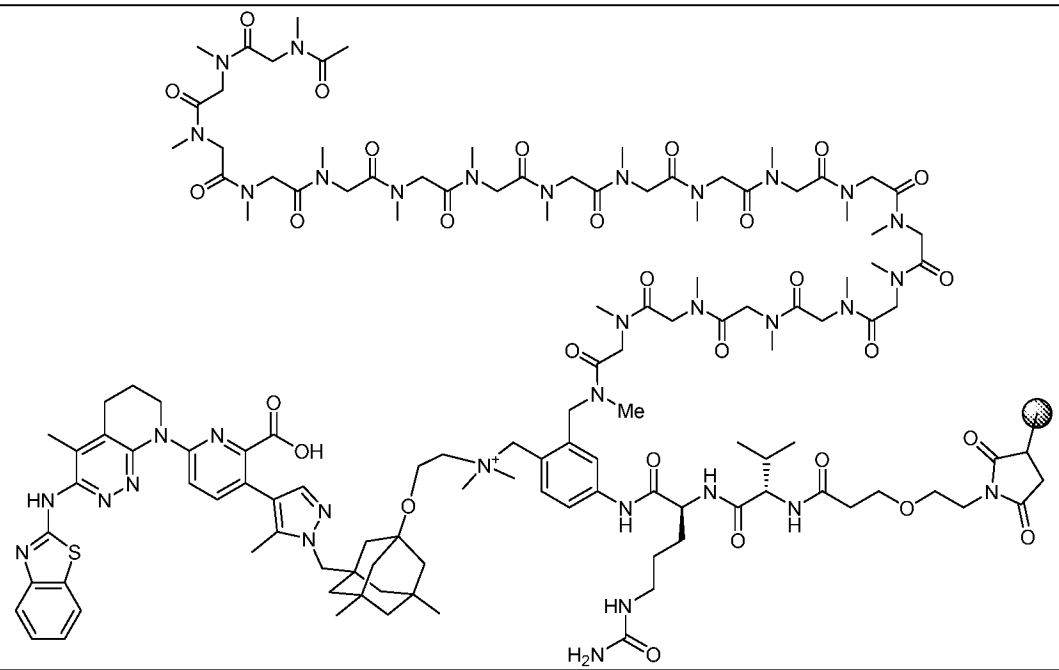
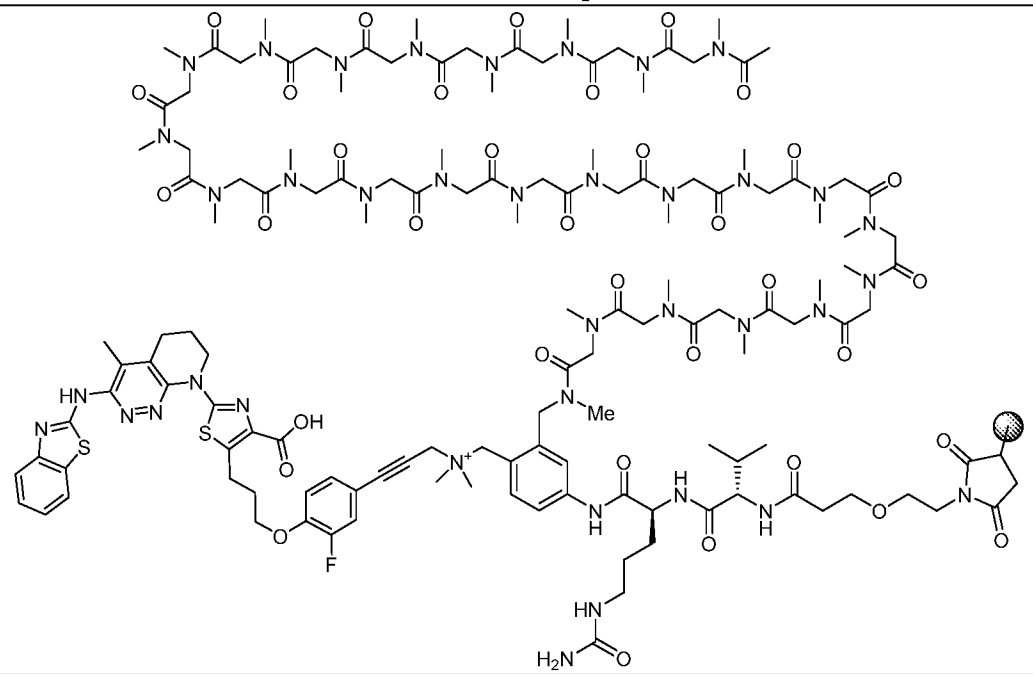
Ab-L39A-P27	
Ab-L40A-P1	

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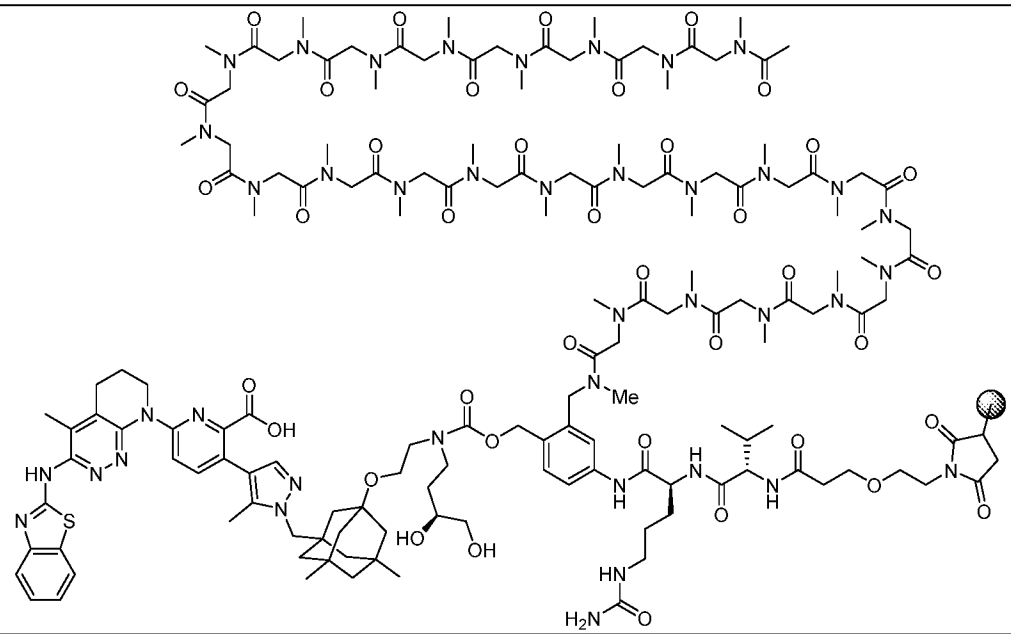
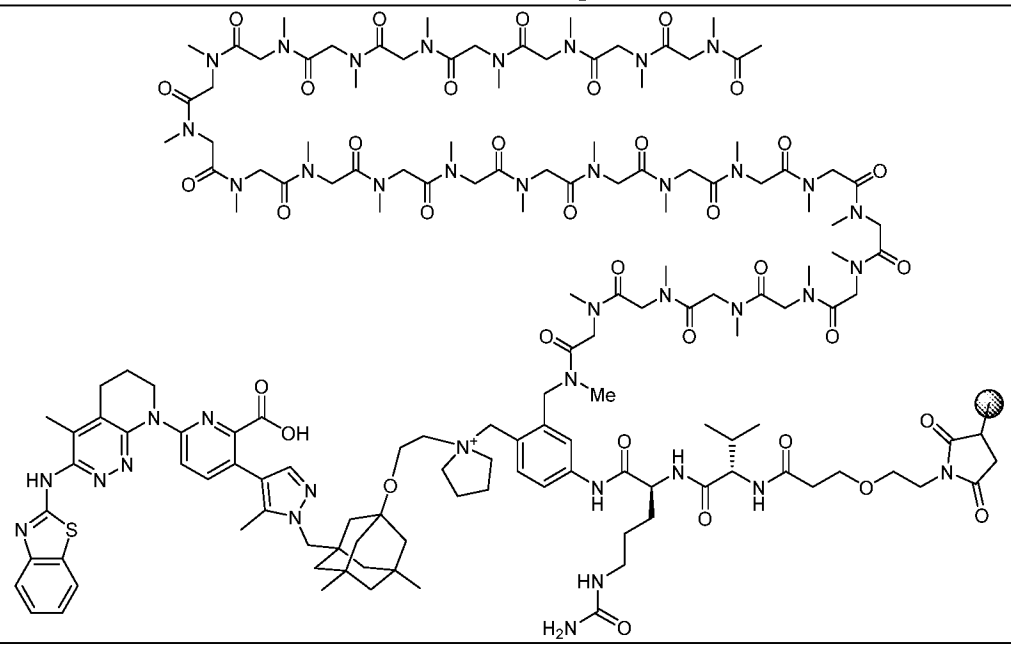
Ab-L40C-P19	
Ab-L40A-P21	

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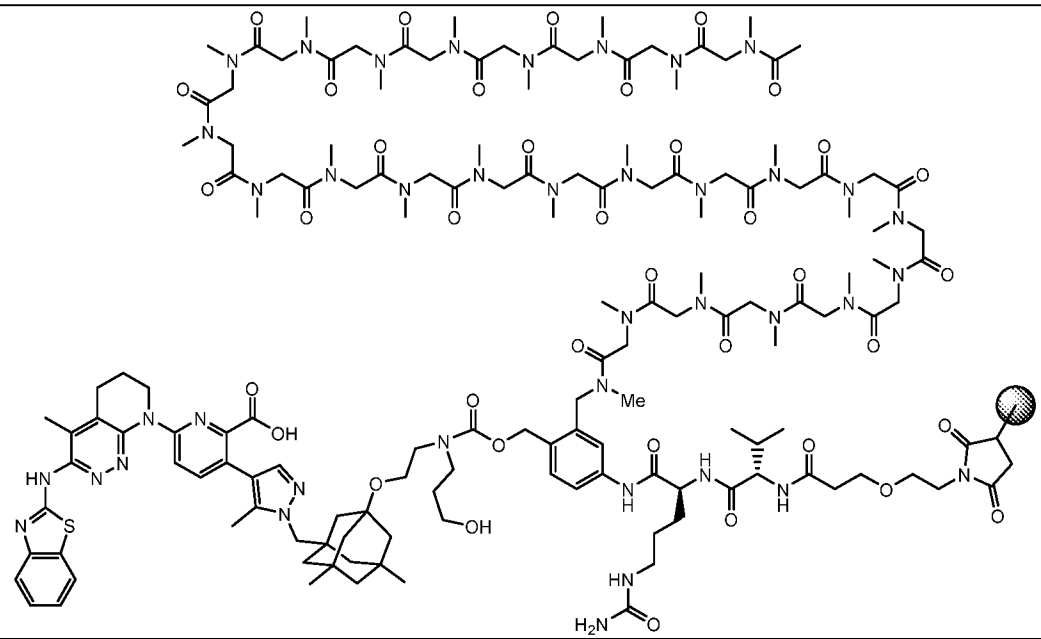
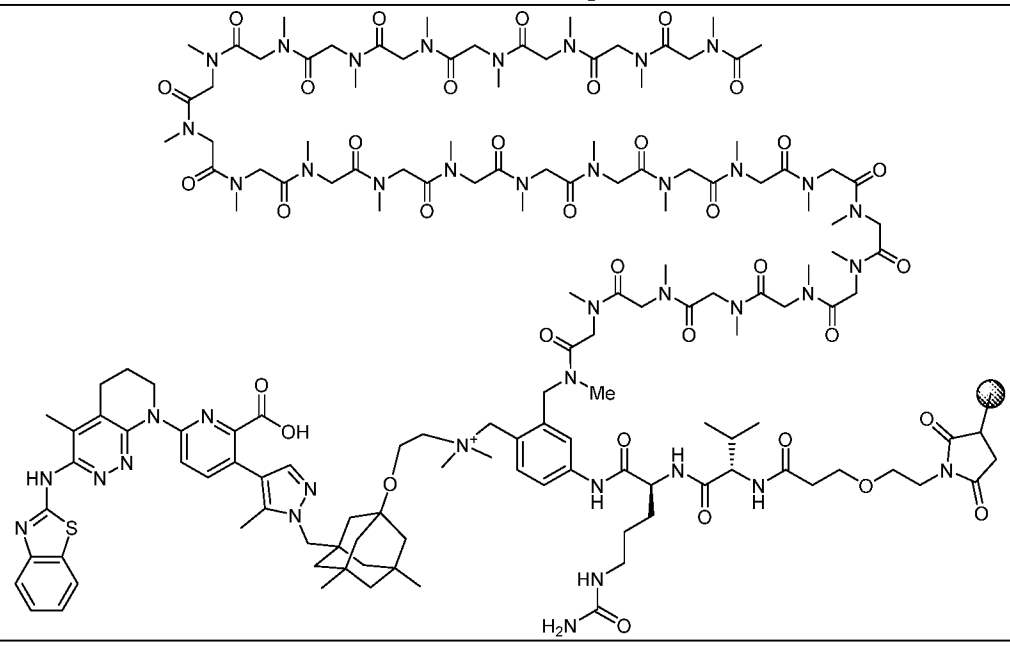
Ab-L40C-P25	
Ab-L40A-P27	

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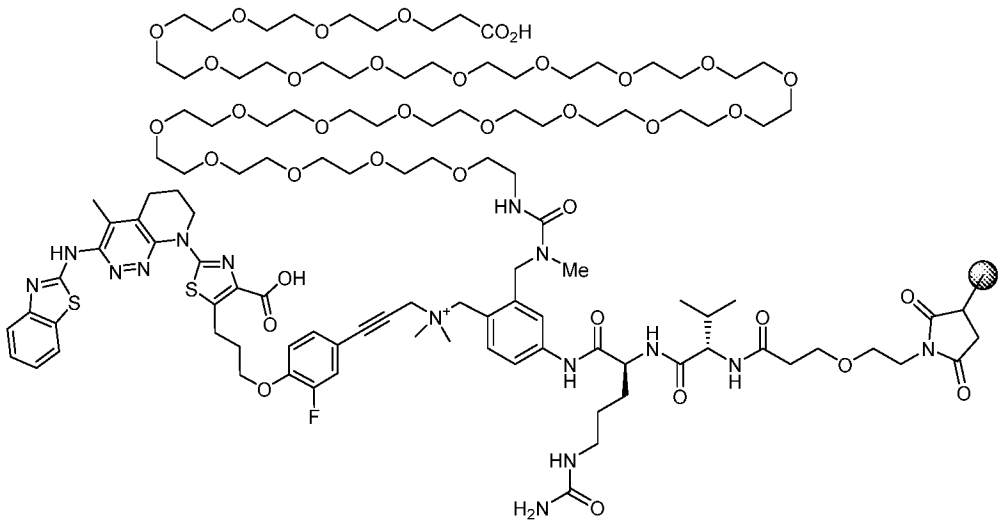
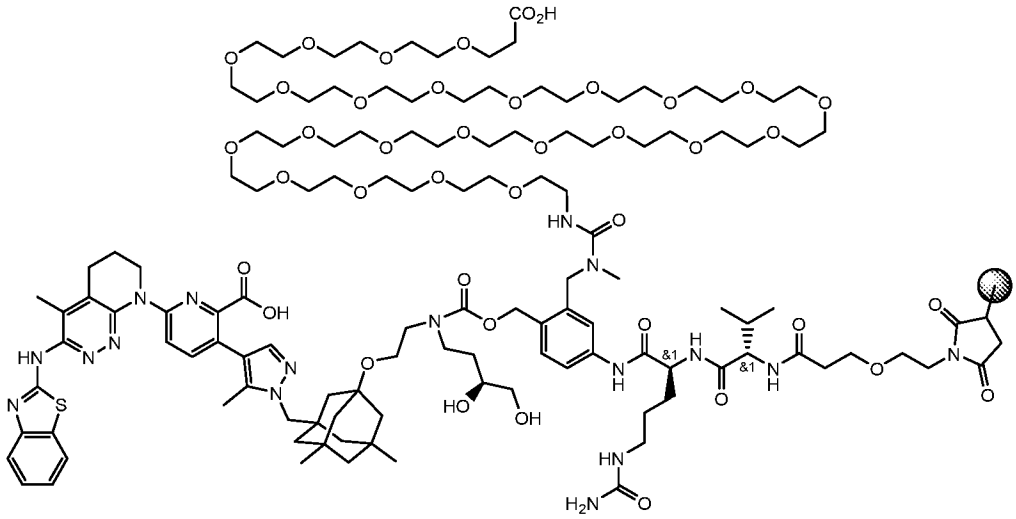
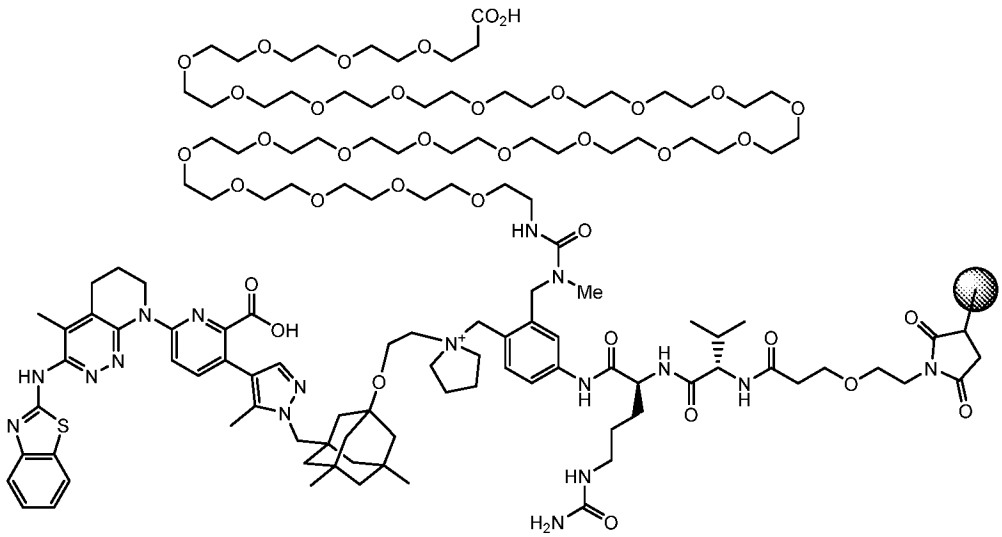
Ab-L42A-P1	
Ab-L42C-P19	
Ab-L42A-P21	

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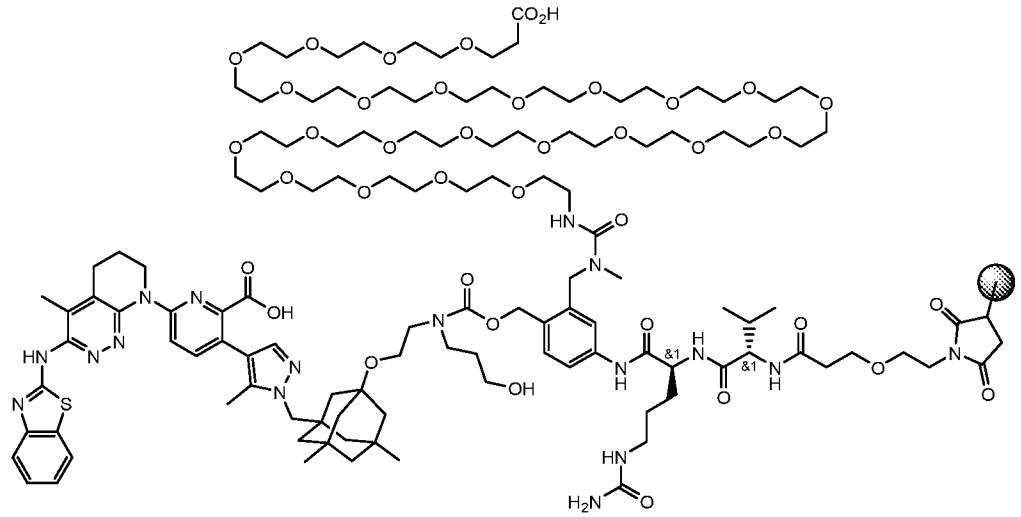
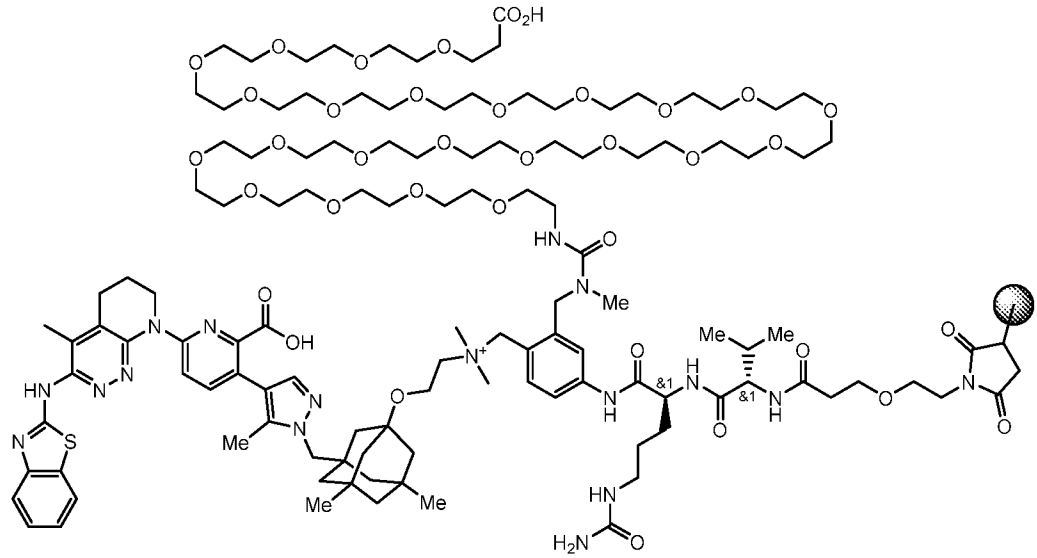
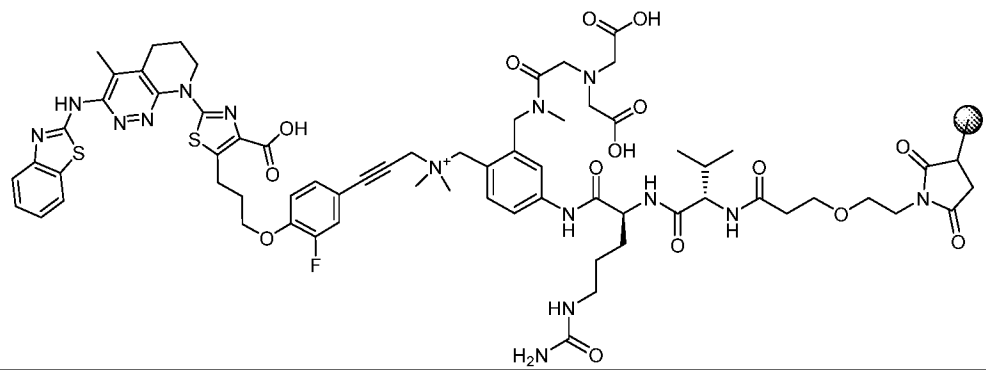
Ab-L42C-P25	
Ab-L42A-P27	
L67A-P1	

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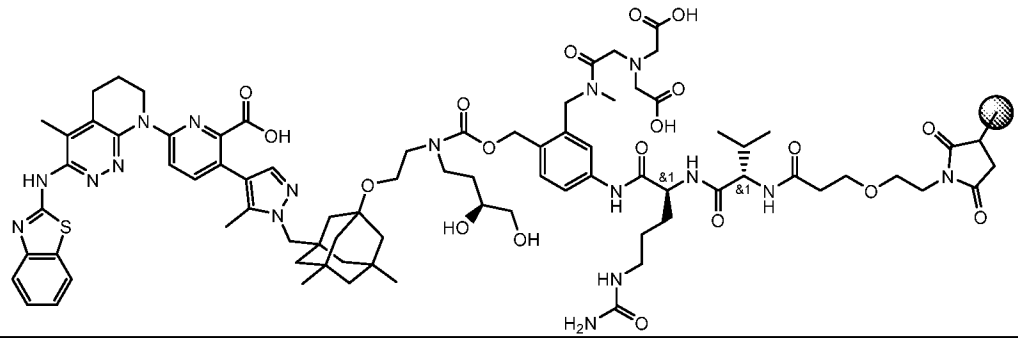
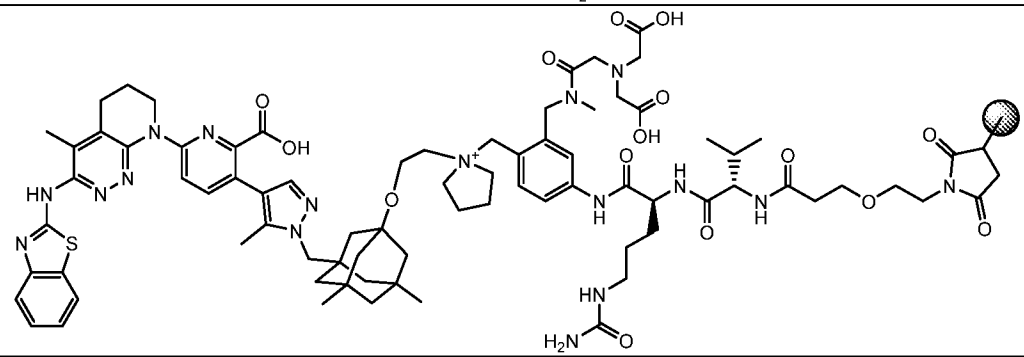
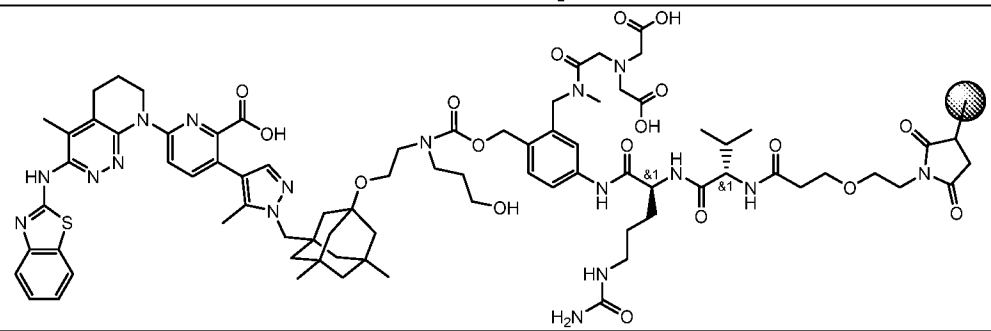
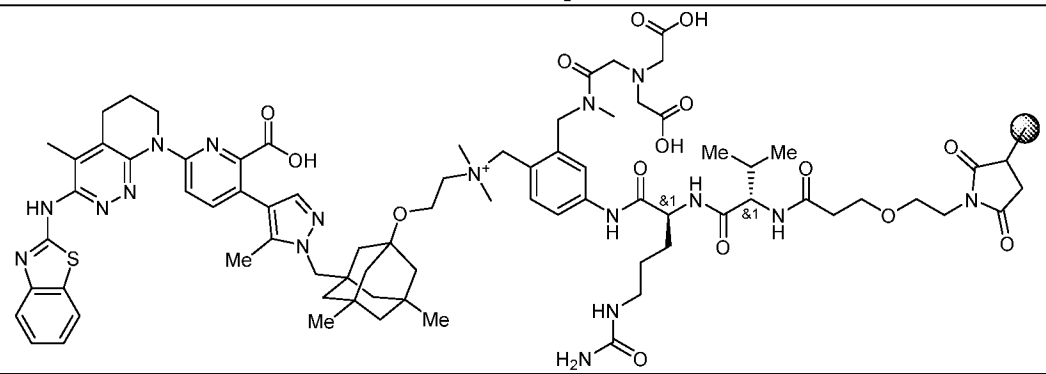
Ab-L67C-P19	
Ab-L67A-P21	
Ab-L67C-P25	
Ab-L67A-P27	

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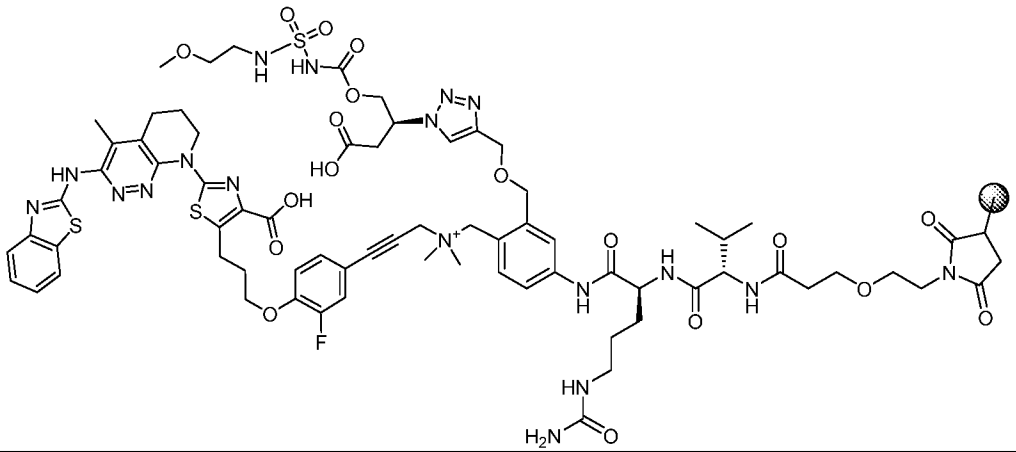
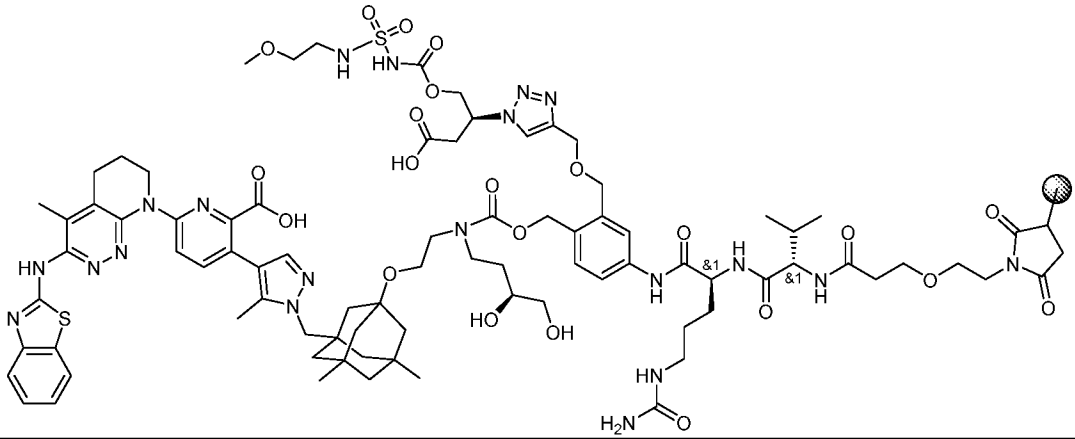
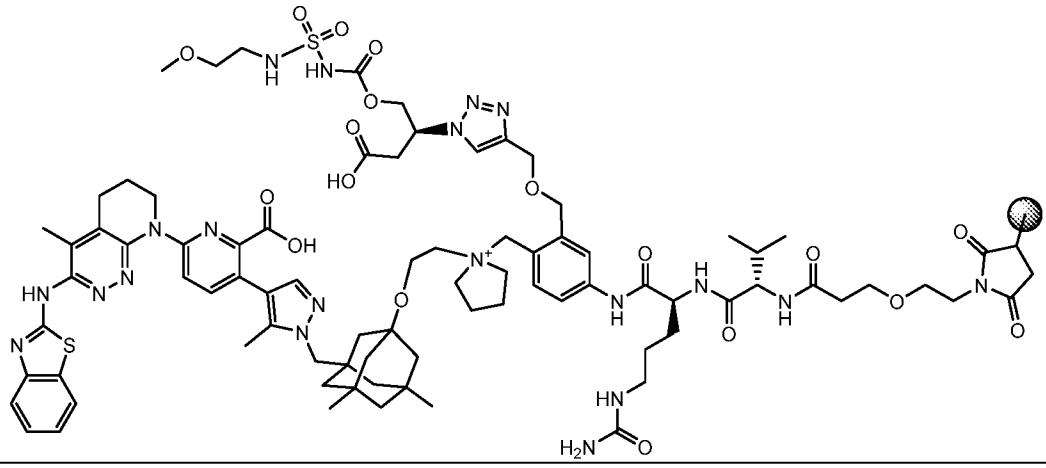
Ab-L100A-P1	
Ab-L100C-P19	
Ab-L100A-P21	

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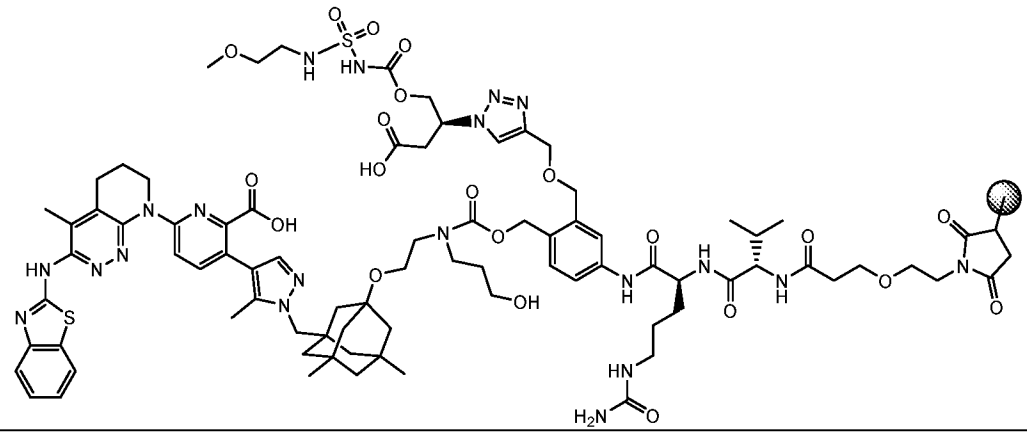
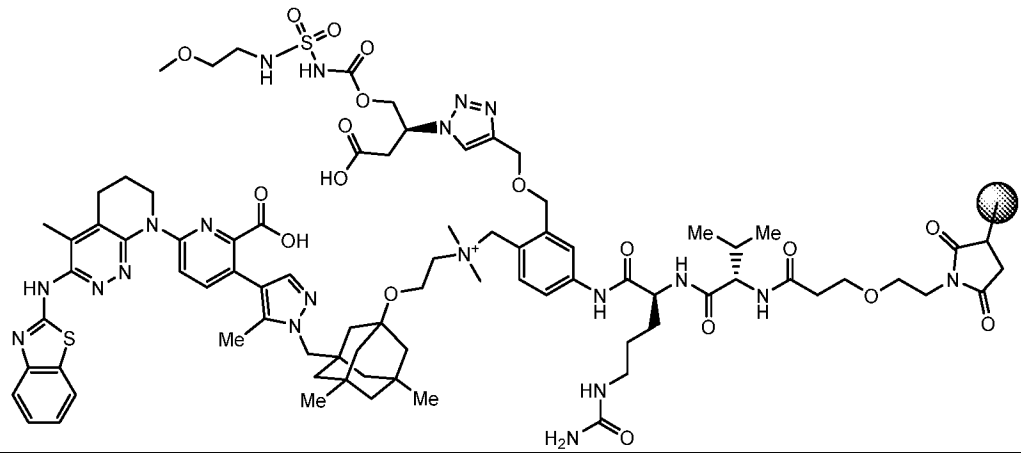
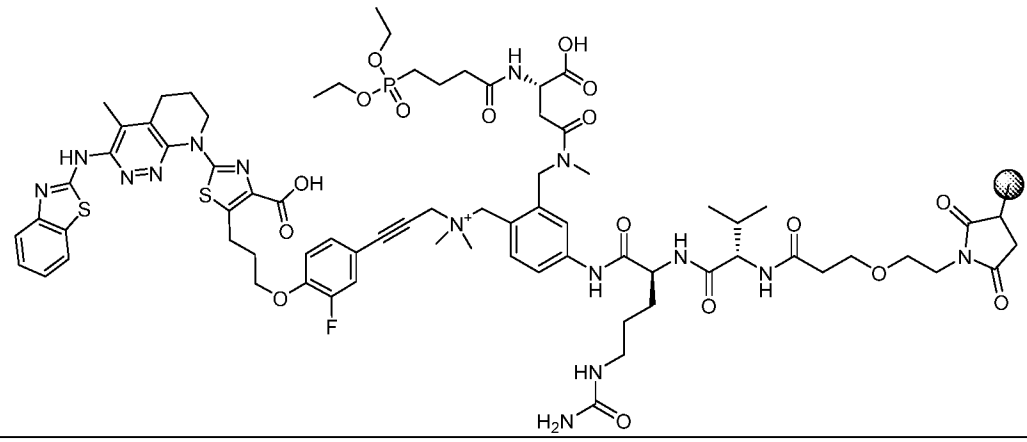
Ab-L100C-P25	
Ab-L100A-P27	
Ab-L103A-P1	

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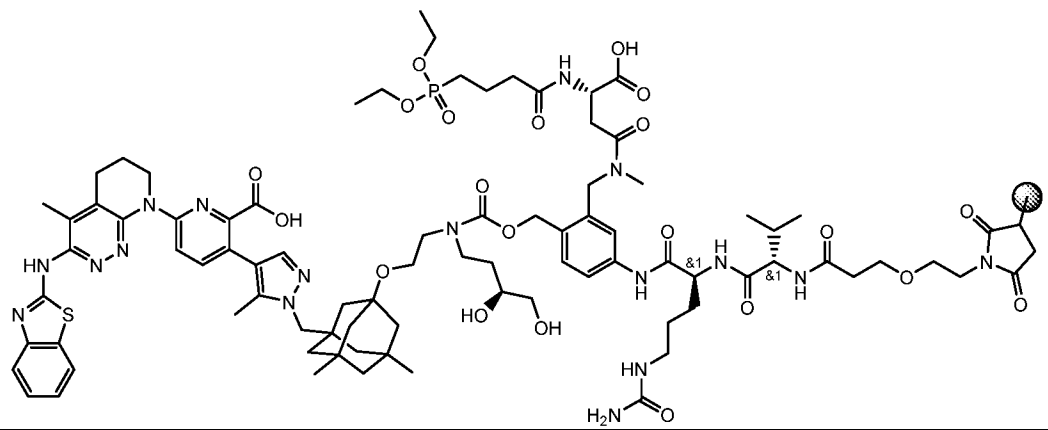
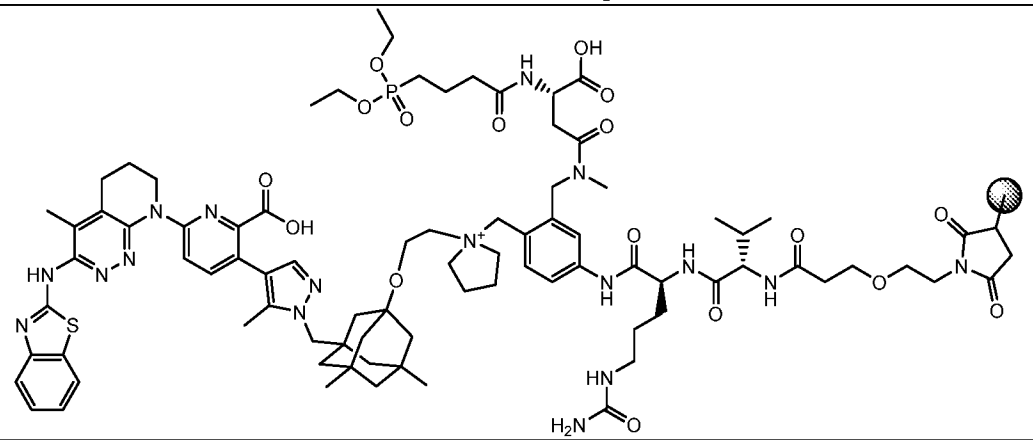
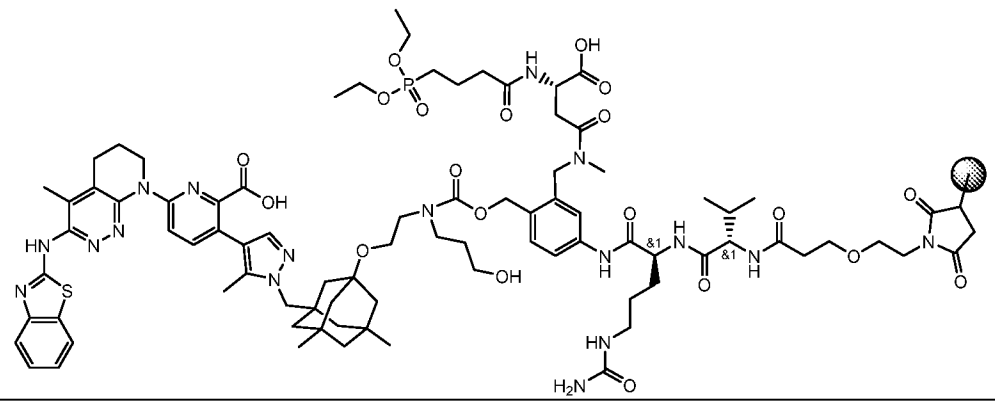
Ab-L103C-P19	
Ab-L103A-P21	
L103C-P25	

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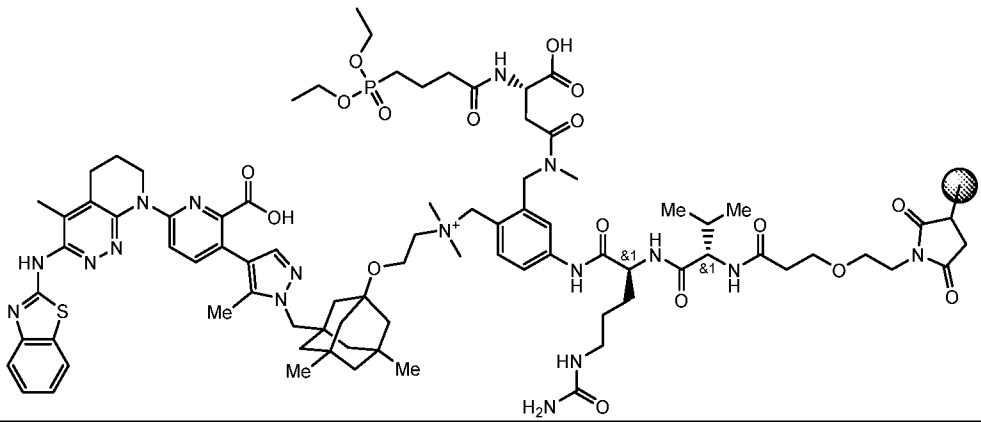
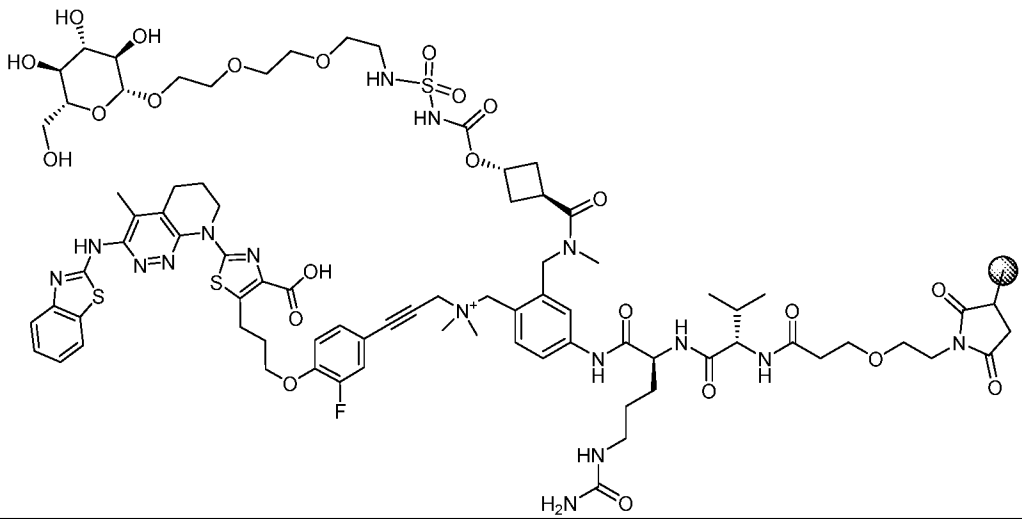
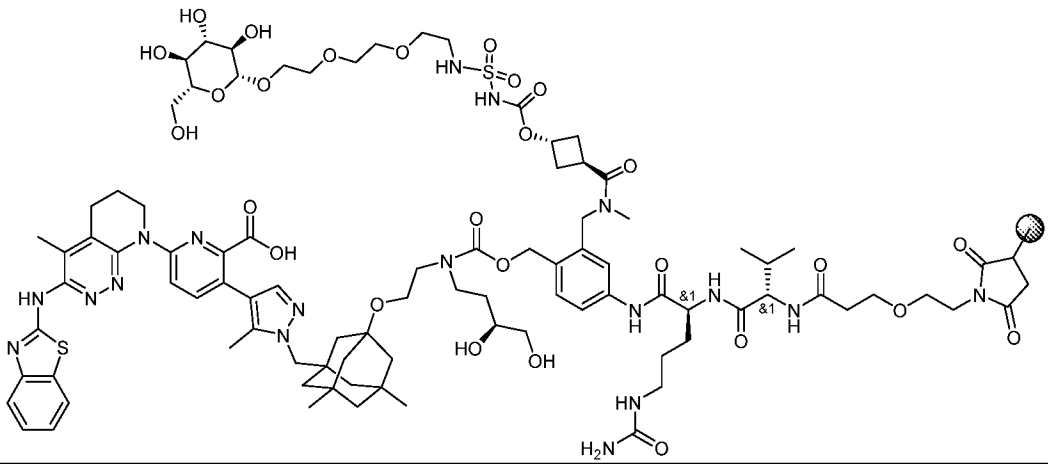
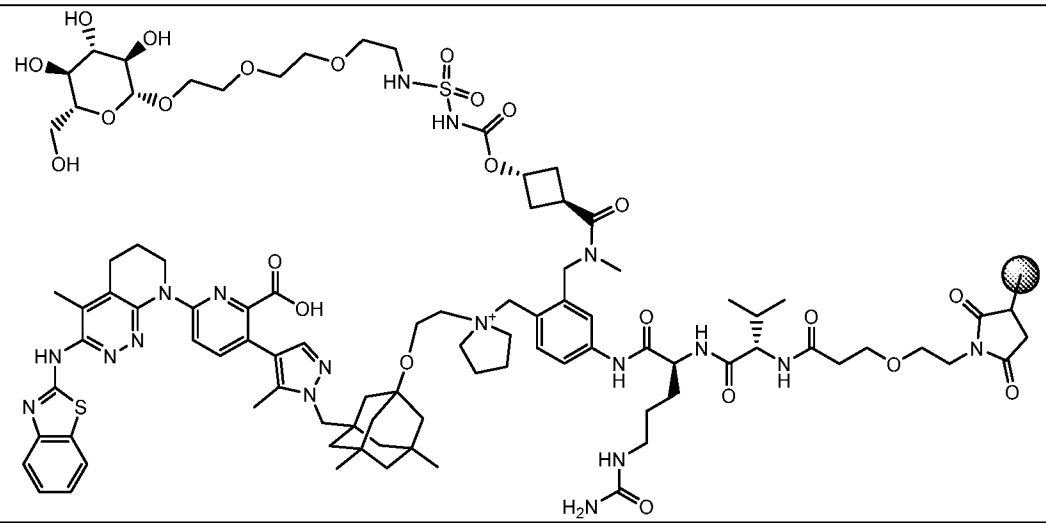
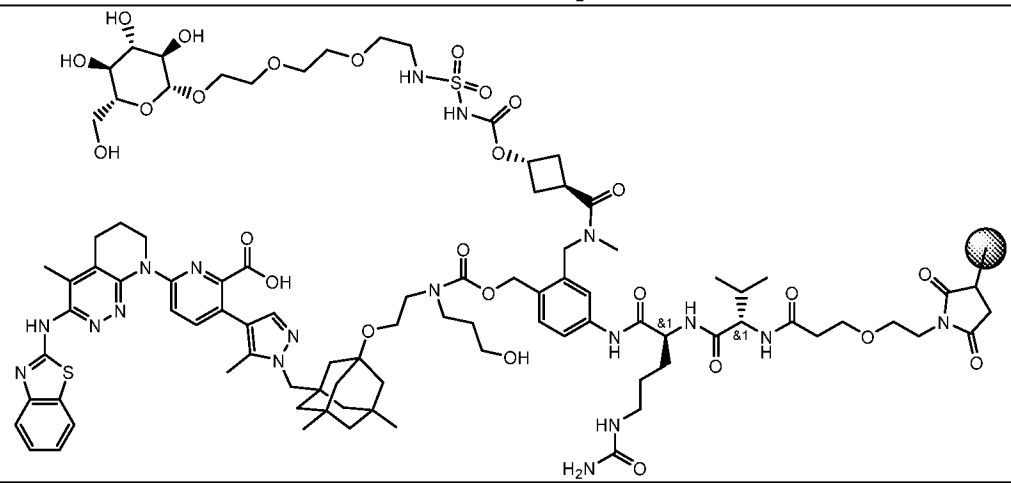
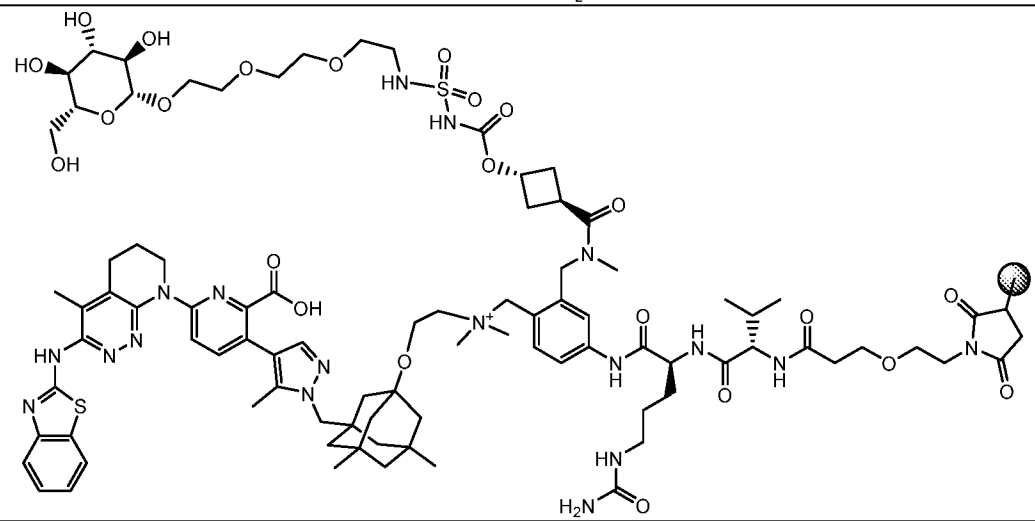
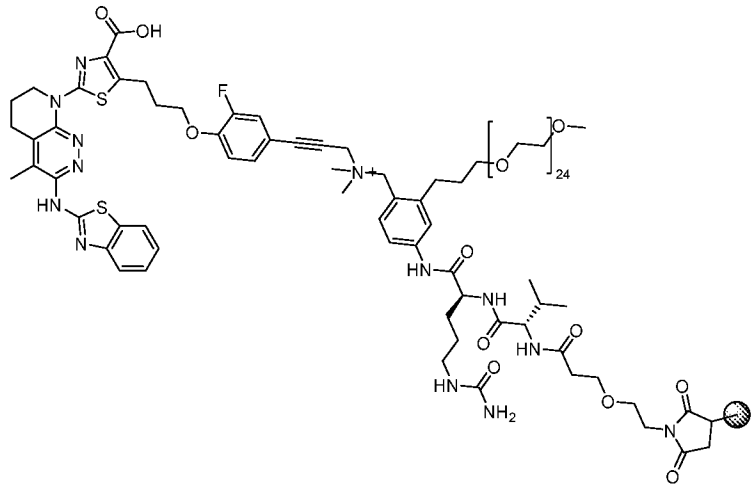
Ab-L103A-P27	
Ab-L111A-P1	
Ab-L111C-P19	

ADC	Structure
Ab-L111A-P21	
Ab-L111C-P25	
Ab-L111A-P27	

ADC	Structure
Ab-L112A-P1	

[97] The ADCs depicted above can also be represented by the following formula:

$$Ab\text{-}(L\text{-}D)_p \ (1),$$

wherein  represents an antibody or an antigen fragment thereof covalently linked to the linker-payload (L/P) depicted above; *p* is an integer from 1 to 16. In some embodiments, *p* is an integer from 1 to 8. In some embodiments, *p* is an integer from 1 to 5. In some embodiments, *p* is an integer from 2 to 4. In some embodiments, *p* is 2. In some embodiments, *p* is 4. In some embodiments, *p* is determined by liquid chromatography-mass spectrometry (LC-MS).

[98] In some embodiments, for ADCs depicted in Table 1, the antibody is an antibody or an antigen fragment thereof described herein. In some embodiments, for ADCs depicted in Table 1, the antibody is an anti-EGFR antibody (*e.g.,* cetuximab or Ab C) . In some embodiments, the antibody is an anti-HER2 antibody (*e.g.*, trastuzumab or Ab T). In other embodiments, the antibody is an anti-CD7 antibody (*e.g.*, Ab D or Ab E). In other embodiments, the antibody is an anti-chicken lysozyme antibody (*e.g.*, Ab F). In some embodiments, the antibody is an anti-CD74 antibody (*e.g.*, Ab G). In some embodiments, the antibody is an anti-CD38 antibody (*e.g.*, Ab H). In some embodiments, the antibody is an anti-CD48 antibody (*e.g.*, Ab I).

[99] As used herein, "L/P" refers to the linker-payloads, linker-drugs, or linker-compounds disclosed herein and the terms "L#-P#" and "L#-C#" are used interchangeably to refer to a specific linker-drug disclosed herein, while the codes "P#" and "C#" are used interchangeably to refer to a specific compound unless otherwise specified. For example, both "L1-C1" and "L1-P1" refer to the same linker-payload structure disclosed herein, while both "C1" and "P1" indicate the same compound disclosed herein, including an enantiomer,

diastereoisomer, atropisomer, deuterated derivative, and/or pharmaceutically acceptable salt of any of the foregoing.

[100] In some embodiments, the antibody or antigen-binding fragment binds to a target antigen on a cancer cell. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2.

[101] In some embodiments, the antibody or antigen-binding fragment are antibodies or antigen-binding fragments disclosed on the internet at go.drugbank.com/drugs/DB00002, in international application publication WO2018/098306, WO2016/179257, WO2011/097627, WO2017/214282, WO2017/214301, WO2017/214233, WO2013/126810, WO2008/056833,

WO2020/236817, WO2017/214335, and WO2012147713, and in U.S. Patent No. US6870034B2, which are incorporated by reference in their entireties.

[102] In some embodiments, the antibody or antigen-binding fragment is an anti-BCMA antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:15 (HCDR1), SEQ ID NO:16 (HCDR2), and SEQ ID NO:17 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:18 (LCDR1), SEQ ID NO:19 (LCDR2), and SEQ ID NO:20 (LCDR3). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:2. In some embodiments, the antibody or antigen-binding fragment comprises an IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at position 152 and position 375. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at position 156 and position 379. In some embodiments, the antibody or antigen-binding fragment comprises an Ig kappa light chain constant domain.

[103] In some embodiments, the antibody or antigen-binding fragment is an anti-CD33 antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:21 (HCDR1), SEQ ID NO:22 (HCDR2), and SEQ ID NO:23 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:24 (LCDR1), SEQ ID NO:25 (LCDR2), and SEQ ID NO:26 (LCDR3). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:3, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises an IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a glutamine residue (Q) at position 297. In some embodiments, the antibody or antigen-binding fragment comprises an Ig kappa light chain constant domain.

[104] In some embodiments, the antibody or antigen-binding fragment is an anti-PCAD antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:33 (HCDR1), SEQ ID NO:34 (HCDR2), and SEQ ID NO:35 (HCDR3); and three light chain complementarity determining

regions (LCDRs) comprising amino acid sequences of SEQ ID NO:36 (LCDR1), SEQ ID NO:37 (LCDR2), and SEQ ID NO:38 (LCDR3). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:7, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:8.

[105] In some embodiments, the antibody or antigen-binding fragment is an anti-HER2 antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:39 (HCDR1), SEQ ID NO:40 (HCDR2), and SEQ ID NO:41 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:42 (LCDR1), SEQ ID NO:43 (LCDR2), and SEQ ID NO:44 (LCDR3). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:10. In some embodiments, the antibody or antigen-binding fragment comprises an IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a glutamine residue (Q) at position 297. In some embodiments, the IgG1 heavy chain constant domain comprises a serine residue (S) at position 297. In some embodiments, the antibody or antigen-binding fragment comprises an Ig kappa light chain constant domain.

[106] In some embodiments, the antibody or antigen-binding fragment is an anti-CD38 antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment is an anti-CD46 antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment is an anti-CD48 antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment is an anti-CD79b antibody or antigen-binding fragment.

[107] Also provided herein, in some embodiments, are compositions comprising multiple copies of an antibody-drug conjugate (e.g., any of the exemplary antibody-drug conjugates described herein). In some embodiments, the average *p* of the antibody-drug conjugates in the composition is from about 2 to about 4.

[108] Also provided herein, in some embodiments, are pharmaceutical compositions comprising an antibody-drug conjugate (e.g., any of the exemplary antibody-drug conjugates described herein) or a composition (e.g., any of the exemplary compositions described herein), and a pharmaceutically acceptable carrier.

[109] Further provided herein, in some embodiments, are therapeutic uses for the described ADC compounds and compositions, e.g., in treating a cancer. In some embodiments, the present disclosure provides methods of treating a cancer (e.g., a cancer

that expresses an antigen targeted by the antibody or antigen-binding fragment of the ADC, such as EGFR, CD7, or HER2). In some embodiments, the present disclosure provides methods of reducing or slowing the expansion of a cancer cell population in a subject. In some embodiments, the present disclosure provides methods of determining whether a subject having or suspected of having a cancer will be responsive to treatment with an ADC compound or composition disclosed herein.

[110] An exemplary embodiment is a method of treating a subject having or suspected of having a cancer, comprising administering to the subject a therapeutically effective amount of an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1,

ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[111] Another exemplary embodiment is a method of reducing or inhibiting the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the tumor expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2,

STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the tumor is a breast cancer, gastric cancer, bladder cancer, brain cancer, cervical cancer, colorectal cancer, esophageal cancer, hepatocellular cancer, melanoma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, or spleen cancer. In some embodiments, the tumor is a gastric cancer. In some embodiments, administration of the antibody-drug conjugate, composition, or pharmaceutical composition reduces or inhibits the growth of the tumor by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%.

[112] Another exemplary embodiment is a method of reducing or slowing the expansion of a cancer cell population in a subject, comprising administering to the subject a therapeutically effective amount of an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the cancer cell population expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human

scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer cell population is from a tumor or a hematological cancer. In some embodiments, the cancer cell population is from a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer cell population is from a lymphoma or gastric cancer. In some embodiments, administration of the antibody-drug conjugate, composition, or pharmaceutical composition reduces the cancer cell population by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%. In some embodiments, administration of the antibody-drug conjugate, composition, or pharmaceutical composition slows the expansion of the cancer cell population by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%.

[113] Another exemplary embodiment is an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein) for use in treating a subject having or suspected of having a cancer. In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56,

DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[114] Another exemplary embodiment is a use of an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein) in treating a subject having or suspected of having a cancer. In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute

lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[115] Another exemplary embodiment is a use of an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein) in a method of manufacturing a medicament for treating a subject having or suspected of having a cancer. In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a

hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[116] Another exemplary embodiment is a method of determining whether a subject having or suspected of having a cancer will be responsive to treatment with an antibody-drug conjugate, composition, or pharmaceutical composition (e.g., any of the exemplary antibody-drug conjugates, compositions, or pharmaceutical compositions disclosed herein) by providing a biological sample from the subject; contacting the sample with the antibody-drug conjugate; and detecting binding of the antibody-drug conjugate to cancer cells in the sample. In some embodiments, the cancer cells in the sample express a target antigen. In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb,

STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer. In some embodiments, the sample is a tissue biopsy sample, a blood sample, or a bone marrow sample.

[117] Methods of producing the described ADC compounds and compositions are also disclosed. An exemplary embodiment is a method of producing an antibody-drug conjugate by reacting an antibody or antigen-binding fragment with a cleavable linker joined or covalently attached to a Bcl-xL inhibitor under conditions that allow conjugation.

BRIEF DESCRIPTION OF THE DRAWINGS

[118] FIG. 1 shows an exemplary site-specific cysteine conjugation.

[119] FIG. 2 shows an exemplary site-specific antibody conjugation using bacterial transglutaminase (BTG).

[120] FIG. 3 shows *in vitro* activity of anti-CD7-Bcl-xL ADCs and payloads in ALL-SIL cell line (CTG 72h).

[121] FIG. 4 shows *in vitro* activity of anti-CD7-BclxL ADCs and payloads in DND-41 cell line (CTG 72h).

[122] FIG. 5A and FIG. 5B show *in vitro* activity of anti-EGFR-BclxL ADCs and payloads in H1650 cell line (3D, CTG 120h).

[123] FIG. 6 shows the effects of anti-HER2-Bcl-xL ADCs as single agent and in combination with paclitaxel in HCC1569 cell viability using CTG assay.

[124] FIGs. 7A, 7B, 7C and 7D show *in vitro* activity of Bcl-xLi payloads and anti-CD7-, anti-CD38-, anti-CD48- and anti-chLys- Bcl-xLi ADCs at single agent or in combination with Vincristine or BCL2 inhibitors in HPB-ALL cell line.

[125] FIG. 8A and FIG. 8B show *in vitro* combination activity of EGFR-AbA-L109A-P1 with inhibitors of the MAP Kinase pathway in various cancer cell lines.

[126] FIG. 9 shows tumor growth inhibition for EGFR2 CysMab DANAPA-L109A-P1 ADC in combination with docetaxel against the H1650 human non-small cell lung carcinoma (NSCLC) model in mice.

[127] FIG. 10 shows body weight change for EGFR2 CysMab DANAPA-L109A-P1 ADC in combination with docetaxel against the H1650 human non-small cell lung carcinoma (NSCLC) model in mice.

[128] FIG. 11 shows the antitumor effect of EGFR-Bcl-xLi-ADCs with different linker payloads in combination with docetaxel. Values are mean ± SEM; sample size, (n=7 mice per group). Statistical analysis on day 21 (vs vehicle control group) and on days 21 and 46 (vs EGFR-L109A-P1) was performed using one way ANOVA post hoc Tukey's multiple comparisons test; Indigo InLife Results analysis in TIBCO Spotfire. #: $p<0.05$.

[129] FIG. 12 shows body weight changes following treatment with EGFR-Bcl-xLi-ADCs with different linker payloads in combination with docetaxel. Changes in body weight (%) represent the ratio between body weight at the evaluation day and body weight at day 0 expressed in percentage for each individual animals. Values are mean ± SEM; sample size, (n=7 mice per group). Statistical analysis on day 21 (vs vehicle control group) and on days 21 and 46 (vs EGFR-L109A-P1) was performed using one way ANOVA post hoc Tukey's multiple comparisons test; Indigo InLife Results analysis in TIBCO Spotfire. #: $p<0.05$.

[130] FIG. 13 shows EBC-1 Growth kinetics of EpCAM-DANAPA-L11C-P25 ADC, 3207-DANAPA-L11C-P25 isotype control ADC, and EpCAM-DANAPA CysMab control antibody in combination with paclitaxel.

[131] FIG. 14 shows body weight changes following treatment with EpCAM-DANAPA-L11C-P25 ADC, 3207-DANAPA-L11C-P25 isotype control ADC, and EpCAM-DANAPA CysMab control antibody in combination with paclitaxel.

[132] FIG. 15 shows tumor volume (mm^3) of ALL-SIL-grafted female NSG mice upon treatment with IgG1 DANAPA-L9A-P21, Ab D DANAPA_L9A-P1, Ab D DANAPA_L9A-P21, Ab D DANAPA_L9C-P25, Ab D DANAPA_L9A-P33 and Ab D DANAPA_L9C-P40 (2.5 and/or 7.5 mg/kg, administered once IV, n=6).

[133] FIG. 16 shows body weight of ALL-SIL-grafted female NSG mice upon treatment with IgG1 DANAPA-L9A-P21, Ab D DANAPA_L9A-P1, Ab D DANAPA_L9A-P21, Ab D DANAPA_L9C-P25, Ab D DANAPA_L9A-P33 and Ab D DANAPA_L9C-P40 (2.5 and/or 7.5 mg/kg, administered once IV, n=6).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[134] The disclosed compositions and methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure.

[135] Throughout this text, the descriptions refer to compositions and methods of using the compositions. Where the disclosure describes or claims a feature or embodiment associated with a composition, such a feature or embodiment is equally applicable to the methods of using the composition. Likewise, where the disclosure describes or claims a feature or embodiment associated with a method of using a composition, such a feature or embodiment is equally applicable to the composition.

[136] When a range of values is expressed, it includes embodiments using any particular value within the range. Further, reference to values stated in ranges includes each and every value within that range. All ranges are inclusive of their endpoints and combinable. When values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. Reference to a particular numerical value includes at least that particular value, unless the context clearly dictates otherwise. The use of "or" will mean "and/or" unless the specific context of its use dictates otherwise. All references cited herein are incorporated by reference for any purpose. Where a reference and the specification conflict, the specification will control.

[137] Unless the context of a description indicates otherwise, e.g., in the absence of symbols indicating specific point(s) of connectivity, when a structure or fragment of a structure is drawn, it may be used on its own or attached to other components of an ADC, and it may do so with any orientation, e.g., with the antibody attached at any suitable attachment point to a chemical moiety such as a linker-drug. Where indicated, however, components of an ADC are attached in the orientation shown in a given formula. For example, if Formula (1) is described as Ab-(L-D)$_p$ and the group "-(L-D)" is described as $-\left(R^1-L_1-E-D\right)$, then the elaborated structure of Formula (1) is $Ab-\left(R^1-L_1-E-D\right)_p$. It is not $Ab-\left(D-E-L_1-R_1\right)_p$.

[138] It is to be appreciated that certain features of the disclosed compositions and methods, which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed compositions and methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any sub-combination.

[139] As used throughout this application, antibody drug conjugates can be identified using a naming convention in the general format of "target antigen/antibody-linker-payload". For example only, if an antibody drug conjugate is referred to as "Target X-L0-P0", such a conjugate would comprise an antibody that binds Target X, a linker designated as L0, and a payload designated as P0. Alternatively, if an antibody drug conjugate is referred to as "anti-Target X-L0-P0", such a conjugate would comprise an antibody that binds Target X, a linker designated as L0, and a payload designated as P0. In another alternative, if an antibody drug conjugate is referred to as "AbX-L0-P0", such a conjugate would comprise the antibody designated as AbX, a linker designated as L0, and a payload designated as P0. A control antibody drug conjugate comprising a non-specific, isotype control antibody may be referenced as "isotype control IgG1-L0-P0" or "IgG1-L0-P0".

[140] Any formula given herein is also intended to represent unlabeled forms as well as isotopically labeled forms of the compounds. Isotopically labeled compounds have structures depicted by the formulae given herein except that one or more atoms are replaced by an atom having a selected atomic mass or mass number. Isotopes that can be incorporated into compounds of the invention include, for example, isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine, and chlorine, such as ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}F, and ^{36}Cl. Accordingly, it should be understood that the present disclosure includes compounds that incorporate one or more of any of the aforementioned isotopes, including for example, radioactive isotopes, such as ^{3}H and ^{14}C, or those into which non-radioactive isotopes, such as ^{2}H and ^{13}C are present. Such isotopically labelled compounds are useful in metabolic studies (with ^{14}C), reaction kinetic studies (with, for example ^{2}H or ^{3}H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. In particular, an ^{18}F or labeled compound may be particularly desirable for PET or SPECT studies. Isotopically-labeled compounds can generally be prepared by conventional techniques known to those skilled in the art, e.g., using an appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

Definitions

[141] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[142] As used herein, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise. The terms "comprising", "having", "being of" as in "being of a chemical formula", "including", and "containing" are to be construed as open terms (i.e., meaning "including but not limited to") unless otherwise noted. Additionally whenever

"comprising" or another open-ended term is used in an embodiment, it is to be understood that the same embodiment can be more narrowly claimed using the intermediate term "consisting essentially of" or the closed term "consisting of".

[143] The term "about" or "approximately," when used in the context of numerical values and ranges, refers to values or ranges that approximate or are close to the recited values or ranges such that the embodiment may perform as intended, as is apparent to the skilled person from the teachings contained herein. In some embodiments, about means plus or minus 20%, 15%, 10%, 5%, 1%, 0.5%, or 0.1% of a numerical amount. In one embodiment, the term "about" refers to a range of values which are 10% more or less than the specified value. In another embodiment, the term "about" refers to a range of values which are 5% more or less than the specified value. In another embodiment, the term "about" refers to a range of values which are 1% more or less than the specified value.

[144] The terms "antibody-drug conjugate," "antibody conjugate," "conjugate," "immunoconjugate," and "ADC" are used interchangeably, and refer to one or more therapeutic compounds (e.g., a Bcl-xL inhibitor) that is linked to one or more antibodies or antigen-binding fragments. In some embodiments, the ADC is defined by the generic formula: Ab-(L-D)$_p$ (Formula 1), wherein Ab = an antibody or antigen-binding fragment, L = a linker moiety, D = a drug moiety (e.g., a Bcl-xL inhibitor drug moiety), and p = the number of drug moieties per antibody or antigen-binding fragment. In ADCs comprising a Bcl-xL inhibitor drug moiety, "p" refers to the number of Bcl-xL inhibitor compounds linked to the antibody or antigen-binding fragment.

[145] The term "antibody" is used in the broadest sense to refer to an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. An antibody can be polyclonal or monoclonal, multiple or single chain, or an intact immunoglobulin, and may be derived from natural sources or from recombinant sources. An "intact" antibody is a glycoprotein that typically comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxyl-terminus in the following order: FR1,

CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. An antibody can be a monoclonal antibody, human antibody, humanized antibody, camelised antibody, or chimeric antibody. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass. An antibody can be an intact antibody or an antigen-binding fragment thereof.

[146] In some embodiments, the antibody or antibody fragment disclosed herein include modified or engineered amino acid residues, e.g., one or more cysteine residues, as sites for conjugation to a drug moiety (Junutula JR, et al., Nat Biotechnol 2008, 26:925-932). In one embodiment, the disclosure provides a modified antibody or antibody fragment comprising a substitution of one or more amino acids with cysteine at the positions described herein. Sites for cysteine substitution are in the constant regions of the antibody or antibody fragment and are thus applicable to a variety of antibody or antibody fragment, and the sites are selected to provide stable and homogeneous conjugates. A modified antibody or fragment can have one, two or more cysteine substitutions, and these substitutions can be used in combination with other modification and conjugation methods as described herein. Methods for inserting cysteine at specific locations of an antibody are known in the art, see, e.g., Lyons et al., (1990) Protein Eng., 3:703-708, WO 2011/005481, WO2014/124316, WO 2015/138615. In certain embodiments, a modified antibody comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 191, 195, 197, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400 and 422 of a heavy chain of the antibody, and wherein the positions are numbered according to the EU system. In some embodiments a modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 107, 108, 109, 114, 129, 142, 143, 145, 152, 154, 156, 159, 161, 165, 168, 169, 170, 182, 183, 197, 199, and 203 of a light chain of the antibody or antibody fragment, wherein the positions are numbered according to the EU system, and wherein the light chain is a human kappa light chain. In certain embodiments a modified antibody or antibody fragment thereof comprises a combination of substitution of two or more amino acids with cysteine on its constant regions wherein the combinations comprise substitutions at positions 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, or position 107 of an antibody light chain and wherein the positions are numbered according to the EU system.

In certain embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine on its constant regions wherein the substitution is position 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, position 107 of an antibody light chain, position 165 of an antibody light chain or position 159 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain. In particular embodiments a modified antibody or antibody fragment thereof comprises a combination of substitution of two amino acids with cysteine on its constant regions wherein the combinations comprise substitutions at positions 375 of an antibody heavy chain and position 152 of an antibody heavy chain, wherein the positions are numbered according to the EU system. In particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 360 of an antibody heavy chain, wherein the positions are numbered according to the EU system. In other particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 107 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain.

[147] The term “antibody fragment” or “antigen-binding fragment” or “functional antibody fragment,” as used herein, refers to at least one portion of an antibody that retains the ability to specifically interact with (e.g., by binding, steric hinderance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen (e.g., EGFR, CD7, or HER2). Antigen-binding fragments may also retain the ability to internalize into an antigen-expressing cell. In some embodiments, antigen-binding fragments also retain immune effector activity. The terms antibody, antibody fragment, antigen-binding fragment, and the like, are intended to embrace the use of binding domains from antibodies in the context of larger macromolecules such as ADCs. It has been shown that fragments of a full-length antibody can perform the antigen binding function of a full-length antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab’, F(ab’)2, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen-binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, bispecific or multi-specific antibody constructs, ADCs, v-NAR and bis-scFv (see, e.g., Holliger and Hudson (2005) Nat Biotechnol. 23(9):1126-36). Antigen-binding fragments can also be grafted into scaffolds

based on polypeptides such as a fibronectin type III (Fn3) (see US Patent No. 6,703,199, which describes fibronectin polypeptide minibodies). The term "scFv" refers to a fusion protein comprising at least one antigen-binding fragment comprising a variable region of a light chain and at least one antigen-binding fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked, e.g., via a synthetic linker, e.g., a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL. Antigen-binding fragments are obtained using conventional techniques known to those of skill in the art, and the binding fragments are screened for utility (e.g., binding affinity, internalization) in the same manner as are intact antibodies. Antigen-binding fragments, for example, may be prepared by cleavage of the intact protein, e.g., by protease or chemical cleavage.

[148] The term "complementarity determining region" or "CDR," as used herein, refers to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991) "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme); Al-Lazikani et al. (1997) J Mol Biol. 273(4):927-48 ("Chothia" numbering scheme); ImMunoGenTics (IMGT) numbering (Lefranc (2001) Nucleic Acids Res. 29(1):207-9; Lefranc et al. (2003) Dev Comp Immunol. 27(1):55-77) ("IMGT" numbering scheme); or a combination thereof. In a combined Kabat and Chothia numbering scheme for a given CDR region (for example, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, or LC CDR3), in some embodiments, the CDRs correspond to the amino acid residues that are defined as part of the Kabat CDR, together with the amino acid residues that are defined as part of the Chothia CDR. As used herein, the CDRs defined according to the "Chothia" number scheme are also sometimes referred to as "hypervariable loops."

[149] In some embodiments, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1) (e.g., insertion(s) after position 35), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1) (e.g., insertion(s) after position 27), 50-56 (LCDR2), and 89-97 (LCDR3). In some embodiments, under Chothia, the CDR amino

acids in the VH are numbered 26-32 (HCDR1) (e.g., insertion(s) after position 31), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1) (e.g., insertion(s) after position 30), 50-52 (LCDR2), and 91-96 (LCDR3). By combining the CDR definitions of both Kabat and Chothia, in some embodiments, the CDRs comprise or consist of, e.g., amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in human VH and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in human VL. In some embodiments, under IMGT, the CDR amino acid residues in the VH are numbered approximately 26-35 (CDR1), 51-57 (CDR2) and 93-102 (CDR3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (CDR1), 50-52 (CDR2), and 89-97 (CDR3). In some embodiments, under IMGT, the CDR regions of an antibody may be determined using the program IMGT/DomainGap Align.

[150] The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler et al. (1975) Nature 256:495, or may be made by recombinant DNA methods (see, e.g., US Patent No. 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al. (1991) Nature 352:624-8, and Marks et al. (1991) J Mol Biol. 222:581-97, for example. The term also includes preparations of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[151] The monoclonal antibodies described herein can be non-human, human, or humanized. The term specifically includes "chimeric" antibodies, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they specifically bind the target antigen and/or exhibit the desired biological activity.

[152] The term "human antibody," as used herein, refers an antibody produced by a human or an antibody having an amino acid sequence of an antibody produced by a human. The term includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region is also derived from such human sequences, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik et al. ((2000) J Mol Biol. 296(1):57-86). The structures and locations of immunoglobulin variable domains, e.g., CDRs, may be defined using well known numbering schemes, e.g., the Kabat numbering scheme, the Chothia numbering scheme, or a combination of Kabat and Chothia, and/or ImMunoGenTics (IMGT) numbering. The human antibodies of the invention may include amino acid residues not encoded by human sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[153] The term "recombinant human antibody," as used herein, refers to a human antibody that is prepared, expressed, created, or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In some embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[154] The term "chimeric antibody," as used herein, refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. In some instances, the variable regions of both heavy and light chains correspond to the variable regions of antibodies derived from one species with the desired specificity, affinity,

and activity while the constant regions are homologous to antibodies derived from another species (e.g., human) to minimize an immune response in the latter species.

[155] As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies are a type of chimeric antibody which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The humanized antibody can be further modified by the substitution of residues, either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or activity.

[156] The term “Fc region,” as used herein, refers to a polypeptide comprising the CH3, CH2 and at least a portion of the hinge region of a constant domain of an antibody. Optionally, an Fc region may include a CH4 domain, present in some antibody classes. An Fc region may comprise the entire hinge region of a constant domain of an antibody. In some embodiments, an antibody or antigen-binding fragment comprises an Fc region and a CH1 region of an antibody. In some embodiments, an antibody or antigen-binding fragment comprises an Fc region CH3 region of an antibody. In some embodiments, an antibody or antigen-binding fragment comprises an Fc region, a CH1 region, and a kappa/lambda region from the constant domain of an antibody. In some embodiments, an antibody or antigen-binding fragment comprises a constant region, e.g., a heavy chain constant region and/or a light chain constant region. In some embodiments, such a constant region is modified compared to a wild-type constant region. That is, the polypeptide may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2, or CH3) and/or to the light chain constant region domain (CL). Example modifications include additions, deletions, or substitutions of one or more amino acids in one or more domains. Such changes may be included to optimize effector function, half-life, etc.

[157] “Internalizing” as used herein in reference to an antibody or antigen-binding fragment refers to an antibody or antigen-binding fragment that is capable of being taken through the cell’s lipid bilayer membrane to an internal compartment (i.e., “internalized”) upon binding to the cell, preferably into a degradative compartment in the cell. For example, an internalizing anti-HER2 antibody is one that is capable of being taken into the cell after binding to HER2 on the cell membrane. In some embodiments, the antibody or antigen-binding fragment used in the ADCs disclosed herein targets a cell surface antigen (e.g.,

EGFR, CD7, or HER2) and is an internalizing antibody or internalizing antigen-binding fragment (i.e., the ADC transfers through the cellular membrane after antigen binding). In some embodiments, the internalizing antibody or antigen-binding fragment binds a receptor on the cell surface. An internalizing antibody or internalizing antigen-binding fragment that targets a receptor on the cell membrane may induce receptor-mediated endocytosis. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment is taken into the cell via receptor-mediated endocytosis.

[158] "Non-internalizing" as used herein in reference to an antibody or antigen-binding fragment refers to an antibody or antigen-binding fragment that remains at the cell surface upon binding to the cell. In some embodiments, the antibody or antigen-binding fragment used in the ADCs disclosed herein targets a cell surface antigen and is a non-internalizing antibody or non-internalizing antigen-binding fragment (i.e., the ADC remains at the cell surface and does not transfer through the cellular membrane after antigen binding). In some embodiments, the non-internalizing antibody or antigen-binding fragment binds a non-internalizing receptor or other cell surface antigen. Exemplary non-internalizing cell surface antigens include but are not limited to CA125 and CEA, and antibodies that bind to non-internalizing antigen targets are also known in the art (see, e.g., Bast et al. (1981) J Clin Invest. 68(5):1331-7; Scholler and Urban (2007) Biomark Med. 1(4):513-23; and Boudousq et al. (2013) PLoS One 8(7):e69613).

[159] The term "B-cell maturation antigen" or "BCMA," as used herein, refers to any native form of human BCMA (also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17)). The term encompasses full-length human BCMA (e.g., UniProt Reference Sequence: Q02223; SEQ ID NO:72), as well as any form of human BCMA that may result from cellular processing. The term also encompasses functional variants or fragments of human BCMA, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human BCMA (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). BCMA can be isolated from human, or may be produced recombinantly or by synthetic methods.

[160] The term "anti-BCMA antibody" or "antibody that binds to BCMA," as used herein, refers to any form of antibody or antigen-binding fragment thereof that binds, e.g., specifically binds, to BCMA. The term encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, and biologically functional antigen-binding fragments so long as they bind, e.g., specifically bind, to BCMA. WO 2012/163805 provides and is incorporated herein by reference for exemplary BCMA-binding sequences, including exemplary anti-BCMA antibody sequences. In some embodiments, the anti-BCMA

antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antigen-binding fragment. J6M0 (WO 2012/163805) is an exemplary anti-BCMA antibody.

[161] The term "myeloid cell surface antigen CD33" or "CD33," as used herein, refers to any native form of human CD33 (also known as sialic acid binding Ig-like lectin 3 (SIGLEC3)). The term encompasses full-length human CD33 (e.g., UniProt Reference Sequence: P20138; SEQ ID NO:73), as well as any form of human CD33 that may result from cellular processing. The term also encompasses functional variants or fragments of human CD33, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human CD33 (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). CD33 can be isolated from human, or may be produced recombinantly or by synthetic methods.

[162] The term "anti-CD33 antibody" or "antibody that binds to CD33," as used herein, refers to any form of antibody or antigen-binding fragment thereof that binds, e.g., specifically binds, to CD33. The term encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, and biologically functional antigen-binding fragments so long as they bind, e.g., specifically bind, to CD33. US 2013/0078241 provides and is incorporated herein by reference for exemplary CD33-binding sequences, including exemplary anti-CD33 antibody sequences. In some embodiments, the anti-CD33 antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antigen-binding fragment. MuMy9-6ch (US 2013/0078241) is an exemplary anti-CD33 antibody.

[163] The term "P-cadherin" or "PCAD," as used herein, refers to any native form of human PCAD (also known as cadherin 3, type 1 or CDH3). The term encompasses full-length human PCAD (e.g., UniProt Reference Sequence: P22223; SEQ ID NO:74), as well as any form of human PCAD that may result from cellular processing. The term also encompasses functional variants or fragments of human PCAD, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human PCAD (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). PCAD can be isolated from human, or may be produced recombinantly or by synthetic methods.

[164] The term "anti-PCAD antibody" or "antibody that binds to PCAD," as used herein, refers to any form of antibody or antigen-binding fragment thereof that binds, e.g., specifically binds, to PCAD. The term encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, and biologically functional antigen-binding fragments so long as they bind, e.g., specifically bind, to PCAD. WO 2016/203432 provides and is incorporated herein by reference for exemplary PCAD-binding sequences,

including exemplary anti-PCAD antibody sequences. In some embodiments, the anti-PCAD antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antigen-binding fragment. NOV169N31Q (WO 2016/203432) is an exemplary anti-PCAD antibody.

[165] The term "human epidermal growth factor receptor 2," "HER2," or "HER2/NEU," as used herein, refers to any native form of human HER2. The term encompasses full-length human HER2 (e.g., UniProt Reference Sequence: P04626; SEQ ID NO:75), as well as any form of human HER2 that may result from cellular processing. The term also encompasses functional variants or fragments of human HER2, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human HER2 (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). HER2 can be isolated from human, or may be produced recombinantly or by synthetic methods.

[166] The term "anti-HER2 antibody" or "antibody that binds to HER2," as used herein, refers to any form of antibody or antigen-binding fragment thereof that binds, e.g., specifically binds, to HER2. The term encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, and biologically functional antigen-binding fragments so long as they bind, e.g., specifically bind, to HER2. US Patent Nos. 5,821,337 and 6,870,034 provide and are incorporated herein by reference for exemplary HER2-binding sequences, including exemplary anti-HER2 antibody sequences. In some embodiments, the anti-HER2 antibody used in the ADCs disclosed herein is an internalizing antibody or internalizing antigen-binding fragment. Trastuzumab (US Patent Nos. 5,821,337 and 6,870,034; see also Molina et al. (2001) Cancer Res. 61(12):4744-9) is an exemplary anti-HER2 antibody.

[167] The term "cluster of differentiation 38" or "CD38," as used herein, refers to any native form of human CD38 (also known as ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase). The term encompasses full-length human CD38 (e.g., UniProt Reference Sequence: P28907; SEQ ID NO:76), as well as any form of human CD38 that may result from cellular processing. The term also encompasses functional variants or fragments of human CD38, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human CD38 (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). CD38 can be isolated from human, or may be produced recombinantly or by synthetic methods.

[168] The term "cluster of differentiation 48" or "CD48," as used herein, refers to any native form of human CD48 (also known as B-lymphocyte activation marker (BLAST-1) or signaling lymphocytic activation molecule 2 (SLAMF2)). The term encompasses full-length human

CD48 (e.g., UniProt Reference Sequence: P09326; SEQ ID NO:77), as well as any form of human CD48 that may result from cellular processing. The term also encompasses functional variants or fragments of human CD48, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human CD48 (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). CD48 can be isolated from human, or may be produced recombinantly or by synthetic methods.

[169] The term “cluster of differentiation 79b” or “CD79b,” as used herein, refers to any native form of human CD79b (also known as B-cell antigen receptor complex-associated protein beta chain). The term encompasses full-length human CD79b (e.g., UniProt Reference Sequence: P40259; SEQ ID NO:78), as well as any form of human CD79b that may result from cellular processing. The term also encompasses functional variants or fragments of human CD79b, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human CD79b (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). CD79b can be isolated from human, or may be produced recombinantly or by synthetic methods.

[170] The term “binding specificity,” as used herein, refers to the ability of an individual antibody or antigen binding fragment to preferentially react with one antigenic determinant over a different antigenic determinant. The degree of specificity indicates the extent to which an antibody or fragment preferentially binds to one antigenic determinant over a different antigenic determinant. Also, as used herein, the term "specific," "specifically binds," and "binds specifically" refers to a binding reaction between an antibody or antigen-binding fragment (e.g., an anti-HER2 antibody) and a target antigen (e.g., HER2) in a heterogeneous population of proteins and other biologics. Antibodies can be tested for specificity of binding by comparing binding to an appropriate antigen to binding to an irrelevant antigen or antigen mixture under a given set of conditions. If the antibody binds to the appropriate antigen with at least 2, 5, 7, 10 or more times more affinity than to the irrelevant antigen or antigen mixture, then it is considered to be specific. A “specific antibody” or a “target-specific antibody” is one that only binds the target antigen (e.g., EGFR, CD7, or HER2), but does not bind (or exhibits minimal binding) to other antigens. In some embodiments, an antibody or antigen-binding fragment that specifically binds a target antigen (e.g., EGFR, CD7, or HER2) has a K_D of less than $1x10^{-6}$ M, less than $1x10^{-7}$ M, less than $1x10^{-8}$ M, less than $1x10^{-9}$ M, less than $1x10^{-10}$ M, less than $1x10^{-11}$ M, less than $1x10^{-12}$ M, or less than $1x10^{-13}$ M. In some embodiments, the K_D is 1 pM to 500 pM. In some embodiments, the K_D is between 500 pM to 1 µM, 1 µM to 100 nM, or 100 mM to 10 nM.

[171] The term “affinity,” as used herein, refers to the strength of interaction between

antibody and antigen at single antigenic sites. Without being bound by theory, within each antigen binding site, the variable region of the antibody "arm" interacts through weak non-covalent forces with the antigen at numerous sites; the more interactions, typically the stronger the affinity. The binding affinity of an antibody is the sum of the attractive and repulsive forces operating between the antigenic determinant and the binding site of the antibody.

[172] The term "k_{on}" or "k_a" refers to the on-rate constant for association of an antibody to the antigen to form the antibody/antigen complex. The rate can be determined using standard assays, such as a surface plasmon resonance, biolayer inferometry, or ELISA assay.

[173] The term "k_{off}" or "k_d" refers to the off-rate constant for dissociation of an antibody from the antibody/antigen complex. The rate can be determined using standard assays, such as a surface plasmon resonance, biolayer inferometry, or ELISA assay.

[174] The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction. K_D is calculated by k_a/k_d. The rate can be determined using standard assays, such as a surface plasmon resonance, biolayer inferometry, or ELISA assay.

[175] The term "epitope" refers to the portion of an antigen capable of being recognized and specifically bound by an antibody (or antigen-binding fragment). Epitope determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. When the antigen is a polypeptide, epitopes can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of the polypeptide. An epitope may be "linear" or "conformational." Conformational and linear epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. The epitope bound by an antibody (or antigen-binding fragment) may be identified using any epitope mapping technique known in the art, including X-ray crystallography for epitope identification by direct visualization of the antigen-antibody complex, as well as monitoring the binding of the antibody to fragments or mutated variations of the antigen, or monitoring solvent accessibility of different parts of the antibody and the antigen. Exemplary strategies used to map antibody epitopes include, but are not limited to, array-based oligo-peptide scanning, limited proteolysis, site-directed mutagenesis, high-throughput mutagenesis mapping, hydrogen-deuterium exchange, and mass spectrometry (see, e.g., Gershoni et al. (2007) BioDrugs 21:145-56; and Hager-Braun and Tomer (2005) Expert Rev Proteomics 2:745-56).

[176] Competitive binding and epitope binning can also be used to determine antibodies sharing identical or overlapping epitopes. Competitive binding can be evaluated using a cross-blocking assay, such as the assay described in "Antibodies, A Laboratory Manual,"

Cold Spring Harbor Laboratory, Harlow and Lane (1st edition 1988, 2nd edition 2014). In some embodiments, competitive binding is identified when a test antibody or binding protein reduces binding of a reference antibody or binding protein to a target antigen such as EGFR, CD7, or HER2 (e.g., a binding protein comprising CDRs and/or variable domains selected from those identified in Tables 3-5), by at least about 50% in the cross-blocking assay (e.g., 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.5%, or more, or any percentage in between), and/or vice versa. In some embodiments, competitive binding can be due to shared or similar (e.g., partially overlapping) epitopes, or due to steric hindrance where antibodies or binding proteins bind at nearby epitopes (see, e.g., Tzartos, Methods in Molecular Biology (Morris, ed. (1998) vol. 66, pp. 55-66)). In some embodiments, competitive binding can be used to sort groups of binding proteins that share similar epitopes. For example, binding proteins that compete for binding can be "binned" as a group of binding proteins that have overlapping or nearby epitopes, while those that do not compete are placed in a separate group of binding proteins that do not have overlapping or nearby epitopes.

[177] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably to refer to a polymer of amino acid residues. The terms encompass amino acid polymers comprising two or more amino acids joined to each other by peptide bonds, amino acid polymers in which one or more amino acid residues is an artificial chemical mimetic of a corresponding naturally-occurring amino acid, as well as naturally-occurring amino acid polymers and non-naturally-occurring amino acid polymers. The terms include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The terms also include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

[178] A "recombinant" protein refers to a protein (e.g., an antibody) made using recombinant techniques, e.g., through the expression of a recombinant nucleic acid.

[179] An "isolated" protein refers to a protein unaccompanied by at least some of the material with which it is normally associated in its natural state. For example, a naturally-occurring polynucleotide or polypeptide present in a living organism is not isolated, but the same polynucleotide or polypeptide separated from some or all of the coexisting materials in the living organism, is isolated. The definition includes the production of an antibody in a wide variety of organisms and/or host cells that are known in the art.

[180] An "isolated antibody," as used herein, is an antibody that has been identified and separated from one or more (e.g., the majority) of the components (by weight) of its source environment, e.g., from the components of a hybridoma cell culture or a different cell culture

that was used for its production. In some embodiments, the separation is performed such that it sufficiently removes components that may otherwise interfere with the suitability of the antibody for the desired applications (e.g., for therapeutic use). Methods for preparing isolated antibodies are known in the art and include, without limitation, protein A chromatography, anion exchange chromatography, cation exchange chromatography, virus retentive filtration, and ultrafiltration.

[181] As used herein, the term "variant" refers to a nucleic acid sequence or an amino acid sequence that differs from a reference nucleic acid sequence or amino acid sequence respectively, but retains one or more biological properties of the reference sequence. A variant may contain one or more amino acid substitutions, deletions, and/or insertions (or corresponding substitution, deletion, and/or insertion of codons) with respect to a reference sequence. Changes in a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid sequence, or may result in amino acid substitutions, additions, deletions, fusions, and/or truncations. In some embodiments, a nucleic acid variant disclosed herein encodes an identical amino acid sequence to that encoded by the unmodified nucleic acid or encodes a modified amino acid sequence that retains one or more functional properties of the unmodified amino acid sequence. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the unmodified peptide and the variant are closely similar overall and, in many regions, identical. In some embodiments, a peptide variant retains one or more functional properties of the unmodified peptide sequence. A variant and unmodified peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination.

[182] A variant of a nucleic acid or peptide can be a naturally-occurring variant or a variant that is not known to occur naturally. Variants of nucleic acids and peptides may be made by mutagenesis techniques, by direct synthesis, or by other techniques known in the art. A variant does not necessarily require physical manipulation of the reference sequence. As long as a sequence contains a different nucleic acid or amino acid as compared to a reference sequence, it is considered a "variant" regardless of how it was synthesized. In some embodiments, a variant has high sequence identity (i.e., 60% nucleic acid or amino acid sequence identity or higher) as compared to a reference sequence. In some embodiments, a peptide variant encompasses polypeptides having amino acid substitutions, deletions, and/or insertions as long as the polypeptide has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% amino acid sequence identity with a reference sequence, or with a corresponding segment (e.g., a functional fragment) of a reference sequence, e.g., those variants that also

retain one or more functions of the reference sequence. In some embodiments, a nucleic acid variant encompasses polynucleotides having amino acid substitutions, deletions, and/or insertions as long as the polynucleotide has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% nucleic acid sequence identity with a reference sequence, or with a corresponding segment (e.g., a functional fragment) of a reference sequence.

[183] The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. For nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence. For polypeptide sequences, conservatively modified variants include individual substitutions, deletions, or additions to a polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitutions providing functionally similar amino acids are well known in the art.

[184] The term "conservative sequence modifications," as used herein, refers to amino acid modifications that do not significantly affect or alter the binding characteristics of, e.g., an antibody or antigen-binding fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions, and deletions. Modifications can be introduced into an antibody or antigen-binding fragment by standard techniques known in the art, such as, e.g., site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side

chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, in some embodiments, one or more amino acid residues within an antibody can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested using the functional assays described herein.

[185] The term "homologous" or "identity," as used herein, refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions. For example, if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are matched or homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

[186] Percentage of "sequence identity" can be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage can be calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. The output is the percent identity of the subject sequence with respect to the query sequence. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. Generally, the amino acid identity or homology between proteins disclosed herein and variants thereof, including variants of target antigens (such as EGFR, CD7, or HER2) and variants of antibody variable domains (including individual variant CDRs), is at least 80% to the sequences depicted herein, e.g., identities or homologies of at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, almost 100%, or 100%.

[187] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In some embodiments, the percent identity between two amino acid sequences is determined using the Needleman

and Wunsch ((1970) J Mol Biol. 48:444-53) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In some embodiments, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. An exemplary set of parameters is a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of Meyers and Miller ((1989) CABIOS 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[188] The term “agent” is used herein to refer to a chemical compound, a mixture of chemical compounds, a biological macromolecule, an extract made from biological materials, or a combination of two or more thereof. The term “therapeutic agent” or “drug” refers to an agent that is capable of modulating a biological process and/or has biological activity. The Bcl-xL inhibitors and the ADCs comprising them, as described herein, are exemplary therapeutic agents.

[189] The term "chemotherapeutic agent" or “anti-cancer agent” is used herein to refer to all agents that are effective in treating cancer (regardless of mechanism of action). Inhibition of metastasis or angiogenesis is frequently a property of a chemotherapeutic agent. Chemotherapeutic agents include antibodies, biological molecules, and small molecules, and encompass the Bcl-xL inhibitors and ADCs comprising them, as described herein. A chemotherapeutic agent may be a cytotoxic or cytostatic agent. The term “cytostatic agent” refers to an agent that inhibits or suppresses cell growth and/or multiplication of cells. The term "cytotoxic agent" refers to a substance that causes cell death primarily by interfering with a cell’s expression activity and/or functioning.

[190] The term “B-cell lymphoma-extra large” or “Bcl-xL,” as used herein, refers to any native form of human Bcl-xL, an anti-apoptotic member of the Bcl-2 protein family. The term encompasses full-length human Bcl-xL (e.g., UniProt Reference Sequence: Q07817-1; SEQ ID NO:71), as well as any form of human Bcl-xL that may result from cellular processing. The term also encompasses functional variants or fragments of human Bcl-xL, including but not limited to splice variants, allelic variants, and isoforms that retain one or more biologic functions of human Bcl-xL (i.e., variants and fragments are encompassed unless the context indicates that the term is used to refer to the wild-type protein only). Bcl-xL can be isolated from human, or may be produced recombinantly or by synthetic methods.

[191] The term "inhibit" or "inhibition" or "inhibiting," as used herein, means to reduce a biological activity or process by a measurable amount, and can include but does not require complete prevention or inhibition. In some embodiments, "inhibition" means to reduce the expression and/or activity of Bcl-xL and/or one or more upstream modulators or downstream targets thereof.

[192] The term "Bcl-xL inhibitor," as used herein, refers to an agent capable of reducing the expression and/or activity of Bcl-xL and/or one or more upstream modulators or downstream targets thereof. Exemplary Bcl-xL modulators (including exemplary inhibitors of Bcl-xL) are described in WO2010/080503, WO2010/080478, WO2013/055897, WO2013/055895, WO2016/094509, WO2016/094517, WO2016/094505, Tao *et al.*, *ACS Medicinal Chemistry Letters* (2014), 5(10), 1088-109, and Wang *et al.*, *ACS Medicinal Chemistry Letters* (2020), 11(10), 1829–1836, each of which are incorporated herein by reference as exemplary Bcl-xL modulators, including exemplary Bcl-xL inhibitors, that can be included as drug moieties in the disclosed ADCs.

[193] As used herein, a "Bcl-xL inhibitor drug moiety", "Bcl-xL inhibitor", and the like refer to the component of an ADC or composition that provides the structure of a Bcl-xL inhibitor compound or a compound modified for attachment to an ADC that retains essentially the same, similar, or enhanced biological function or activity as compared to the original compound. In some embodiments, Bcl-xL inhibitor drug moiety is component (D) in an ADC of Formula (1).

[194] The term "cancer," as used herein, refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and/or certain morphological features. Often, cancer cells can be in the form of a tumor or mass, but such cells may exist alone within a subject, or may circulate in the blood stream as independent cells, such as leukemic or lymphoma cells. The term "cancer" includes all types of cancers and cancer metastases, including hematological cancers, solid tumors, sarcomas, carcinomas and other solid and non-solid tumor cancers. Hematological cancers may include B-cell malignancies, cancers of the blood (leukemias), cancers of plasma cells (myelomas, e.g., multiple myeloma), or cancers of the lymph nodes (lymphomas). Exemplary B-cell malignancies include chronic lymphocytic leukemia (CLL), follicular lymphoma, mantle cell lymphoma, and diffuse large B-cell lymphoma. Leukemias may include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), acute monocytic leukemia (AMoL), etc. The terms "acute lymphoblastic leukemia" and "acute lymphocytic leukemia" can be used interchangeably to describe ALL. Lymphomas may include Hodgkin's

lymphoma, non-Hodgkin's lymphoma, etc. Other hematologic cancers may include myelodysplasia syndrome (MDS). Solid tumors may include carcinomas such as adenocarcinoma, e.g., breast cancer, pancreatic cancer, prostate cancer, colon or colorectal cancer, lung cancer, gastric cancer, cervical cancer, endometrial cancer, ovarian cancer, cholangiocarcinoma, glioma, melanoma, etc. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[195] As used herein, the term “tumor” refers to any mass of tissue that results from excessive cell growth or proliferation, either benign or malignant, including precancerous lesions. In some embodiments, the tumor is a breast cancer, gastric cancer, bladder cancer, brain cancer, cervical cancer, colorectal cancer, esophageal cancer, hepatocellular cancer, melanoma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, or spleen cancer. In some embodiments, the tumor is a gastric cancer.

[196] The terms “tumor cell” and “cancer cell” may be used interchangeably herein and refer to individual cells or the total population of cells derived from a tumor or cancer, including both non-tumorigenic cells and cancer stem cells. The terms “tumor cell” and “cancer cell” will be modified by the term “non-tumorigenic” when referring solely to those cells lacking the capacity to renew and differentiate to distinguish those cells from cancer stem cells.

[197] The term “target-negative,” “target antigen-negative,” or “antigen-negative,” as used herein, refers to the absence of target antigen expression by a cell or tissue. The term “target-positive,” “target antigen-positive,” or “antigen-positive” refers to the presence of target antigen expression. For example, a cell or a cell line that does not express a target antigen may be described as target-negative, whereas a cell or cell line that expresses a target antigen may be described as target-positive.

[198] The terms “subject” and “patient” are used interchangeably herein to refer to any human or non-human animal in need of treatment. Non-human animals include all vertebrates (e.g., mammals and non-mammals) such as any mammal. Non-limiting examples of mammals include humans, chimpanzees, apes, monkeys, cattle, horses, sheep, goats, swine, rabbits, dogs, cats, rats, mice, and guinea pigs. Non-limiting examples of non-mammals include birds and fish. In some embodiments, the subject is a human.

[199] The term “a subject in need of treatment,” as used herein, refers to a subject that would benefit biologically, medically, or in quality of life from a treatment (e.g., a treatment with any one or more of the exemplary ADC compounds described herein).

[200] As used herein, the term “treat,” “treating,” or “treatment” refers to any improvement of any consequence of disease, disorder, or condition, such as prolonged survival, less morbidity, and/or a lessening of side effects which result from an alternative therapeutic modality. In some embodiments, treatment comprises delaying or ameliorating a disease, disorder, or condition (i.e., slowing or arresting or reducing the development of a disease or at least one of the clinical symptoms thereof). In some embodiments, treatment comprises delaying, alleviating, or ameliorating at least one physical parameter of a disease, disorder, or condition, including those which may not be discernible by the patient. In some embodiments, treatment comprises modulating a disease, disorder, or condition, either physically (e.g., stabilization of a discernible symptom), physiologically (e.g., stabilization of a physical parameter), or both. In some embodiments, treatment comprises administration of a described ADC compound or composition to a subject, e.g., a patient, to obtain a treatment benefit enumerated herein. The treatment can be to cure, heal, alleviate, delay, prevent, relieve, alter, remedy, ameliorate, palliate, improve, or affect a disease, disorder, or condition (e.g., a cancer), the symptoms of a disease, disorder, or condition (e.g., a cancer), or a predisposition toward a disease, disorder, or condition (e.g., a cancer). In some embodiments, in addition to treating a subject having a disease, disorder, or condition, a composition disclosed herein can also be provided prophylactically to prevent or reduce the likelihood of developing that disease, disorder, or condition.

[201] As used herein, the term “prevent”, “preventing," or “prevention” of a disease, disorder, or condition refers to the prophylactic treatment of the disease, disorder, or condition; or delaying the onset or progression of the disease, disorder, or condition.

[202] As used herein, a "pharmaceutical composition" refers to a preparation of a composition, e.g., an ADC compound or composition, in addition to at least one other (and optionally more than one other) component suitable for administration to a subject, such as a pharmaceutically acceptable carrier, stabilizer, diluent, dispersing agent, suspending agent, thickening agent, and/or excipient. The pharmaceutical compositions provided herein are in such form as to permit administration and subsequently provide the intended biological activity of the active ingredient(s) and/or to achieve a therapeutic effect. The pharmaceutical compositions provided herein preferably contain no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[203] As used herein, the terms "pharmaceutically acceptable carrier" and "physiologically acceptable carrier," which may be used interchangeably, refer to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the biological activity

and properties of the administered ADC compound or composition and/or any additional therapeutic agent in the composition. Pharmaceutically acceptable carriers may enhance or stabilize the composition or can be used to facilitate preparation of the composition. Pharmaceutically acceptable carriers can include solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drug stabilizers, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, and the like and combinations thereof, as would be known to those skilled in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289- 1329). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated. The carrier may be selected to minimize adverse side effects in the subject, and/or to minimize degradation of the active ingredient(s). An adjuvant may also be included in any of these formulations.

[204] As used herein, the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Formulations for parenteral administration can, for example, contain excipients such as sterile water or saline, polyalkylene glycols such as polyethylene glycol, vegetable oils, or hydrogenated napthalenes. Other exemplary excipients include, but are not limited to, calcium bicarbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, ethylene-vinyl acetate co-polymer particles, and surfactants, including, for example, polysorbate 20.

[205] The term “pharmaceutically acceptable salt,” as used herein, refers to a salt which does not abrogate the biological activity and properties of the compounds of the invention, and does not cause significant irritation to a subject to which it is administered. Examples of such salts include, but are not limited to: (a) acid addition salts formed with inorganic acids, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (b) salts formed from elemental anions such as chlorine, bromine, and iodine. See, e.g., Haynes et al., “Commentary: Occurrence of Pharmaceutically Acceptable Anions and Cations in the Cambridge Structural Database,” J. Pharmaceutical Sciences, vol. 94, no. 10 (2005), and Berge et al., “Pharmaceutical Salts,” J. Pharmaceutical Sciences, vol. 66, no. 1 (1977), which are incorporated by reference herein.

[206] In some embodiments, depending on their electronic charge, the antibody-drug conjugates (ADCs), linkers, payloads and linker-payloads described herein can contain a monovalent anionic counterion M_1^-. Any suitable anionic counterion can be used. In certain embodiments, the monovalent anionic counterion is a pharmaceutically acceptable monovalent anionic counterion. In certain embodiments, the monovalent anionic counterion M_1^- can be selected from bromide, chloride, iodide, acetate, trifluoroacetate, benzoate, mesylate, tosylate, triflate, formate, or the like. In some embodiments, the monovalent anionic counterion M_1^- is trifluoroacetate or formate.

[207] As used herein, the term "therapeutically effective amount" or "therapeutically effective dose," refers to an amount of a compound described herein, e.g., an ADC compound or composition described herein, to effect the desired therapeutic result (i.e., reduction or inhibition of an enzyme or a protein activity, amelioration of symptoms, alleviation of symptoms or conditions, delay of disease progression, a reduction in tumor size, inhibition of tumor growth, prevention of metastasis). In some embodiments, a therapeutically effective amount does not induce or cause undesirable side effects. In some embodiments, a therapeutically effective amount induces or causes side effects but only those that are acceptable by a treating clinician in view of a patient's condition. In some embodiments, a therapeutically effective amount is effective for detectable killing, reduction, and/or inhibition of the growth or spread of cancer cells, the size or number of tumors, and/or other measure of the level, stage, progression and/or severity of a cancer. The term also applies to a dose that will induce a particular response in target cells, e.g., a reduction, slowing, or inhibition of cell growth. A therapeutically effective amount can be determined by first administering a low dose, and then incrementally increasing that dose until the desired effect is achieved. A therapeutically effective amount can also vary depending upon the intended application (*in vitro* or *in vivo*), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The specific amount may vary depending on, for example, the particular pharmaceutical composition, the subject and their age and existing health conditions or risk for health conditions, the dosing regimen to be followed, the severity of the disease, whether it is administered in combination with other agents, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried. In the case of cancer, a therapeutically effective amount of an ADC may reduce the number of cancer cells, reduce tumor size, inhibit (e.g., slow or stop) tumor metastasis, inhibit (e.g., slow or stop) tumor growth, and/or relieve one or more symptoms.

[208] As used herein, the term "prophylactically effective amount" or "prophylactically effective dose," refers to an amount of a compound disclosed herein, e.g., an ADC

compound or composition described herein, that is effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount. In some embodiments, a prophylactically effective amount can prevent the onset of disease symptoms, including symptoms associated with a cancer.

[209] The term "*p*" or "drug loading" or "drug:antibody ratio" or "drug-to-antibody ratio" or "DAR" refers to the number of drug moieties per antibody or antigen-binding fragment, i.e., drug loading, or the number of -L-D moieties per antibody or antigen-binding fragment (Ab) in ADCs of Formula (1). In ADCs comprising a Bcl-xL inhibitor drug moiety, "*p*" refers to the number of Bcl-xL inhibitor compounds linked to the antibody or antigen-binding fragment. For example, if two Bcl-xL inhibitor compounds are linked to an antibody or antigen-binding fragment, $p = 2$. In compositions comprising multiple copies of ADCs of Formula (1), "average *p*" refers to the average number of -L-D moieties per antibody or antigen-binding fragment, also referred to as "average drug loading."

Antibody-Drug Conjugates

[210] The antibody-drug conjugate (ADC) compounds of the present disclosure include those with anti-cancer activity. In particular, the ADC compounds include an antibody or antigen-binding fragment conjugated (i.e., covalently attached by a linker) to a drug moiety (e.g., a Bcl-xL inhibitor), wherein the drug moiety when not conjugated to an antibody or antigen-binding fragment has a cytotoxic or cytostatic effect. In some embodiments, the drug moiety when not conjugated to an antibody or antigen-binding fragment is capable of reducing the expression and/or activity of Bcl-xL and/or one or more upstream modulators or downstream targets thereof. Without being bound by theory, by targeting Bcl-xL expression and/or activity, in some embodiments, the ADCs disclosed herein may provide potent anti-cancer agents. Also, without being bound by theory, by conjugating the drug moiety to an antibody that binds an antigen associated with expression in a tumor cell or cancer, the ADC may provide improved activity, better cytotoxic specificity, and/or reduced off-target killing as compared to the drug moiety when administered alone.

[211] In some embodiments, therefore, the components of the ADC are selected to (i) retain one or more therapeutic properties exhibited by the antibody and drug moieties in isolation, (ii) maintain the specific binding properties of the antibody or antigen-binding fragment; (iii) optimize drug loading and drug-to-antibody ratios; (iv) allow delivery, e.g., intracellular delivery, of the drug moiety via stable attachment to the antibody or antigen-binding fragment; (v) retain ADC stability as an intact conjugate until transport or delivery to a target site; (vi) minimize aggregation of the ADC prior to or after administration; (vii) allow

for the therapeutic effect, e.g., cytotoxic effect, of the drug moiety after cleavage or other release mechanism in the cellular environment; (viii) exhibit *in vivo* anti-cancer treatment efficacy comparable to or superior to that of the antibody and drug moieties in isolation; (ix) minimize off-target killing by the drug moiety; and/or (x) exhibit desirable pharmacokinetic and pharmacodynamics properties, formulatability, and toxicologic/immunologic profiles. Each of these properties may provide for an improved ADC for therapeutic use (Ab et al. (2015) Mol Cancer Ther. 14:1605-13).

[212] The ADC compounds of the present disclosure may selectively deliver an effective dose of a cytotoxic or cytostatic agent to cancer cells or to tumor tissue. In some embodiments, the cytotoxic and/or cytostatic activity of the ADC is dependent on target antigen expression in a cell. In some embodiments, the disclosed ADCs are particularly effective at killing cancer cells expressing a target antigen while minimizing off-target killing. In some embodiments, the disclosed ADCs do not exhibit a cytotoxic and/or cytostatic effect on cancer cells that do not express a target antigen.

[213] Exemplary BCMA-expressing cancers include but are not limited to multiple myeloma (Cho et al. (2018) Front Immunol. 9:1821).

[214] Exemplary CD33-expressing cancers include but are not limited to colorectal cancer, pancreatic cancer, lymphoma, and leukemia (e.g., acute myeloid leukemia) (Human Protein Atlas; Walter (2014) Expert Opin Ther Targets 18(7):715-8).

[215] Exemplary PCAD-expressing cancers include but are not limited to breast cancer, gastric cancer, endometrial cancer, ovarian cancer, pancreatic cancer, bladder cancer, prostate cancer, and melanoma (Vieira and Paredes (2015) Mol Cancer 14:178).

[216] Exemplary HER2-expressing cancers include but are not limited to breast cancer, gastric cancer, bladder cancer, urothelial cell carcinoma, esophageal cancer, lung cancer (e.g., lung adenocarcinoma), uterine cancer (e.g., uterine serous endometrial carcinoma), salivary duct carcinoma, cervical cancer, endometrial cancer, and ovarian cancer (English et al. (2013) Mol Diagn Ther. 17:85-99).

[217] Provided herein, in certain aspects, are ADC compounds comprising an antibody or antigen-binding fragment thereof (Ab), a Bcl-xL inhibitor drug moiety (D), and a linker moiety (L) that covalently attaches Ab to D. In some embodiments, provided herein, are ADC compounds comprising an antibody or antigen-binding fragment thereof (Ab) which targets a cancer cell, a Bcl-xL inhibitor drug moiety (D), and a linker moiety (L) that covalently attaches Ab to D. In some embodiments, the antibody or antigen-binding fragment is able to bind to a tumor-associated antigen (e.g., EGFR, CD7, or HER2), e.g., with high specificity and high affinity. In some embodiments, the antibody or antigen-binding fragment is internalized into a target cell upon binding, e.g., into a degradative compartment in the cell. In some embodiments, the ADCs internalize upon binding to a target cell, undergo

degradation, and release the Bcl-xL inhibitor drug moiety to kill cancer cells. The Bcl-xL inhibitor drug moiety may be released from the antibody and/or the linker moiety of the ADC by enzymatic action, hydrolysis, oxidation, or any other mechanism.

[218] An exemplary ADC has Formula (1):

$$Ab\text{-}(L\text{-}D)_p \qquad (1)$$

wherein Ab = an antibody or antigen-binding fragment, L = a linker moiety, D = a Bcl-xL inhibitor drug moiety, and p = the number of Bcl-xL inhibitor drug moieties per antibody or antigen-binding fragment.

Antibodies

[219] The antibody or antigen-binding fragment (Ab) of Formula (1) includes within its scope any antibody or antigen-binding fragment that specifically binds to a target antigen on a cell. In some embodiment, the antibody or antigen-binding fragment (Ab) of Formula (1) includes within its scope any antibody or antigen-binding fragment that specifically binds to a target antigen on a cancer cell. The antibody or antigen-binding fragment may bind to a target antigen with a dissociation constant (K_D) of ≤1 mM, ≤100 nM or ≤10 nM, or any amount in between, as measured by, e.g., BIAcore® analysis. In some embodiments, the K_D is 1 pM to 500 pM. In some embodiments, the K_D is between 500 pM to 1 μM, 1 μM to 100 nM, or 100 mM to 10 nM.

[220] In some embodiments, the antibody or antigen-binding fragment is a four-chain antibody (also referred to as an immunoglobulin or a full-length or intact antibody), comprising two heavy chains and two light chains. In some embodiments, the antibody or antigen-binding fragment is an antigen-binding fragment of an immunoglobulin. In some embodiments, the antibody or antigen-binding fragment is an antigen-binding fragment of an immunoglobulin that retains the ability to bind a target cancer antigen and/or provide at least one function of the immunoglobulin.

[221] In some embodiments, the antibody or antigen-binding fragment is an internalizing antibody or internalizing antigen-binding fragment thereof. In some embodiments, the internalizing antibody or internalizing antigen-binding fragment thereof binds to a target cancer antigen expressed on the surface of a cell and enters the cell upon binding. In some embodiments, the Bcl-xL inhibitor drug moiety of the ADC is released from the antibody or antigen-binding fragment of the ADC after the ADC enters and is present in a cell expressing the target cancer antigen (i.e., after the ADC has been internalized), e.g., by cleavage, by degradation of the antibody or antigen-binding fragment, or by any other suitable release mechanism.

[222] In some embodiments, the antibodies comprise mutations that mediate reduced or no antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity

(CDC). In some embodiments, these mutations are known as Fc Silencing, Fc Silent, or Fc Silenced mutations. In some embodiments, amino acid residues L234 and L235 of the IgG1 constant region are substituted to A234 and A235 (also known as “LALA”). In some embodiments, amino acid residue N297 of the IgG1 constant region is substituted to A297 (also known as “N297A”). In some embodiments, amino acid residues D265 and P329 of the IgG1 constant region are substituted to A265 and A329 (also known as “DAPA”). Other antibody Fc silencing mutations may also be used. In some embodiments, the Fc silencing mutations are used in combination, for example D265A, N297A and P329A (also known as “DANAPA”).

[223] Amino acid sequences of exemplary antibodies of the present disclosure, in addition to exemplary antigen targets, are set forth in Tables 2-6.

Table 2. Antibodies Exemplified

Antibody Target	Antibody Code	mAb Reference
BCMA	BCMA or Ab B	J6M0
CD33	CD33ch or Ab G	MuMy9-6ch
CD33	CD33	gemtuzumab
PCAD	PCAD	NOV169N31Q
HER2/NEU	HER2 or Ab T	trastuzumab
CD38	CD38	daratumumab
CD46	CD46	Anti-CD46
CD48	CD48	SGN-CD48A
CD79b	CD79b	polatuzumab
EGFR	Ab C or EGFR1 CysMab	cetuximab
CD7	Ab D	Anti-CD7
TFRC	TFRC CysMab	CD71 (CX-2029)
EPCAM	EPCAM CysMab	oportuzumab
FOLR1	FOLR1 CysMab	Mirvetuximab
ENPP3	ENPP3 CysMab	ENPP3 (AGS16-7.8)
MET	MET CysMab	Telisotuzumab
AXL	AXL CysMab	Enapotamab
SLC34A2	SLC34A2 CysMab	Lifastuzumab
NECTIN4	NECTIN4 CysMab	Enfortumab
TACSTD2	TACSTD2 CysMab	Sacituzumab
SLC39A6	SLC39A6 CysMab	Ladiratuzumab
GPNMB	GPNMB CysMab	Glembatumumab
MSLN	MSLN CysMab	Anetumab
CD74	CD74 CysMab	Milatuzumab
CD74	VHmil x VK1aNQ	VHmil x VK1aNQ
F3/TF	F3 CysMab	Tisotumab
MUC16	MUC16 CysMab	Sofituzumab
EGFR	EGFR2 CysMab	Aba
CD56	CD56 CysMab	Lorvotuzumab
SEZ6	SEZ6 CysMab	Anti-SEZ6 (Stemcentrx 17.46)
DLL3	DLL3 CysMab	Rovalpituzumab
DLK1	DLK1 CysMab	Anti-DLK1 (DI-2-14)
B7-H3	B7-H3 CysMab	ABBV-155

B7-H3	B7-H3	DS-5573a
IgG	IgG	anti-chiLysozyme (3207)

Table 3. Amino acid sequences of mAb variable regions

mAb	IgG chain	SEQ ID NO	Amino acid sequence
J6M0	VH	1	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS NYWMHWVRQAPGQGLEWMGATYRGHSDTYY NQKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARGAIYNGYDVLDNWGQGTLVTVS S
J6M0	VL	2	DIQMTQSPSSLSASVGDRVTITCSASQDIS NYLNWYQQKPGKAPKLLIYYTSNLHSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ YRKLPWTFGQGTKLEIK
MuMy9-6ch	VH	3	QVQLQQPGAEVVKPGASVKMSCKASGYTFT SYYIHWIKQTPGQGLEWVGVIYPGNDDISY NQKFKGKATLTADKSSTTAYMQLSSLTSED SAVYYCAREVRLRYFDVWGAGTTVTVSS
MuMy9-6ch	VL	4	NIMLTQSPSSLAVSAGEKVTMSCKSSQSVF FSSSQKNYLAWYQQIPGQSPKLLIYWASTR ESGVPDRFTGSGSGTDFTLIISSVQSEDLA IYYCHQYLSSRTFGGGTKLEIK
gemtuzumab	VH	5	EVQLVQSGAEVKKPGSSVKVSCKASGYTIT DSNIHWVRQAPGQSLEWIGYIYPYNGGTDY NQKFKNRATLTVDNPTNTAYMELSSLRSED TAFYYCVNGNPWLAYWGQGTLVTVSS
gemtuzumab	VL	6	DIQLTQSPSTLSASVGDRVTITCRASESLD NYGIRFLTWFQQKPGKAPKLLMYAASNQGS GVPSRFSGSGSGTEFTLTISSLQPDDFATY YCQQTKEVPWSFGQGTKVEVK
NOV169N31Q	VH	7	QVQLQQSGPGLVKPSQTLSLTCAISGDSVS SQSAAWNWIRQSPSRGLEWLGRIYYRSKWY NDYALSVKSRITINPDTSKNQFSLQLNSVT PEDTAVYYCARGEGYGREGFAIWGQGTLVT VSS
NOV169N31Q	VL	8	DIQMTQSPSSLSASVGDRVTITCRASQTIS NTLAWYQQKPGKAPKLLIYAASNLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ YLSWFTFGQGTKVEIK
trastuzumab	VH	9	EVQLVESGGGLVQPGGSLRLSCAASGFNIK DTYIHWVRQAPGKGLEWVARIYPTNGYTRY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
trastuzumab	VL	10	DIQMTQSPSSLSASVGDRVTITCRASQDVN TAVAWYQQKPGKAPKLLIYSASFLYSGVPS RFSGSRSGTDFTLTISSLQPEDFATYYCQQ HYTTPPTFGQGTKVEIKRT
daratumumab	VH	11	EVQLLESGGGLVQPGGSLRLSCAVSGFTFN SFAMSWVRQAPGKGLEWVSAISGSGGGTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYFCAKDKILWFGEPVFDYWGQGTLVTV SS

daratumumab	VL	12	EIVLTQSPATLSLSPGERATLSCRASQSVS SYLAWYQQKPGQAPRLLIYDASNRATGIPA RFSGSGSGTDFTLTISSLEPEDFAVYYCQQ RSNWPPTFGQGTKVEI
SGN-48A	VH	13	QVQLVQSGSELKKPGASVKVSCKASGYTFT DFGMNWVRQAPGQGLEWMGWINTFTGEPSY GNVFKGRFVFSLDTSVSTAYLQISSLKAED TAVYYCARRHGNGNVFDSWGQGTLVTVSS
SGN-48A	VL	14	EIVLTQSPDFQSVTPKEKVTITCRASQSIG SNIHWYQQKPDQSPKLLIKYTSESISGVPS RFSGSGSGTDFTLTINSLEAEDAATYYCQQ SNSWPLTFGGGTKVEIK
polatuzumab	VH	80	EVQLVESGGGLVQPGGSLRLSCAASGYTFS SYWIEWVRQAPGKGLEWIGEILPGGGDTNY NEIFKGRATFSADTSKNTAYLQMNSLRAED TAVYYCTRRVPIRLDYWGQGTLVTVSS
polatuzumab	VL	81	DIQLTQSPSSLSASVGDRVTITCKASQSVD YEGDSFLNWYQQKPGKAPKLLIYAASNLES GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSNEDPLTFGQGTKVEIK
Anti-CD46	VH	90	QVQLVQSGGGVVQPGRSLRLACAASGLTVN NYAMHWVRQAPGKGLEWVAVISYDGNNKYY ADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGGGYFDLWGRGTLV TVSS
Anti-CD46	VL	91	QSVLTQPPSVSGAPGQRVTISCTGSSSNIG AGYDVHWYQQLPGTAPKLLIYGNNNRP SGVPDRFSGSKSGTSASLAI TGLQAEDEA DYYCSSYTSGTWLFG GGTKLTVL
Cetuximab	VH	151	EVQLQESGPGLVKPSQTLSLTCTVSGYSIS RDFAWNWIRQPPGKGLEWMGYISYNGNTRY QPSLKSRITISRDTSKNQFFLKLNSVTAAD TATYYCVTASRGFPYWGQGTLVTVSS
Cetuximab	VL	152	DIQMTQSPSSMSVSVGDRVTITCHSSQDIN SNIGWLQQKPGKSFKGLIYHGTNLDDGVPS RFSGSGSGTDYTLTISSLQPEDFATYYCVQ YAQFPWTFGGGTKLEIK
VHmil x VK1aNQ	VH	153	QVQLQQSGSELKKPGASVKVSCKASGYTFT NYGVNWIKQAPGQGLQWMGWINPNTGEPTF DDDFKGRFAFSLDTSVSTAYLQISSLKADD TAVYFCSRSRGKNEAWFAYWGQGTLVTVSS
VHmil x VK1aNQ	VL	154	DIVMTQTPLSLPVTPGEPASISCRSSQSLV HRNQNTYLHWYLQKPGQSPQLLIYTVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YFCSQSSHVPPTFGQGTKLEIK
SEZ6	VH	155	QVQLVQSGAEVKKPGASVKVSCKASGYTFT SYWINWVRQAPGQGLEWIGNIFPDTTTTNY NEKFKGRVTLTRDTSISTAYMELSRLRSDD TAVYYCAREYYDGTYDAMDYWGQGTLVTVS S
SEZ6	VL	156	AIQMTQSPSSLSASVGDRVTITCKASQSVN NDVAWYQQKPGKAPKLLIYYASNRYTGVPS RFSGSGSGTDFTLTISSLQPEDFATYFCQQ DYSSPRTFGQGTKLEIK

CD56	VH	157	QVQLVESGGGVVQPGRSLRLSCAASGFTFS SFGMHWVRQAPGKGLEWVAYISSGSFTIYY ADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARMRKGYAMDYWGQGTLVTVSS
CD56	VL	158	DVVMTQSPLSLPVTLGQPASISCRSSQIII HSDGNTYLEWFQQRPGQSPRRLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQGSHVPHTFGQGTKVEIK
DLL3	VH	159	QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMNWVRQAPGQGLEWMGWINTYTGEPTY ADDFKGRVTMTTDTSTSTAYMELRSLRSDD TAVYYCARIGDSSPSDYWGQGTLVTVSS
DLL3	VL	160	EIVMTQSPATLSVSPGERATLSCKASQSVS NDVVWYQQKPGQAPRLLIYYASNRYTGIPA RFSGSGSGTEFTLTISSLQSEDFAVYYCQQ DYTSPWTFGQGTKLEIK
DLK1	VH	161	EVQLQQSGAELVKPGASVKLSCTASGFNIR DTYIHWVKQRPEQGLEWIGRIDPPNGNLKY DPKFQGKATITADTSSNTAYLQFSSLTSED TAVYYCARSDGYSFAYWGQGTLVTVSS
DLK1	VL	162	DIVMTQAAPSVPVTPGESVSISCRSSKSLL HSNGNTYLYWFLQRPGQSPQLLIYRMSNLA SGVPDRFSGSGSGTAFTLRISRVEAEDVGV YYCMQHVEYPFTFGSGTKLEIK
B7-H3 ABBV-155	VH	163	EVQLVQSGAEVKKPGSSVKVSCKASGYTFS SYWMHWVRQAPGQGLEWIGLIHPESGSTNY NEMFKNRATLTVDRSTSTAYMELSSLRSED TAVYYCAGGGRLYFDYWGQGTTVTVSS
B7-H3 ABBV-155	VL	164	DIVMTQSPLSLPVTPGEPASISCRSSQSLV HSNRDTYLRWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCSQSTHVPYTFGGGTKVEIK
B7-H3 DS-5573a	VH	165	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT NYVMHWVRQAPGQGLEWMGYINPYNDDVKY NEKFKGRVTITADESTSTAYMELSSLRSED TAVYYCARWGYYGSPLYYFDYWGQGTLVTV SS
B7-H3 DS-5573a	VL	166	EIVLTQSPATLSLSPGERATLSCRASSRLI YMHWYQQKPGQAPRPLIYATSNLASGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQW NSNPPTFGQGTKVEIK
IgG	VH	167	QVQLQQSGPGLVKPSQTLSLTCAISGDSVS SNSAAWSWIRQSPGRGLEWLGRIYYRSKWY NDYAVSVKSRITINPDTSKNQFSLQLNSVT PEDTAVYYCARLDHRYHEDTVYPGMDVWGQ GTLVTVSS
IgG	VL	168	DIELTQPPSVSVAPGQTARISCSGDNLPAY TVTWYQQKPGQAPVLVIYDDSDRPSGIPER FSGSNSGNTATLTISGTQAEDEADYYCASW DPSSGVVFGGGTKLTVL

Table 4. Amino acid sequences of mAb CDRs (Combined)

mAb	IgG chain	SEQ ID NO	Amino acid sequence

J6M0	HCDR1	15	GGTFSNYWMH
J6M0	HCDR2	16	ATYRGHSDTYYNQKFKG
J6M0	HCDR3	17	GAIYNGYDVLDN
J6M0	LCDR1	18	SASQDISNYLN
J6M0	LCDR2	19	YTSNLHS
J6M0	LCDR3	20	QQYRKLPWT
MuMy9-6ch	HCDR1	21	GYTFTSYYIH
MuMy9-6ch	HCDR2	22	VIYPGNDDISYNQKFKG
MuMy9-6ch	HCDR3	23	EVRLRYFDV
MuMy9-6ch	LCDR1	24	KSSQSVFFSSSQKNYLA
MuMy9-6ch	LCDR2	25	WASTRES
MuMy9-6ch	LCDR3	26	HQYLSSRT
gemtuzumab	HCDR1	27	GYTITDSNIH
gemtuzumab	HCDR2	28	YIYPYNGGTDYNQKFKN
gemtuzumab	HCDR3	29	GNPWLAY
gemtuzumab	LCDR1	30	RASESLDNYGIRFLT
gemtuzumab	LCDR2	31	AASNQGS
gemtuzumab	LCDR3	32	QQTKEVPWS
NOV169N31Q	HCDR1	33	TCAISGDSVSSQSAAWN
NOV169N31Q	HCDR2	34	RIYYRSKWYNDYALSVKS
NOV169N31Q	HCDR3	35	GEGYGREGFAI
NOV169N31Q	LCDR1	36	RASQTISNTLA
NOV169N31Q	LCDR2	37	AASNLQS
NOV169N31Q	LCDR3	38	QQYLSWFT
trastuzumab	HCDR1	39	GFNIKDTYIH
trastuzumab	HCDR2	40	RIYPTNGYTRYADSVKG
trastuzumab	HCDR3	41	WGGDGFYAMDV
trastuzumab	LCDR1	42	RASQDVNTAVAW
trastuzumab	LCDR2	43	SASFLES
trastuzumab	LCDR3	44	QQHYTTPPT
daratumumab	HCDR1	45	GFTFNSFAMS
daratumumab	HCDR2	46	AISGSGGGTYYADSVKG
daratumumab	HCDR3	47	DKILWFGEPVFDY
daratumumab	LCDR1	48	RASQSVSSYLA
daratumumab	LCDR2	49	DASNRAT

daratumumab	LCDR3	50	QQRSNWPPT
SGN-48A	HCDR1	51	GYTFTDFGMN
SGN-48A	HCDR2	52	WINTFTGEPSYGNVFKG
SGN-48A	HCDR3	53	RHGNGNVFDS
SGN-48A	LCDR1	54	RASQSIGSNIH
SGN-48A	LCDR2	55	YTSESIS
SGN-48A	LCDR3	56	QQSNSWPLT
polatuzumab	HCDR1	82	GYTFSSYWIE
polatuzumab	HCDR2	83	EILPGGGDTNYNEIFKG
polatuzumab	HCDR3	84	RVPIRLDY
polatuzumab	LCDR1	85	ITCKASQSVDYEGDSFLN
polatuzumab	LCDR2	86	AASNLES
polatuzumab	LCDR3	87	QQSNEDPLT
VHmil x VK1aNQ	HCDR1	169	GYTFTNYGVN
VHmil x VK1aNQ	HCDR2	170	WINPNTGEPTFDDDFKG
VHmil x VK1aNQ	HCDR3	171	SRGKNEAWFAY
VHmil x VK1aNQ	LCDR1	172	RSSQSLVHRNQNTYLH
VHmil x VK1aNQ	LCDR2	173	TVSNRFS
VHmil x VK1aNQ	LCDR3	174	SQSSHVPPT
SEZ6	HCDR1	175	GYTFTSYWIN
SEZ6	HCDR2	176	NIFPDTTTTNYNEKFKG
SEZ6	HCDR3	177	EYYDGTYDAMDY
SEZ6	LCDR1	178	KASQSVNNDVA
SEZ6	LCDR2	179	YASNRYT
SEZ6	LCDR3	180	QQDYSSPRT
CD56	HCDR1	181	GFTFSSFGMH
CD56	HCDR2	182	YISSGSFTIYYADSVKG
CD56	HCDR3	183	MRKGYAMDY
CD56	LCDR1	184	RSSQIIIHSDGNTYLE
CD56	LCDR2	185	KVSNRFS
CD56	LCDR3	186	FQGSHVPHT
DLL3	HCDR1	187	GYTFTNYGMN
DLL3	HCDR2	188	WINTYTGEPTYADDFKG
DLL3	HCDR3	189	IGDSSPSDY
DLL3	LCDR1	190	KASQSVSNDVV

DLL3	LCDR2	179	YASNRYT
DLL3	LCDR3	191	QQDYTSPWT
DLK1	HCDR1	192	GFNIRDTYIH
DLK1	HCDR2	193	RIDPPNGNLKYDPKFQG
DLK1	HCDR3	194	SDGYSFAY
DLK1	LCDR1	195	RSSKSLLHSNGNTYLY
DLK1	LCDR2	196	RMSNLAS
DLK1	LCDR3	197	MQHVEYPFT
B7-H3 ABBV-155	HCDR1	198	GYTFSSYWMH
B7-H3 ABBV-155	HCDR2	199	LIHPESGSTNYNEMFKN
B7-H3 ABBV-155	HCDR3	200	GGRLYFDY
B7-H3 ABBV-155	LCDR1	201	RSSQSLVHSNRDTYLR
B7-H3 ABBV-155	LCDR2	185	KVSNRFS
B7-H3 ABBV-155	LCDR3	202	SQSTHVPYT
B7-H3 DS-5573a	HCDR1	203	GYTFTNYVMH
B7-H3 DS-5573a	HCDR2	204	YINPYNDDVKYNEKFKG
B7-H3 DS-5573a	HCDR3	205	WGYYGSPLYYFDY
B7-H3 DS-5573a	LCDR1	206	RASSRLIYMH
B7-H3 DS-5573a	LCDR2	207	ATSNLAS
B7-H3 DS-5573a	LCDR3	208	QQWNSNPPT
IgG	HCDR1	209	GDSVSSNSAAWS
IgG	HCDR2	210	RIYYRSKWYNDYAVSVKS
IgG	HCDR3	211	LDHRYHEDTVYPGMDV
IgG	LCDR1	212	SGDNLPAYTVT
IgG	LCDR2	213	DDSDRPS
IgG	LCDR3	214	ASWDPSSGVV

Table 5. Amino acid sequences of full-length mAb Ig chains

mAb	IgG chain	SEQ ID NO	Amino acid sequence
J6M0	Heavy chain	57	QVQLVQSGAEVKKPGSSVKVSCKASGGTF SNYWMHWVRQAPGQGLEWMGATYRGHSDT YYNQKFKGRVTITADKSTSTAYMELSSLR SEDTAVYYCARGAIYNGYDVLDNWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPCPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
J6M0	Light chain	58	DIQMTQSPSSLSASVGDRVTITCSASQDI SNYLNWYQQKPGKAPKLLIYYTSNLHSGV PSRFSGSGSGTDFTLTISSLQPEDFATYY CQQYRKLPWTFGQGTKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
MuMy9-6ch	Heavy chain	59	QVQLQQPGAEVVKPGASVKMSCKASGYTF TSYYIHWIKQTPGQGLEWVGVIYPGNDDI SYNQKFKGKATLTADKSSTTAYMQLSSLT SEDSAVYYCAREVRLRYFDVWGAGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPCPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
MuMy9-6ch	Light chain	60	NIMLTQSPSSLAVSAGEKVTMSCKSSQSV FFSSSQKNYLAWYQQIPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLIISSVQSE DLAIYYCHQYLSSRTFGGGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
gemtuzumab	Heavy chain	61	EVQLVQSGAEVKKPGSSVKVSCKASGYTI TDSNIHWVRQAPGQSLEWIGYIYPYNGGT DYNQKFKNRATLTVDNPTNTAYMELSSLR SEDTAFYYCVNGNPWLAYWGQGTLVTVSS

			ASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPCPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPCDIA VEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
gemtuzumab	Light chain	62	DIQLTQSPSTLSASVGDRVTITCRASESL DNYGIRFLTWFQQKPGKAPKLLMYAASNQ GSGVPSRFSGSGSGTEFTLTISSLQPDDF ATYYCQQTKEVPWSFGQGTKVEVKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
NOV169N31Q	Heavy chain	63	QVQLQQSGPGLVKPSQTLSLTCAISGDSV SSQSAAWNWIRQSPSRGLEWLGRIYYRSK WYNDYALSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARGEGYGREGFAIWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPCPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
NOV169N31Q	Light chain	64	DIQMTQSPSSLSASVGDRVTITCRASQTI SNTLAWYQQKPGKAPKLLIYAASNLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYY CQQYLSWFTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
trastuzumab	Heavy chain	65	EVQLVESGGGLVQPGGSLRLSCAASGFNI KDTYIHWVRQAPGKGLEWVARIYPTNGYT RYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCSRWGGDGFYAMDYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLN

			GKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
trastuzumab	Light chain	66	DIQMTQSPSSLSASVGDRVTITCRASQDV NTAVAWYQQKPGKAPKLLIYSASFLYSGV PSRFSGSRSGTDFTLTISSLQPEDFATYY CQQHYTTPPTFGQGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
daratumumab	Heavy chain	67	EVQLLESGGGLVQPGGSLRLSCAVSGFTF NSFAMSWVRQAPGKGLEWVSAISGSGGGT YYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYFCAKDKILWFGEPVFDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPCPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPCDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
daratumumab	Light chain	68	EIVLTQSPATLSLSPGERATLSCRASQSV SSYLAWYQQKPGQAPRLLIYDASNRATGI PARFSGSGSGTDFTLTISSLEPEDFAVYY CQQRSNWPPTFGQGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
SGN-48A	Heavy chain	69	QVQLVQSGSELKKPGASVKVSCKASGYTF TDFGMNWVRQAPGQGLEWMGWINTFTGEP SYGNVFKGRFVFSLDTSVSTAYLQISSLK AEDTAVYYCARRHGNGNVFDSWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK

SGN-48A	Light chain	70	EIVLTQSPDFQSVTPKEKVTITCRASQSI GSNIHWYQQKPDQSPKLLIKYTSESISGV PSRFSGSGSGTDFTLTINSLEAEDAATYY CQQSNSWPLTFGGGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
Polatuzimab	Heavy Chain	88	EVQLVESGGGLVQPGGSLRLSCAASGYTF SSYWIEWVRQAPGKGLEWIGEILPGGGDT NYNEIFKGRATFSADTSKNTAYLQMNSLR AEDTAVYYCTRRVPIRLDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPCPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPCDI AVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
Polatuzimab	Light Chain	89	DIQLTQSPSSLSASVGDRVTITCKASQSV DYEGDSFLNWYQQKPGKAPKLLIYAASNL ESGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSNEDPLTFGQGTKVEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
VHmil x VK1aNQ	Heavy Chain	215	QVQLQQSGSELKKPGASVKVSCKASGYTF TNYGVNWIKQAPGQGLQWMGWINPNTGEP TFDDDFKGRFAFSLDTSVSTAYLQISSLK ADDTAVYFCSRSRGKNEAWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPCPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
	Light Chain	216	DIVMTQTPLSLPVTPGEPASISCRSSQSL VHRNQNTYLHWYLQKPGQSPQLLIYTVSN RFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYFCSQSSHVPPTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSK

			DSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
SEZ6	Heavy chain	217	QVQLVQSGAEVKKPGASVKVSCKASGYTF TSYWINWVRQAPGQGLEWIGNIFPDTTTT NYNEKFKGRVTLTRDTSISTAYMELSRLR SDDTAVYYCAREYYDGTYDAMDYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPCPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
SEZ6	Light chain	218	AIQMTQSPSSLSASVGDRVTITCKASQSV NNDVAWYQQKPGKAPKLLIYYASNRYTGV PSRFSGSGSGTDFTLTISSLQPEDFATYF CQQDYSSPRTFGQGTKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
CD56	Heavy chain	219	QVQLVESGGGVVQPGRSLRLSCAASGFTF SSFGMHWVRQAPGKGLEWVAYISSGSFTI YYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCARMRKGYAMDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPCPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
CD56	Light chain	220	DVVMTQSPLSLPVTLGQPASISCRSSQII IHSDGNTYLEWFQQRPGQSPRRLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCFQGSHVPHTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
DLL3	Heavy chain	221	QVQLVQSGAEVKKPGASVKVSCKASGYTF TNYGMNWVRQAPGQGLEWMGWINTYTGEP TYADDFKGRVTMTTDTSTSTAYMELRSLR SDDTAVYYCARIGDSSPSDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGC

			LVKDYFPCPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
DLL3	Light chain	222	EIVMTQSPATLSVSPGERATLSCKASQSV SNDVVWYQQKPGQAPRLLIYYASNRYTGI PARFSGSGSGTEFTLTISSLQSEDFAVYY CQQDYTSPWTFGQGTKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
DLK1	Heavy chain	223	EVQLQQSGAELVKPGASVKLSCTASGFNI RDTYIHWVKQRPEQGLEWIGRIDPPNGNL KYDPKFQGKATITADTSSNTAYLQFSSLT SEDTAVYYCARSDGYSFAYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPCPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPCDI AVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
DLK1	Light chain	224	DIVMTQAAPSVPVTPGESVSISCRSSKSL LHSNGNTYLYWFLQRPGQSPQLLIYRMSN LASGVPDRFSGSGSGTAFTLRISRVEAED VGVYYCMQHVEYPFTFGSGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
B7-H3 ABBV-155	Heavy chain	225	EVQLVQSGAEVKKPGSSVKVSCKASGYTF SSYWMHWVRQAPGQGLEWIGLIHPESGST NYNEMFKNRATLTVDRSTSTAYMELSSLR SEDTAVYYCAGGGRLYFDYWGQGTTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPCPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQV

			YTLPPSREEMTKNQVSLTCLVKGFYPCDI AVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
B7-H3 ABBV-155	Light chain	226	DIVMTQSPLSLPVTPGEPASISCRSSQSL VHSNRDTYLRWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCSQSTHVPYTFGGGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
B7-H3 DS-5573a	Heavy Chain	227	QVQLVQSGAEVKKPGSSVKVSCKASGYTF TNYVMHWVRQAPGQGLEWMGYINPYNDDV KYNEKFKGRVTITADESTSTAYMELSSLR SEDTAVYYCARWGYYGSPLYYFDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPCPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGF YPCDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
B7-H3 DS-5573a	Light Chain	228	EIVLTQSPATLSLSPGERATLSCRASSRL IYMHWYQQKPGQAPRPLIYATSNLASGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYC QQWNSNPPTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
IgG	Heavy Chain	229	QVQLQQSGPGLVKPSQTLSLTCAISGDSV SSNSAAWSWIRQSPGRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARLDHRYHEDTVYPGMD VWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG
IgG	Light Chain	230	DIELTQPPSVSVAPGQTARISCSGDNLPA YTVTWYQQKPGQAPVLVIYDDSDRPSGIP ERFSGSNSGNTATLTISGTQAEDEADYYC

			ASWDPSSGVVFGGGTKLTVLGQPKAAPSV TLFPPSSEELQANKATLVCLISDFYPGAV TVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTV EKTVAPTECS

Table 6. Exemplary Bcl-xLand target antigen amino acid sequences

Bcl-xL/Antigen	SEQ ID NO	Amino acid sequence
Bcl-xL	71	MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEM ETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQAL REAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFRDGV NWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLNDHLEP WIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAGVVL LGSLFSRK
BCMA	72	MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASV TNSVKGTNAILWTCLGLSLIISLAVFVLMFLLRKINSEPLKDEFK NTGSGLLGMANIDLEKSRTGDEIILPRGLEYTVEECTCEDCIKSK PKVDSDHCFPLPAMEEGATILVTTKTNDYCKSLPAALSATEIEKS ISAR
CD33	73	MPLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLVPCTFFH PIPYYDKNSPVHGYWFREGAIISRDSPVATNKLDQEVQEETQGRF RLLGDPSRNNCSLSIVDARRRDNGSYFFRMERGSTKYSYKSPQLS VHVTDLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPPIFSWL SAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQVKFAGAGVTTER TIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGAIGGAGVTAL LALCLCLIFFIVKTHRRKAARTAVGRNDTHPTTGSASPKHQKKSK LHGPTETSSCSGAAPTVEMDEELHYASLNFHGMNPSKDTSTEYSE VRTQ
PCAD	74	MGLPRGPLASLLLLQVCWLQCAASEPCRAVFREAEVTLEAGGAEQ EPGQALGKVFMGCPGQEPALFSTDNDDFTVRNGETVQERRSLKER NPLKIFPSKRILRRHKRDWVVAPISVPENGKGPFPQRLNQLKSNK DRDTKIFYSITGPGADSPPEGVFAVEKETGWLLLNKPLDREEIAK YELFGHAVSENGASVEDPMNISIIVTDQNDHKPKFTQDTFRGSVL EGVLPGTSVMQVTATDEDDAIYTYNGVVAYSIHSQEPKDPHDLMF TIHRSTGTISVISSGLDREKVPEYTLTIQATDMDGDGSTTTAVAV VEILDANDNAPMFDPQKYEAHVPENAVGHEVQRLTVTDLDAPNSP AWRATYLIMGGDDGDHFTITTHPESNQGILTTRKGLDFEAKNQHT LYVEVTNEAPFVLKLPTSTATIVVHVEDVNEAPVFVPPSKVVEVQ EGIPTGEPVCVYTAEDPDKENQKISYRILRDPAGWLAMDPDSGQV TAVGTLDREDEQFVRNNIYEVMVLAMDNGSPPTTGTGTLLLTLID VNDHGPVPEPRQITICNQSPVRQVLNITDKDLSPHTSPFQAQLTD DSDIYWTAEVNEEGDTVVLSLKKFLKQDTYDVHLSLSDHGNKEQL TVIRATVCDCHGHVETCPGPWKGGFILPVLGAVLALLFLLLVLLL LVRKKRKIKEPLLLPEDDTRDNVFYYGEEGGGEEDQDYDITQLHR GLEARPEVVLRNDVAPTIIPTPMYRPRPANPDEIGNFIIENLKAA NTDPTAPPYDTLLVFDYEGSGSDAASLSSLTSSASDQDQDYDYLN EWGSRFKKLADMYGGGEDD
HER2/NEU	75	MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDM LRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQ VRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPG GLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLA LTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCA RCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPA LVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVC PLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSAN IQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEE ITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGI SWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPHQALLH TANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQEC VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCV

		ACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINC THSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILI KRRQQKIRKYTMRRLLQETELVEPLTPSGAMPNQAQMRILKETEL RKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANK EILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHV RENRGRLGSQDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVLVKS PNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFT HQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPP ICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVVIQ NEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDP APGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEG AGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETD GYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERP KTLSPGKNGVVKDVFAFGGAVENPEYLTPQGGAAPQPHPPPAFSP AFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV
CD38	76	MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPR WRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFK GAFISKHPCNITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAHQF TQVQRDMFTLEDTLLGYLADDLTWCGEFNTSKINYQSCPDWRKDC SNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDKNSTFGSV EVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRN IQFSCKNIYRPDKFLQCVKNPEDSSCTSEI
CD48	77	MCSRGWDSCLALELLLLPLSLLVTSIQGHLVHMTVVSGSNVTLNI SESLPENYKQLTWFYTFDQKIVEWDSRKSKYFESKFKGRVRLDPQ SGALYISKVQKEDNSTYIMRVLKKTGNEQEWKIKLQVLDPVPKPV IKIEKIEDMDDNCYLKLSCVIPGESVNYTWYGDKRPFPKELQNSV LETTLMPHNYSRCYTCQVSNSVSSKNGTVCLSPPCTLARSFGVEW IASWLVVTVPTILGLLLT
CD79b	78	MARLALSPVPSHWMVALLLLLSAEPVPAARSEDRYRNPKGSACSR IWQSPRFIARKRGFTVKMHCYMNSASGNVSWLWKQEMDENPQQLK LEKGRMEESQNESLATLTIQGIRFEDNGIYFCQQKCNNTSEVYQG CGTELRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFLL LDKDDSKAGMEEDHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEH PGQE
EGFR (NP_005219.2)	126	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFE DHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVL IALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKEL PMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF QNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRG KSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLY NPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGADS YEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFK NCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFL LIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGL RSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRG ENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCN LLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHY IDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTG PGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVR KRTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGS GAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYV MASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIG SQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITD FGLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSY GVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYM

		IMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLP SPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLL SSLSATSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTED SIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHY QDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNP DYQQDFFPKEAKPNGIFKGSTAENAEYLRVAPQSSEFIGA
CD7 (NP_006128.1)	127	MAGPPRLLLLPLLLALARGLPGALAAQEVQQSPHCTTVPVGASVN ITCSTSGGLRGIYLRQLGPQPQDIIYYEDGVVPTTDRRFRGRIDF SGSQDNLTITMHRLQLSDTGTYTCQAITEVNVYGSGTLVLVTEEQ SQGWHRCSDAPPRASALPAPPTGSALPDPQTASALPDPPAASALP AALAVISFLLGLGLGVACVLARTQIKKLCSWRDKNSAACVVYEDM SHSRCNTLSSPNQYQ
TFRC (NP_003225.2)	128	MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDEE ENADNNTKANVTKPKRCSGSICYGTIAVIVFFLIGFMIGYLGYCK GVEPKTECERLAGTESPVREEPGEDFPAARRLYWDDLKRKLSEKL DSTDFTGTIKLLNENSYVPREAGSQKDENLALYVENQFREFKLSK VWRDQHFVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKA ATVTGKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFAEKVAN AESLNAIGVLIYMDQTKFPIVNAELSFFGHAHLGTGDPYTPGFPS FNHTQFPPSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKTD STCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHYVVV GAQRDAWGPGAAKSGVGTALLLKLAQMFSDMVLKDGFQPSRSIIF ASWSAGDFGSVGATEWLEGYLSSLHLKAFTYINLDKAVLGTSNFK VSASPLLYTLIEKTMQNVKHPVTGQFLYQDSNWASKVEKLTLDNA AFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELIERIPELNK VARAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSFVRDLNQYRA DIKEMGLSLQWLYSARGDFFRATSRLTTDFGNAEKTDRFVMKKLN DRVMRVEYHFLSPYVSPKESPFRHVFWGSGSHTLPALLENLKLRK QNNGAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF
EPCAM (NP_002345.2)	129	MAPPQVLAFGLLLAAATATFAAAQEECVCENYKLAVNCFVNNNRQ CQCTSVGAQNTVICSKLAAKCLVMKAEMNGSKLGRRAKPEGALQN NDGLYDPDCDESGLFKAKQCNGTSMCWCVNTAGVRRTDKDTEITC SERVRTYWIIIELKHKAREKPYDSKSLRTALQKEITTRYQLDPKF ITSILYENNVITIDLVQNSSQKTQNDVDIADVAYYFEKDVKGESL FHSKKMDLTVNGEQLDLDPGQTLIYYVDEKAPEFSMQGLKAGVIA VIVVVVIAVVAGIVVLVISRKKRMAKYEKAEIKEMGEMHRELNA
FOLR1 (NP_057936.1)	130	MAQRMTTQLLLLLVWVAVVGEAQTRIAWARTELLNVCMNAKHHKE KPGPEDKLHEQCRPWRKNACCSTNTSQEAHKDVSYLYRFNWNHCG EMAPACKRHFIQDTCLYECSPNLGPWIQQVDQSWRKERVLNVPLC KEDCEQWWEDCRTSYTCKSNWHKGWNWTSGFNKCAVGAACQPFHF YFPTPTVLCNEIWTHSYKVSNYSRGSGRCIQMWFDPAQGNPNEEV ARFYAAAMSGAGPWAAWPFLLSLALMLLWLLS
ENPP3 (NP_005012.2)	131	MESTLTLATEQPVKKNTLKKYKIACIVLLALLVIMSLGLGLGLGL RKLEKQGSCRKKCFDASFRGLENCRCDVACKDRGDCCWDFEDTCV ESTRIWMCNKFRCGETRLEASLCSCSDDCLQRKDCCADYKSVCQG ETSWLEENCDTAQQSQCPEGFDLPPVILFSMDGFRAEYLYTWDTL MPNINKLKTCGIHSKYMRAMYPTKTFPNHYTIVTGLYPESHGIID NNMYDVNLNKNFSLSSKEQNNPAWWHGQPMWLTAMYQGLKAATYF WPGSEVAINGSFPSIYMPYNGSVPFEERISTLLKWLDLPKAERPR FYTMYFEEPDSSGHAGGPVSARVIKALQVVDHAFGMLMEGLKQRN LHNCVNIILLADHGMDQTYCNKMEYMTDYFPRINFFYMYEGPAPR IRAHNIPHDFFSFNSEEIVRNLSCRKPDQHFKPYLTPDLPKRLHY AKNVRIDKVHLFVDQQWLAVRSKSNTNCGGGNHGYNNEFRSMEAI FLAHGPSFKEKTEVEPFENIEVYNLMCDLLRIQPAPNNGTHGSLN HLLKVPFYEPSHAEEVSKFSVCGFANPLPTESLDCFCPHLQNSTQ

		LEQVNQMLNLTQEEITATVKVNLPFGRPRVLQKNVDHCLLYHREY VSGFGKAMRMPMWSSYTVPQLGDTSPLPPTVPDCLRADVRVPPSE SQKCSFYLADKNITHGFLYPPASNRTSDSQYDALITSNLVPMYEE FRKMWDYFHSVLLIKHATERNGVNVVSGPIFDYNYDGHFDAPDEI TKHLANTDVPIPTHYFVVLTSCKNKSHTPENCPGWLDVLPFIIPH RPTNVESCPEGKPEALWVEERFTAHIARVRDVELLTGLDFYQDKV QPVSEILQLKTYLPTFETTI
MET (NP_001120972.1)	132	MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPN FTAETPIQNVILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVL EHPDCFPCQDCSSKANLSGGVWKDNINMALVVDTYYDDQLISCGS VNRGTCQRHVFPHNHTADIQSEVHCIFSPQIEEPSQCPDCVVSAL GAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLKETKDG FMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLD AQTFHTRIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKEVFN ILQAAYVSKPGAQLARQIGASLNDDILFGVFAQSKPDSAEPMDRS AMCAFPIKYVNDFFNKIVNKNNVRCLQHFYGPNHEHCFNRTLLRN SSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLTSISTFIKGDL TIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHT LNQNGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGW CHDKCVRSEECLSGTWTQQICLPAIYKVFPNSAPLEGGTRLTICG WDFGFRRNNKFDLKKTRVLLGNESCTLTLSESTMNTLKCTVGPAM NKHFNMSIIISNGHGTTQYSTFSYVDPVITSISPKYGPMAGGTLL TLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEF AVKLKIDLANRETSIFSYREDPIVYEIHPTKSFISTWWKEPLNIV SFLFCFASGGSTITGVGKNLNSVSVPRMVINVHEAGRNFTVACQH RSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIYVH NPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSC ENIHLHSEAVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQP DQNFTGLIAGVVSISTALLLLLGFFLWLKKRKQIKDLGSELVRYD ARVHTPHLDRLVSARSVSPTTEMVSNESVDYRATFPEDQFPNSSQ NGSCRQVQYPLTDMSPILTSGDSDISSPLLQNTVHIDLSALNPEL VQAVQHVVIGPSSLIVHFNEVIGRGHFGCVYHGTLLDNDGKKIHC AVKSLNRITDIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEGS PLVVLPYMKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLAS KKFVHRDLAARNCMLDEKFTVKVADFGLARDMYDKEYYSVHNKTG AKLPVKWMALESLQTQKFTTKSDVWSFGVLLWELMTRGAPPYPDV NTFDITVYLLQGRRLLQPEYCPDPLYEVMLKCWHPKAEMRPSFSE LVSRISAIFSTFIGEHYVHVNATYVNVKCVAPYPSLLSSEDNADD EVDTRPASFWETS
AXL (NP_068713.2)	133	MAWRCPRMGRVPLAWCLALCGWACMAPRGTQAEESPFVGNPGNIT GARGLTGTLRCQLQVQGEPPEVHWLRDGQILELADSTQTQVPLGE DEQDDWIVVSQLRITSLQLSDTGQYQCLVFLGHQTFVSQPGYVGL EGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLWLQDAVPL ATAPGHGPQRSLHVPGLNKTSSFSCEAHNAKGVTTSRTATITVLP QQPRNLHLVSRQPTELEVAWTPGLSGIYPLTHCTLQAVLSDDGMG IQAGEPDPPEEPLTSQASVPPHQLRLGSLHPHTPYHIRVACTSSQ GPSSWTHWLPVETPEGVPLGPPENISATRNGSQAFVHWQEPRAPL QGTLLGYRLAYQGQDTPEVLMDIGLRQEVTLELQGDGSVSNLTVC VAAYTAAGDGPWSLPVPLEAWRPGQAQPVHQLVKEPSTPAFSWPW WYVLLGAVVAAACVLILALFLVHRRKKETRYGEVFEPTVERGELV VRYRVRKSYSRRTTEATLNSLGISEELKEKLRDVMVDRHKVALGK TLGEGEFGAVMEGQLNQDDSILKVAVKTMKIAICTRSELEDFLSE AVCMKEFDHPNVMRLIGVCFQGSERESFPAPVVILPFMKHGDLHS FLLYSRLGDQPVYLPTQMLVKFMADIASGMEYLSTKRFIHRDLAA RNCMLNENMSVCVADFGLSKKIYNGDYYRQGRIAKMPVKWIAIES

		LADRVYTSKSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYLRQG NRLKQPADCLDGLYALMSRCWELNPQDRPSFTELREDLENTLKAL PPAQEPDEILYVNMDEGGGYPEPPGAAGGADPPTQPDPKDSCSCL TAAEVHPAGRYVLCPSTTPSPAQPADRGSPAAPGQEDGA
SLC34A2 (NP_006415.3)	134	MAPWPELGDAQPNPDKYLEGAAGQQPTAPDKSKETNKTDNTEAPV TKIELLPSYSTATLIDEPTEVDDPWNLPTLQDSGIKWSERDTKGK ILCFFQGIGRLILLLGFLYFFVCSLDILSSAFQLVGGKMAGQFFS NSSIMSNPLLGLVIGVLVTVLVQSSSTSTSIVVSMVSSSLLTVRA AIPIIMGANIGTSITNTIVALMQVGDRSEFRRAFAGATVHDFFNW LSVLVLLPVEVATHYLEIITQLIVESFHFKNGEDAPDLLKVITKP FTKLIVQLDKKVISQIAMNDEKAKNKSLVKIWCKTFTNKTQINVT VPSTANCTSPSLCWTDGIQNWTMKNVTYKENIAKCQHIFVNFHLP DLAVGTILLILSLLVLCGCLIMIVKILGSVLKGQVATVIKKTINT DFPFPFAWLTGYLAILVGAGMTFIVQSSSVFTSALTPLIGIGVIT IERAYPLTLGSNIGTTTTAILAALASPGNALRSSLQIALCHFFFN ISGILLWYPIPFTRLPIRMAKGLGNISAKYRWFAVFYLIIFFFLI PLTVFGLSLAGWRVLVGVGVPVVFIIILVLCLRLLQSRCPRVLPK KLQNWNFLPLWMRSLKPWDAVVSKFTGCFQMRCCCCCRVCCRACC LLCDCPKCCRCSKCCEDLEEAQEGQDVPVKAPETFDNITISREAQ GEVPASDSKTECTAL
NECTIN4 (NP_112178.2)	135	MPLSLGAEMWGPEAWLLLLLLLASFTGRCPAGELETSDVVTVVLG QDAKLPCFYRGDSGEQVGQVAWARVDAGEGAQELALLHSKYGLHV SPAYEGRVEQPPPPRNPLDGSVLLRNAVQADEGEYECRVSTFPAG SFQARLRLRVLVPPLPSLNPGPALEEGQGLTLAASCTAEGSPAPS VTWDTEVKGTTSSRSFKHSRSAAVTSEFHLVPSRSMNGQPLTCVV SHPGLLQDQRITHILHVSFLAEASVRGLEDQNLWHIGREGAMLKC LSEGQPPPSYNWTRLDGPLPSGVRVDGDTLGFPPLTTEHSGIYVC HVSNEFSSRDSQVTVDVLDPQEDSGKQVDLVSASVVVVGVIAALL FCLLVVVVVLMSRYHRRKAQQMTQKYEEELTLTRENSIRRLHSHH TDPRSQPEESVGLRAEGHPDSLKDNSSCSVMSEEPEGRSYSTLTT VREIETQTELLSPGSGRAEEEEDQDEGIKQAMNHFVQENGTLRAK PTGNGIYINGRGHLV
TACSTD2 (NP_002344.2)	136	MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCS PDGPGGRCQCRALGSGMAVDCSTLTSKCLLLKARMSAPKNARTLV RPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVNSVGVRR TDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDAELRRLF RERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYY FERDIKGESLFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFSM KRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSGKYKKVEIKELG ELRKEPSL
SLC39A6 (NP_036451.4)	137	MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGIN VDLAISTRQYHLQQLFYRYGENNSLSVEGFRKLLQNIGIDKIKRI HIHHDHDHHSDHEHHSDHERHSDHEHHSEHEHHSDHDHHSHHNHA ASGKNKRKALCPDHDSDSSGKDPRNSQGKGAHRPEHASGRRNVKD SVSASEVTSTVYNTVSEGTHFLETIETPRPGKLFPKDVSSSTPPS VTSKSRVSRLAGRKTNESVSEPRKGFMYSRNTNENPQECFNASKL LTSHGMGIQVPLNATEFNYLCPAIINQIDARSCLIHTSEKKAEIP PKTYSLQIAWVGGFIAISIISFLSLLGVILVPLMNRVFFKFLLSF LVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEMKRGPLFS HLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFKDK KKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLR ADSQEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKC HSHFHDTLGQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSRE ELKDAGVATLAWMVIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSV AVFCHELPHELGDFAVLLKAGMTVKQAVLYNALSAMLAYLGMATG

		IFIGHYAENVSMWIFALTAGLFMYVALVDMVPEMLHNDASDHGCS RWGYFFLQNAGMLLGFGIMLLISIFEHKIVFRINF
GPNMB (NP_001005340.1)	138	MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLN GWSSDENDWNEKLYPVWKRGDMRWKNSWKGGRVQAVLTSDSPALV GSNITFAVNLIFPRCQKEDANGNIVYEKNCRNEAGLSADPYVYNW TAWSEDSDGENGTGQSHHNVFPDGKPFPHHPGWRRWNFIYVFHTL GQYFQKLGRCSVRVSVNTANVTLGPQLMEVTVYRRHGRAYVPIAQ VKDVYVVTDQIPVFVTMFQKNDRNSSDETFLKDLPIMFDVLIHDP SHFLNYSTINYKWSFGDNTGLFVSTNHTVNHTYVLNGTFSLNLTV KAAAPGPCPPPPPPPRPSKPTPSLATTLKSYDSNTPGPAGDNPLE LSRIPDENCQINRYGHFQATITIVEGILEVNIIQMTDVLMPVPWP ESSLIDFVVTCQGSIPTEVCTIISDPTCEITQNTVCSPVDVDEMC LLTVRRTFNGSGTYCVNLTLGDDTSLALTSTLISVPDRDPASPLR MANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVRSKG LSVFLNRAKAVFFPGNQEKDPLLKNQEFKGVS
MSLN (NP_005814.2)	139	MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEA APLDGVLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALA QKNVKLSTEQLRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQ ACTRFFSRITKANVDLLPRGAPERQRLLPAALACWGVRGSLLSEA DVRALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARA ALQGGGPPYGPPSTWSVSTMDALRGLLPVLGQPIIRSIPQGIVAA WRQRSSRDPSWRQPERTILRPRFRREVEKTACPSGKKAREIDESL IFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELY PQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGH EMSPQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLCSLSPEELS SVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEYFVK IQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQ KLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNG YLVLDLSMQEALSGTPCLLGPGPVLTVLALLLASTLA
CD74 (NP_001020330.1)	140	MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCS RGALYTGFSILVTLLLAGQATTAYFLYQQQGRLDKLTVTSQNLQL ENLRMKLPKPPKPVSKMRMATPLLMQALPMGALPQGPMQNATKYG NMTEDHVMHLLQNADPLKVYPPLKGSFPENLRHLKNTMETIDWKV FESWMHHWLLFEMSRHSLEQKPTDAPPKVLTKCQEEVSHIPAVHP GSFRPKCDENGNYLPLQCYGSIGYCWCVFPNGTEVPNTRSRGHHN CSESLELEDPSSGLGVTKQDLGPVPM
F3 (NP_001984.1)	141	METPAWPRVPRPETAVARTLLLGWVFAQVAGASGTTNTVAAYNLT WKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECD LTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPY LETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFG KDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVI PSRTVNRKSTDSPVECMGQEKGEFREIFYIIGAVVFVVIILVIIL AISLHKCRKAGVGQSWKENSPLNVS
MUC16 (NP_078966.2)	142	MLKPSGLPGSSSPTRSLMTGSRSTKATPEMDSGLTGATLSPKTST GAIVVTEHTLPFTSPDKTLASPTSSVVGRTTQSLGVMSSALPEST SRGMTHSEQRTSPSLSPQVNGTPSRNYPATSMVSGLSSPRTRTSS TEGNFTKEASTYTLTVETTSGPVTEKYTVPTETSTTEGDSTETPW DTRYIPVKITSPMKTFADSTASKENAPVSMTPAETTVTDSHTPGR TNPSFGTLYSSFLDLSPKGTPNSRGETSLELILSTTGYPFSSPEP GSAGHSRISTSAPLSSSASVLDNKISETSIFSGQSLTSPLSPGVP EARASTMPNSAIPFSMTLSNAETSAERVRSTISSLGTPSISTKQT AETILTFHAFAETMDIPSTHIAKTLASEWLGSPGTLGGTSTSALT TTSPSTTLVSEETNTHHSTSGKETEGTLNTSMTPLETSAPGEESE MTATLVPTLGFTTLDSKIRSPSQVSSSHPTRELRTTGSTSGRQSS STAAHGSSDILRATTSSTSKASSWTSESTAQQFSEPQHTQWVETS

		PSMKTERPPASTSVAAPITTSVPSVVSGFTTLKTSSTKGIWLEET SADTLIGESTAGPTTHQFAVPTGISMTGGSSTRGSQGTTHLLTRA TASSETSADLTLATNGVPVSVSPAVSKTAAGSSPPGGTKPSYTMV SSVIPETSSLQSSAFREGTSLGLTPLNTRHPFSSPEPDSAGHTKI STSIPLLSSASVLEDKVSATSTFSHHKATSSITTGTPEISTKTKP SSAVLSSMTLSNAATSPERVRNATSPLTHPSPSGEETAGSVLTLS TSAETTDSPNIHPTGTLTSESSESPSTLSLPSVSGVKTTFSSSTP STHLFTSGEETEETSNPSVSQPETSVSRVRTTLASTSVPTPVFPT MDTWPTRSAQFSSSHLVSELRATSSTSVTNSTGSALPKISHLTGT ATMSQTNRDTFNDSAAPQSTTWPETSPRFKTGLPSATTTVSTSAT SLSATVMVSKFTSPATSSMEATSIREPSTTILTTETTNGPGSMAV ASTNIPIGKGYITEGRLDTSHLPIGTTASSETSMDFTMAKESVSM SVSPSQSMDAAGSSTPGRTSQFVDTFSDDVYHLTSREITIPRDGT SSALTPQMTATHPPSPDPGSARSTWLGILSSSPSSPTPKVTMSST FSTQRVTTSMIMDTVETSRWNMPNLPSTTSLTPSNIPTSGAIGKS TLVPLDTPSPATSLEASEGGLPTLSTYPESTNTPSIHLGAHASSE SPSTIKLTMASVVKPGSYTPLTFPSIETHIHVSTARMAYSSGSSP EMTAPGETNTGSTWDPTTYITTTDPKDTSSAQVSTPHSVRTLRTT ENHPKTESATPAAYSGSPKISSSPNLTSPATKAWTITDTTEHSTQ LHYTKLAEKSSGFETQSAPGPVSVVIPTSPTIGSSTLELTSDVPG EPLVLAPSEQTTITLPMATWLSTSLTEEMASTDLDISSPSSPMST FAIFPPMSTPSHELSKSEADTSAIRNTDSTTLDQHLGIRSLGRTG DLTTVPITPLTTTWTSVIEHSTQAQDTLSATMSPTHVTQSLKDQT SIPASASPSHLTEVYPELGTQGRSSSEATTFWKPSTDTLSREIET GPTNIQSTPPMDNTTTGSSSSGVTLGIAHLPIGTSSPAETSTNMA LERRSSTATVSMAGTMGLLVTSAPGRSISQSLGRVSSVLSESTTE GVTDSSKGSSPRLNTQGNTALSSSLEPSYAEGSQMSTSIPLTSSP TTPDVEFIGGSTFWTKEVTTVMTSDISKSSARTESSSATLMSTAL GSTENTGKEKLRTASMDLPSPTPSMEVTPWISLTLSNAPNTTDSL DLSHGVHTSSAGTLATDRSLNTGVTRASRLENGSDTSSKSLSMGN STHTSMTYTEKSEVSSSIHPRPETSAPGAETTLTSTPGNRAISLT LPFSSIPVEEVISTGITSGPDINSAPMTHSPITPPTIVWTSTGTI EQSTQPLHAVSSEKVSVQTQSTPYVNSVAVSASPTHENSVSSGSS TSSPYSSASLESLDSTISRRNAITSWLWDLTTSLPTTTWPSTSLS EALSSGHSGVSNPSSTTTEFPLFSAASTSAAKQRNPETETHGPQN TAASTLNTDASSVTGLSETPVGASISSEVPLPMAITSRSDVSGLT SESTANPSLGTASSAGTKLTRTISLPTSESLVSFRMNKDPWTVSI PLGSHPTTNTETSIPVNSAGPPGLSTVASDVIDTPSDGAESIPTV SFSPSPDTEVTTISHFPEKTTHSFRTISSLTHELTSRVTPIPGDW MSSAMSTKPTGASPSITLGERRTITSAAPTTSPIVLTASFTETST VSLDNETTVKTSDILDARKTNELPSDSSSSSDLINTSIASSTMDV TKTASISPTSISGMTASSSPSLFSSDRPQVPTSTTETNTATSPSV SSNTYSLDGGSNVGGTPSTLPPFTITHPVETSSALLAWSRPVRTF STMVSTDTASGENPTSSNSVVTSVPAPGTWTSVGSTTDLPAMGFL KTSPAGEAHSLLASTIEPATAFTPHLSAAVVTGSSATSEASLLTT SESKAIHSSPQTPTTPTSGANWETSATPESLLVVTETSDTTLTSK ILVTDTILFSTVSTPPSKFPSTGTLSGASFPTLLPDTPAIPLTAT EPTSSLATSFDSTPLVTIASDSLGTVPETTLTMSETSNGDALVLK TVSNPDRSIPGITIQGVTESPLHPSSTSPSKIVAPRNTTYEGSIT VALSTLPAGTTGSLVFSQSSENSETTALVDSSAGLERASVMPLTT GSQGMASSGGIRSGSTHSTGTKTFSSLPLTMNPGEVTAMSEITTN RLTATQSTAPKGIPVKPTSAESGLLTPVSASSSPSKAFASLTTAP PTWGIPQSTLTFEFSEVPSLDTKSASLPTPGQSLNTIPDSDASTA SSSLSKSPEKNPRARMMTSTKAISASSFQSTGFTETPEGSASPSM AGHEPRVPTSGTGDPRYASESMSYPDPSKASSAMTSTSLASKLTT

		LFSTGQAARSGSSSSPISLSTEKETSFLSPTASTSRKTSLFLGPS MARQPNILVHLQTSALTLSPTSTLNMSQEEPPELTSSQTIAEEEG TTAETQTLTFTPSETPTSLLPVSSPTEPTARRKSSPETWASSISV PAKTSLVETTDGTLVTTIKMSSQAAQGNSTWPAPAEETGSSPAGT SPGSPEMSTTLKIMSSKEPSISPEIRSTVRNSPWKTPETTVPMET TVEPVTLQSTALGSGSTSISHLPTGTTSPTKSPTENMLATERVSL SPSPPEAWTNLYSGTPGGTRQSLATMSSVSLESPTARSITGTGQQ SSPELVSKTTGMEFSMWHGSTGGTTGDTHVSLSTSSNILEDPVTS PNSVSSLTDKSKHKTETWVSTTAIPSTVLNNKIMAAEQQTSRSVD EAYSSTSSWSDQTSGSDITLGASPDVTNTLYITSTAQTTSLVSLP SGDQGITSLTNPSGGKTSSASSVTSPSIGLETLRANVSAVKSDIA PTAGHLSQTSSPAEVSILDVTTAPTPGISTTITTMGTNSISTTTP NPEVGMSTMDSTPATERRTTSTEHPSTWSSTAASDSWTVTDMTSN LKVARSPGTISTMHTTSFLASSTELDSMSTPHGRITVIGTSLVTP SSDASAVKTETSTSERTLSPSDTTASTPISTFSRVQRMSISVPDI LSTSWTPSSTEAEDVPVSMVSTDHASTKTDPNTPLSTFLFDSLST LDWDTGRSLSSATATTSAPQGATTPQELTLETMISPATSQLPFSI GHITSAVTPAAMARSSGVTFSRPDPTSKKAEQTSTQLPTTTSAHP GQVPRSAATTLDVIPHTAKTPDATFQRQGQTALTTEARATSDSWN EKEKSTPSAPWITEMMNSVSEDTIKEVTSSSSVLRTLNTLDINLE SGTTSSPSWKSSPYERIAPSESTTDKEAIHPSTNTVETTGWVTSS EHASHSTIPAHSASSKLTSPVVTTSTREQAIVSMSTTTWPESTRA RTEPNSFLTIELRDVSPYMDTSSTTQTSIISSPGSTAITKGPRTE ITSSKRISSSFLAQSMRSSDSPSEAITRLSNFPAMTESGGMILAM QTSPPGATSLSAPTLDTSATASWTGTPLATTQRFTYSEKTTLFSK GPEDTSQPSPPSVEETSSSSSLVPIHATTSPSNILLTSQGHSPSS TPPVTSVFLSETSGLGKTTDMSRISLEPGTSLPPNLSSTAGEALS TYEASRDTKAIHHSADTAVTNMEATSSEYSPIPGHTKPSKATSPL VTSHIMGDITSSTSVFGSSETTEIETVSSVNQGLQERSTSQVASS ATETSTVITHVSSGDATTHVTKTQATFSSGTSISSPHQFITSTNT FTDVSTNPSTSLIMTESSGVTITTQTGPTGAATQGPYLLDTSTMP YLTETPLAVTPDFMQSEKTTLISKGPKDVSWTSPPSVAETSYPSS LTPFLVTTIPPATSTLQGQHTSSPVSATSVLTSGLVKTTDMLNTS MEPVTNSPQNLNNPSNEILATLAATTDIETIHPSINKAVTNMGTA SSAHVLHSTLPVSSEPSTATSPMVPASSMGDALASISIPGSETTD IEGEPTSSLTAGRKENSTLQEMNSTTESNIILSNVSVGAITEATK MEVPSFDATFIPTPAQSTKFPDIFSVASSRLSNSPPMTISTHMTT TQTGSSGATSKIPLALDTSTLETSAGTPSVVTEGFAHSKITTAMN NDVKDVSQTNPPFQDEASSPSSQAPVLVTTLPSSVAFTPQWHSTS SPVSMSSVLTSSLVKTAGKVDTSLETVTSSPQSMSNTLDDISVTS AATTDIETTHPSINTVVTNVGTTGSAFESHSTVSAYPEPSKVTSP NVTTSTMEDTTISRSIPKSSKTTRTETETTSSLTPKLRETSISQE ITSSTETSTVPYKELTGATTEVSRTDVTSSSSTSFPGPDQSTVSL DISTETNTRLSTSPIMTESAEITITTQTGPHGATSQDTFTMDPSN TTPQAGIHSAMTHGFSQLDVTTLMSRIPQDVSWTSPPSVDKTSSP SSFLSSPAMTTPSLISSTLPEDKLSSPMTSLLTSGLVKITDILRT RLEPVTSSLPNFSSTSDKILATSKDSKDTKEIFPSINTEETNVKA NNSGHESHSPALADSETPKATTQMVITTTVGDPAPSTSMPVHGSS ETTNIKREPTYFLTPRLRETSTSQESSFPTDTSFLLSKVPTGTIT EVSSTGVNSSSKISTPDHDKSTVPPDTFTGEIPRVFTSSIKTKSA EMTITTQASPPESASHSTLPLDTSTTLSQGGTHSTVTQGFPYSEV TTLMGMGPGNVSWMTTPPVEETSSVSSLMSSPAMTSPSPVSSTSP QSIPSSPLPVTALPTSVLVTTTDVLGTTSPESVTSSPPNLSSITH ERPATYKDTAHTEAAMHHSTNTAVTNVGTSGSGHKSQSSVLADSE TSKATPLMSTTSTLGDTSVSTSTPNISQTNQIQTEPTASLSPRLR

		ESSTSEKTSSTTETNTAFSYVPTGAITQASRTEISSSRTSISDLD RPTIAPDISTGMITRLFTSPIMTKSAEMTVTTQTTTPGATSQGIL PWDTSTTLFQGGTHSTVSQGFPHSEITTLRSRTPGDVSWMTTPPV EETSSGFSLMSPSMTSPSPVSSTSPESIPSSPLPVTALLTSVLVT TTNVLGTTSPEPVTSSPPNLSSPTQERLTTYKDTAHTEAMHASMH TNTAVANVGTSISGHESQSSVPADSHTSKATSPMGITFAMGDTSV STSTPAFFETRIQTESTSSLIPGLRDTRTSEEINTVTETSTVLSE VPTTTTTEVSRTEVITSSRTTISGPDHSKMSPYISTETITRLSTF PFVTGSTEMAITNQTGPIGTISQATLTLDTSSTASWEGTHSPVTQ RFPHSEETTTMSRSTKGVSWQSPPSVEETSSPSSPVPLPAITSHS SLYSAVSGSSPTSALPVTSLLTSGRRKTIDMLDTHSELVTSSLPS ASSFSGEILTSEASTNTETIHFSENTAETNMGTTNSMHKLHSSVS IHSQPSGHTPPKVTGSMMEDAIVSTSTPGSPETKNVDRDSTSPLT PELKEDSTALVMNSTTESNTVFSSVSLDAATEVSRAEVTYYDPTF MPASAQSTKSPDISPEASSSHSNSPPLTISTHKTIATQTGPSGVT SLGQLTLDTSTIATSAGTPSARTQDFVDSETTSVMNNDLNDVLKT SPFSAEEANSLSSQAPLLVTTSPSPVTSTLQEHSTSSLVSVTSVP TPTLAKITDMDTNLEPVTRSPQNLRNTLATSEATTDTHTMHPSIN TAVANVGTTSSPNEFYFTVSPDSDPYKATSAVVITSTSGDSIVST SMPRSSAMKKIESETTFSLIFRLRETSTSQKIGSSSDTSTVFDKA FTAATTEVSRTELTSSSRTSIQGTEKPTMSPDTSTRSVTMLSTFA GLTKSEERTIATQTGPHRATSQGTLTWDTSITTSQAGTHSAMTHG FSQLDLSTLTSRVPEYISGTSPPSVEKTSSSSSLLSLPAITSPSP VPTTLPESRPSSPVHLTSLPTSGLVKTTDMLASVASLPPNLGSTS HKIPTTSEDIKDTEKMYPSTNIAVTNVGTTTSEKESYSSVPAYSE PPKVTSPMVTSFNIRDTIVSTSMPGSSEITRIEMESTFSLAHGLK GTSTSQDPIVSTEKSAVLHKLTTGATETSRTEVASSRRTSIPGPD HSTESPDISTEVIPSLPISLGITESSNMTIITRTGPPLGSTSQGT FTLDTPTTSSRAGTHSMATQEFPHSEMTTVMNKDPEILSWTIPPS IEKTSFSSSLMPSPAMTSPPVSSTLPKTIHTTPSPMTSLLTPSLV MTTDTLGTSPEPTTSSPPNLSSTSHEILTTDEDTTAIEAMHPSTS TAATNVETTSSGHGSQSSVLADSEKTKATAPMDTTSTMGHTTVST SMSVSSETTKIKRESTYSLTPGLRETSISQNASFSTDTSIVLSEV PTGTTAEVSRTEVTSSGRTSIPGPSQSTVLPEISTRTMTRLFASP TMTESAEMTIPTQTGPSGSTSQDTLTLDTSTTKSQAKTHSTLTQR FPHSEMTTLMSRGPGDMSWQSSPSLENPSSLPSLLSLPATTSPPP ISSTLPVTISSSPLPVTSLLTSSPVTTTDMLHTSPELVTSSPPKL SHTSDERLTTGKDTTNTEAVHPSTNTAASNVEIPSSGHESPSSAL ADSETSKATSPMFITSTQEDTTVAISTPHFLETSRIQKESISSLS PKLRETGSSVETSSAIETSAVLSEVSIGATTEISRTEVTSSSRTS ISGSAESTMLPEISTTRKIIKFPTSPILAESSEMTIKTQTSPPGS TSESTFTLDTSTTPSLVITHSTMTQRLPHSEITTLVSRGAGDVPR PSSLPVEETSPPSSQLSLSAMISPSPVSSTLPASSHSSSASVTSL LTPGQVKTTEVLDASAEPETSSPPSLSSTSVEILATSEVTTDTEK IHPFSNTAVTKVGTSSSGHESPSSVLPDSETTKATSAMGTISIMG DTSVSTLTPALSNTRKIQSEPASSLTTRLRETSTSEETSLATEAN TVLSKVSTGATTEVSRTEAISFSRTSMSGPEQSTMSQDISIGTIP RISASSVLTESAKMTITTQTGPSESTLESTLNLNTATTPSWVETH SIVIQGFPHPEMTTSMGRGPGGVSWPSPPFVKETSPPSSPLSLPA VTSPHPVSTTFLAHIPPSPLPVTSLLTSGPATTTDILGTSTEPGT SSSSSLSTTSHERLTTYKDTAHTEAVHPSTNTGGTNVATTSSGYK SQSSVLADSSPMCTTSTMGDTSVLTSTPAFLETRRIQTELASSLT PGLRESSGSEGTSSGTKMSTVLSKVPTGATTEISKEDVTSIPGPA QSTISPDISTRTVSWFSTSPVMTESAEITMNTHTSPLGATTQGTS TLDTSSTTSLTMTHSTISQGFSHSQMSTLMRRGPEDVSWMSPPLL

		EKTRPSFSLMSSPATTSPSPVSSTLPESISSSPLPVTSLLTSGLA KTTDMLHKSSEPVTNSPANLSSTSVEILATSEVTTDTEKTHPSSN RTVTDVGTSSSGHESTSFVLADSQTSKVTSPMVITSTMEDTSVST STPGFFETSRIQTEPTSSLTLGLRKTSSSEGTSLATEMSTVLSGV PTGATAEVSRTEVTSSSRTSISGFAQLTVSPETSTETITRLPTSS IMTESAEMMIKTQTDPPGSTPESTHTVDISTTPNWVETHSTVTQR FSHSEMTTLVSRSPGDMLWPSQSSVEETSSASSLLSLPATTSPSP VSSTLVEDFPSASLPVTSLLNPGLVITTDRMGISREPGTSSTSNL SSTSHERLTTLEDTVDTEDMQPSTHTAVTNVRTSISGHESQSSVL SDSETPKATSPMGTTYTMGETSVSISTSDFFETSRIQIEPTSSLT SGLRETSSSERISSATEGSTVLSEVPSGATTEVSRTEVISSRGTS MSGPDQFTISPDISTEAITRLSTSPIMTESAESAITIETGSPGAT SEGTLTLDTSTTTFWSGTHSTASPGFSHSEMTTLMSRTPGDVPWP SLPSVEEASSVSSSLSSPAMTSTSFFSTLPESISSSPHPVTALLT LGPVKTTDMLRTSSEPETSSPPNLSSTSAEILATSEVTKDREKIH PSSNTPVVNVGTVIYKHLSPSSVLADLVTTKPTSPMATTSTLGNT SVSTSTPAFPETMMTQPTSSLTSGLREISTSQETSSATERSASLS GMPTGATTKVSRTEALSLGRTSTPGPAQSTISPEISTETITRIST PLTTTGSAEMTITPKTGHSGASSQGTFTLDTSSRASWPGTHSAAT HRSPHSGMTTPMSRGPEDVSWPSRPSVEKTSPPSSLVSLSAVTSP SPLYSTPSESSHSSPLRVTSLFTPVMMKTTDMLDTSLEPVTTSPP SMNITSDESLATSKATMETEAIQLSENTAVTQMGTISARQEFYSS YPGLPEPSKVTSPVVTSSTIKDIVSTTIPASSEITRIEMESTSTL TPTPRETSTSQEIHSATKPSTVPYKALTSATIEDSMTQVMSSSRG PSPDQSTMSQDISTEVITRLSTSPIKTESTEMTITTQTGSPGATS RGTLTLDTSTTFMSGTHSTASQGFSHSQMTALMSRTPGDVPWLSH PSVEEASSASFSLSSPVMTSSSPVSSTLPDSIHSSSLPVTSLLTS GLVKTTELLGTSSEPETSSPPNLSSTSAEILAITEVTTDTEKLEM TNVVTSGYTHESPSSVLADSVTTKATSSMGITYPTGDTNVLTSTP AFSDTSRIQTKSKLSLTPGLMETSISEETSSATEKSTVLSSVPTG ATTEVSRTEAISSSRTSIPGPAQSTMSSDTSMETITRISTPLTRK ESTDMAITPKTGPSGATSQGTFTLDSSSTASWPGTHSATTQRFPQ SVVTTPMSRGPEDVSWPSPLSVEKNSPPSSLVSSSSVTSPSPLYS TPSGSSHSSPVPVTSLFTSIMMKATDMLDASLEPETTSAPNMNIT SDESLAASKATTETEAIHVFENTAASHVETTSATEELYSSSPGFS EPTKVISPVVTSSSIRDNMVSTTMPGSSGITRIEIESMSSLTPGL RETRTSQDITSSTETSTVLYKMPSGATPEVSRTEVMPSSRTSIPG PAQSTMSLDISDEVVTRLSTSPIMTESAEITITTQTGYSLATSQV TLPLGTSMTFLSGTHSTMSQGLSHSEMTNLMSRGPESLSWTSPRF VETTRSSSSLTSLPLTTSLSPVSSTLLDSSPSSPLPVTSLILPGL VKTTEVLDTSSEPKTSSSPNLSSTSVEIPATSEIMTDTEKIHPSS NTAVAKVRTSSSVHESHSSVLADSETTITIPSMGITSAVDDTTVF TSNPAFSETRRIPTEPTFSLTPGFRETSTSEETTSITETSAVLYG VPTSATTEVSMTEIMSSNRIHIPDSDQSTMSPDIITEVITRLSSS SMMSESTQMTITTQKSSPGATAQSTLTLATTTAPLARTHSTVPPR FLHSEMTTLMSRSPENPSWKSSLFVEKTSSSSSLLSLPVTTSPSV SSTLPQSIPSSSFSVTSLLTPGMVKTTDTSTEPGTSLSPNLSGTS VEILAASEVTTDTEKIHPSSSMAVTNVGTTSSGHELYSSVSIHSE PSKATYPVGTPSSMAETSISTSMPANFETTGFEAEPFSHLTSGFR KTNMSLDTSSVTPTNTPSSPGSTHLLQSSKTDFTSSAKTSSPDWP PASQYTEIPVDIITPFNASPSITESTGITSFPESRFTMSVTESTH HLSTDLLPSAETISTGTVMPSLSEAMTSFATTGVPRAISGSGSPF SRTESGPGDATLSTIAESLPSSTPVPFSSSTFTTTDSSTIPALHE ITSSSATPYRVDTSLGTESSTTEGRLVMVSTLDTSSQPGRTSSSP ILDTRMTESVELGTVTSAYQVPSLSTRLTRTDGIMEHITKIPNEA

		AHRGTIRPVKGPQTSTSPASPKGLHTGGTKRMETTTTALKTTTTA LKTTSRATLTTSVYTPTLGTLTPLNASMQMASTIPTEMMITTPYV FPDVPETTSSLATSLGAETSTALPRTTPSVFNRESETTASLVSRS GAERSPVIQTLDVSSSEPDTTASWVIHPAETIPTVSKTTPNFFHS ELDTVSSTATSHGADVSSAIPTNISPSELDALTPLVTISGTDTST TFPTLTKSPHETETRTTWLTHPAETSSTIPRTIPNFSHHESDATP SIATSPGAETSSAIPIMTVSPGAEDLVTSQVTSSGTDRNMTIPTL TLSPGEPKTIASLVTHPEAQTSSAIPTSTISPAVSRLVTSMVTSL AAKTSTTNRALTNSPGEPATTVSLVTHPAQTSPTVPWTTSIFFHS KSDTTPSMTTSHGAESSSAVPTPTVSTEVPGVVTPLVTSSRAVIS TTIPILTLSPGEPETTPSMATSHGEEASSAIPTPTVSPGVPGVVT SLVTSSRAVTSTTIPILTFSLGEPETTPSMATSHGTEAGSAVPTV LPEVPGMVTSLVASSRAVTSTTLPTLTLSPGEPETTPSMATSHGA EASSTVPTVSPEVPGVVTSLVTSSSGVNSTSIPTLILSPGELETT PSMATSHGAEASSAVPTPTVSPGVSGVVTPLVTSSRAVTSTTIPI LTLSSSEPETTPSMATSHGVEASSAVLTVSPEVPGMVTSLVTSSR AVTSTTIPTLTISSDEPETTTSLVTHSEAKMISAIPTLAVSPTVQ GLVTSLVTSSGSETSAFSNLTVASSQPETIDSWVAHPGTEASSVV PTLTVSTGEPFTNISLVTHPAESSSTLPRTTSRFSHSELDTMPST VTSPEAESSSAISTTISPGIPGVLTSLVTSSGRDISATFPTVPES PHESEATASWVTHPAVTSTTVPRTTPNYSHSEPDTTPSIATSPGA EATSDFPTITVSPDVPDMVTSQVTSSGTDTSITIPTLTLSSGEPE TTTSFITYSETHTSSAIPTLPVSPGASKMLTSLVISSGTDSTTTF PTLTETPYEPETTAIQLIHPAETNTMVPRTTPKFSHSKSDTTLPV AITSPGPEASSAVSTTTISPDMSDLVTSLVPSSGTDTSTTFPTLS ETPYEPETTATWLTHPAETSTTVSGTIPNFSHRGSDTAPSMVTSP GVDTRSGVPTTTIPPSIPGVVTSQVTSSATDTSTAIPTLTPSPGE PETTASSATHPGTQTGFTVPIRTVPSSEPDTMASWVTHPPQTSTP VSRTTSSFSHSSPDATPVMATSPRTEASSAVLTTISPGAPEMVTS QITSSGAATSTTVPTLTHSPGMPETTALLSTHPRTETSKTFPAST VFPQVSETTASLTIRPGAETSTALPTQTTSSLFTLLVTGTSRVDL SPTASPGVSAKTAPLSTHPGTETSTMIPTSTLSLGLLETTGLLAT SSSAETSTSTLTLTVSPAVSGLSSASITTDKPQTVTSWNTETSPS VTSVGPPEFSRTVTGTTMTLIPSEMPTPPKTSHGEGVSPTTILRT TMVEATNLATTGSSPTVAKTTTTFNTLAGSLFTPLTTPGMSTLAS ESVTSRTSYNHRSWISTTSSYNRRYWTPATSTPVTSTFSPGISTS SIPSSTAATVPFMVPFTLNFTITNLQYEEDMRHPGSRKFNATERE LQGLLKPLFRNSSLEYLYSGCRLASLRPEKDSSATAVDAICTHRP DPEDLGLDRERLYWELSNLTNGIQELGPYTLDRNSLYVNGFTHRS SMPTTSTPGTSTVDVGTSGTPSSSPSPTTAGPLLMPFTLNFTITN LQYEEDMRRTGSRKFNTMESVLQGLLKPLFKNTSVGPLYSGCRLT LLRPEKDGAATGVDAICTHRLDPKSPGLNREQLYWELSKLTNDIE ELGPYTLDRNSLYVNGFTHQSSVSTTSTPGTSTVDLRTSGTPSSL SSPTIMAAGPLLVPFTLNFTITNLQYGEDMGHPGSRKFNTTERVL QGLLGPIFKNTSVGPLYSGCRLTSLRSEKDGAATGVDAICIHHLD PKSPGLNRERLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHRTS VPTSSTPGTSTVDLGTSGTPFSLPSPATAGPLLVLFTLNFTITNL KYEEDMHRPGSRKFNTTERVLQTLLGPMFKNTSVGLLYSGCRLTL LRSEKDGAATGVDAICTHRLDPKSPGVDREQLYWELSQLTNGIKE LGPYTLDRNSLYVNGFTHWIPVPTSSTPGTSTVDLGSGTPSSLPS PTTAGPLLVPFTLNFTITNLKYEEDMHCPGSRKFNTTERVLQSLL GPMFKNTSVGPLYSGCRLTLLRSEKDGAATGVDAICTHRLDPKSP GVDREQLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHQTSAPNT STPGTSTVDLGTSGTPSSLPSPTSAGPLLVPFTLNFTITNLQYEE DMHHPGSRKFNTTERVLQGLLGPMFKNTSVGLLYSGCRLTLLRPE

		KNGAATGMDAICSHRLDPKSPGLNREQLYWELSQLTHGIKELGPY TLDRNSLYVNGFTHRSSVAPTSTPGTSTVDLGTSGTPSSLPSPTT AVPLLVPFTLNFTITNLQYGEDMRHPGSRKFNTTERVLQGLLGPL FKNSSVGPLYSGCRLISLRSEKDGAATGVDAICTHHLNPQSPGLD REQLYWQLSQMTNGIKELGPYTLDRNSLYVNGFTHRSSGLTTSTP WTSTVDLGTSGTPSPVPSPTTTGPLLVPFTLNFTITNLQYEENMG HPGSRKFNITESVLQGLLKPLFKSTSVGPLYSGCRLTLLRPEKDG VATRVDAICTHRPDPKIPGLDRQQLYWELSQLTHSITELGPYTLD RDSLYVNGFTQRSSVPTTSTPGTFTVQPETSETPSSLPGPTATGP VLLPFTLNFTITNLQYEEDMRRPGSRKFNTTERVLQGLLMPLFKN TSVSSLYSGCRLTLLRPEKDGAATRVDAVCTHRPDPKSPGLDRER LYWKLSQLTHGITELGPYTLDRHSLYVNGFTHQSSMTTTRTPDTS TMHLATSRTPASLSGPMTASPLLVLFTINFTITNLRYEENMHHPG SRKFNTTERVLQGLLRPVFKNTSVGPLYSGCRLTLLRPKKDGAAT KVDAICTYRPDPKSPGLDREQLYWELSQLTHSITELGPYTLDRDS LYVNGFTQRSSVPTTSIPGTPTVDLGTSGTPVSKPGPSAASPLLV LFTLNFTITNLRYEENMQHPGSRKFNTTERVLQGLLRSLFKSTSV GPLYSGCRLTLLRPEKDGTATGVDAICTHHPDPKSPRLDREQLYW ELSQLTHNITELGPYALDNDSLFVNGFTHRSSVSTTSTPGTPTVY LGASKTPASIFGPSAASHLLILFTLNFTITNLRYEENMWPGSRKF NTTERVLQGLLRPLFKNTSVGPLYSGCRLTLLRPEKDGEATGVDA ICTHRPDPTGPGLDREQLYLELSQLTHSITELGPYTLDRDSLYVN GFTHRSSVPTTSTGVVSEEPFTLNFTINNLRYMADMGQPGSLKFN ITDNVMQHLLSPLFQRSSLGARYTGCRVIALRSVKNGAETRVDLL CTYLQPLSGPGLPIKQVFHELSQQTHGITRLGPYSLDKDSLYLNG YNEPGPDEPPTTPKPATTFLPPLSEATTAMGYHLKTLTLNFTISN LQYSPDMGKGSATFNSTEGVLQHLLRPLFQKSSMGPFYLGCQLIS LRPEKDGAATGVDTTCTYHPDPVGPGLDIQQLYWELSQLTHGVTQ LGFYVLDRDSLFINGYAPQNLSIRGEYQINFHIVNWNLSNPDPTS SEYITLLRDIQDKVTTLYKGSQLHDTFRFCLVTNLTMDSVLVTVK ALFSSNLDPSLVEQVFLDKTLNASFHWLGSTYQLVDIHVTEMESS VYQPTSSSSTQHFYLNFTITNLPYSQDKAQPGTTNYQRNKRNIED ALNQLFRNSSIKSYFSDCQVSTFRSVPNRHHTGVDSLCNFSPLAR RVDRVAIYEEFLRMTRNGTQLQNFTLDRSSVLVDGYSPNRNEPLT GNSDLPFWAVILIGLAGLLGVITCLICGVLVTTRRRKKEGEYNVQ QQCPGYYQSHLDLEDLQ
SEZ6	231	MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAA PTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEGLEKGDEELRP ALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQS KEGPWSPESESPMLRITAPLPPGPSMAVPTLGPGEIASTTPPSRA WTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETT TTTTIITTTITTVQTPGPCSWNFSGPEGSLDSPTDLSSPTDVGLD CFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQS FLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFP RRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNATQPFW DSKEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAP EGQRLHLHFEKVSLAEDDDRLIIRNGDNVEAPPVYDSYEVEYLPI EGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYG NFSSSTPTYPVGTTVEFSCDPGYTLEQGSIIIECVDPHDPQWNET EPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKR IMLDIRVLRIGPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSM ADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGW KSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSC QRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICDQGFVLMGSSI LTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQL

		HPAGATIHFSCAPGYVLKGQASIKCVPGHPSHWSDPPPICRAASL DGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVY FYFSRLQGKSSLQLPRPRPRPYNRITIESAFDNPTYETGSLSFAG DERI
CD56	232	MLQTKDLIWTLFFLGTAVSLQVDIVPSQGEISVGESKFFLCQVAG DAKDKDISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDD AGIYKCVVTGEDGSESEATVNVKIFQKLMFKNAPTPQEFREGEDA VIVCDVVSSLPPTIIWKHKGRDVILKKDVRFIVLSNNYLQIRGIK KTDEGTYRCEGRILARGEINFKDIQVIVNVPPTIQARQNIVNATA NLGQSVTLVCDAEGFPEPTMSWTKDGEQIEQEEDDEKYIFSDDSS QLTIKKVDKNDEAEYICIAENKAGEQDATIHLKVFAKPKITYVEN QTAMELEEQVTLTCEASGDPIPSITWRTSTRNISSEEKASWTRPE KQETLDGHMVVRSHARVSSLTLKSIQYTDAGEYICTASNTIGQDS QSMYLEVQYAPKLQGPVAVYTWEGNQVNITCEVFAYPSATISWFR DGQLLPSSNYSNIKIYNTPSASYLEVTPDSENDFGNYNCTAVNRI GQESLEFILVQADTPSSPSIDQVEPYSSTAQVQFDEPEATGGVPI LKYKAEWRAVGEEVWHSKWYDAKEASMEGIVTIVGLKPETTYAVR LAALNGKGLGEISAASEFKTQPVQGEPSAPKLEGQMGEDGNSIKV NLIKQDDGGSPIRHYLVRYRALSSEWKPEIRLPSGSDHVMLKSLD WNAEYEVYVVAENQQGKSKAAHFVFRTSAQPTAIPANGSPTSGLS TGAIVGILIVIFVLLLVVVDITCYFLNKCGLFMCIAVNLCGKAGP GAKGKDMEEGKAAFSKDESKEPIVEVRTEEERTPNHDGGKHTEPN ETTPLTEPEKGPVEAKPECQETETKPAPAEVKTVPNDATQTKENE SKA
DLL3	233	MVSPRMSGLLSQTVILALIFLPQTRPAGVFELQIHSFGPGPGPGA PRSPCSARLPCRLFFRVCLKPGLSEEAAESPCALGAALSARGPVY TEQPGAPAPDLPLPDGLLQVPFRDAWPGTFSFIIETWREELGDQI GGPAWSLLARVAGRRRLAAGGPWARDIQRAGAWELRFSYRARCEP PAVGTACTRLCRPRSAPSRCGPGLRPCAPLEDECEAPLVCRAGCS PEHGFCEQPGECRCLEGWTGPLCTVPVSTSSCLSPRGPSSATTGC LVPGPGPCDGNPCANGGSCSETPRSFECTCPRGFYGLRCEVSGVT CADGPCFNGGLCVGGADPDSAYICHCPPGFQGSNCEKRVDRCSLQ PCRNGGLCLDLGHALRCRCRAGFAGPRCEHDLDDCAGRACANGGT CVEGGGAHRCSCALGFGGRDCRERADPCAARPCAHGGRCYAHFSG LVCACAPGYMGARCEFPVHPDGASALPAAPPGLRPGDPQRYLLPP ALGLLVAAGVAGAALLLVHVRRRGHSQDAGSRLLAGTPEPSVHAL PDALNNLRTQEGSGDGPSSSVDWNRPEDVDPQGIYVISAPSIYAR EVATPLFPPLHTGRAGQRQHLLFPYPSSILSVK
DLK1	234	MTATEALLRVLLLLLAFGHSTYGAECFPACNPQNGFCEDDNVCRC QPGWQGPLCDQCVTSPGCLHGLCGEPGQCICTDGWDGELCDRDVR ACSSAPCANNRTCVSLDDGLYECSCAPGYSGKDCQKKDGPCVING SPCQHGGTCVDDEGRASHASCLCPPGFSGNFCEIVANSCTPNPCE NDGVCTDIGGDFRCRCPAGFIDKTCSRPVTNCASSPCQNGGTCLQ HTQVSYECLCKPEFTGLTCVKKRALSPQQVTRLPSGYGLAYRLTP GVHELPVQQPEHRILKVSMKELNKKTPLLTEGQAICFTILGVLTS LVVLGTVGIVFLNKCETWVSNLRYNHMLRKKKNLLLQYNSGEDLA VNIIFPEKIDMTTFSKEAGDEEI
B7H3	79	MLRRRGSPGMGVHVGAALGALWFCLTGALEVQVPEDPVVALVGTD ATLCCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYA NRTALFPDLLAQGNASLRLQRVRVADEGSFTCFVSIRDFGSAAVS LQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYQGYPEAEVFWQD GQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNP VLQQDAHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCSF SPEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFP DLLAQGNASLRLQRVRVADEGSFTCFVSIRDFGSAAVSLQVAAPY

		SKPSMTLEPNKDLRPGDTVTITCSSYRGYPEAEVFWQDGQGVPLT GNVTTSQMANEQGLFDVHSVLRVVLGANGTYSCLVRNPVLQQDAH GSVTITGQPMTFPPEALWVTVGLSVCLIALLVALAFVCWRKIKQS CEEENAGAEDQDGEGEGSKTALQPLKHSDSKEDDGQEIA

[224] In some embodiments, the antibody or antigen-binding fragment of an ADC disclosed herein may comprise any set of heavy and light chain variable domains listed in the tables above or a set of six CDRs from any set of heavy and light chain variable domains listed in the tables above. In some embodiments, the antibody or antigen-binding fragment of an ADC disclosed herein may comprise amino acid sequences that are conservatively modified and/or homologous to the sequences listed in the tables above, so long as the ADC retains the ability to bind to its target cancer antigen (e.g., with a K_D of less than $1x10^{-8}$ M) and retains one or more functional properties of the ADCs disclosed herein (e.g., ability to internalize, bind to an antigen target, e.g., an antigen expressed on a tumor or other cancer cell, etc.).

[225] In some embodiments, the antibody or antigen-binding fragment of an ADC disclosed herein further comprises human heavy and light chain constant domains or fragments thereof. For instance, the antibody or antigen-binding fragment of the described ADCs may comprise a human IgG heavy chain constant domain (such as an IgG1) and a human kappa or lambda light chain constant domain. In some embodiments, the antibody or antigen-binding fragment of the described ADCs comprises a human immunoglobulin G subtype 1 (IgG1) heavy chain constant domain with a human Ig kappa light chain constant domain.

[226] In some embodiments, the target cancer antigen for an ADC is BCMA.

[227] In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:15, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:16, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:17; light chain CDR1 (LCDR1) consisting of SEQ ID NO:18, light chain CDR2 (LCDR2) consisting of SEQ ID NO:19, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:20.

[228] In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:1 and the light chain variable region amino acid sequence of SEQ ID NO:2, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ

ID NO:1 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:2.

[229] In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-BCMA antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 156 and 379 in a wild-type (unmodified) IgG1 heavy chain constant domain. In some embodiments, the anti-BCMA antibody comprises a human Ig kappa light chain constant domain or a modified Ig kappa light chain constant domain.

[230] In some embodiments, the anti-BCMA antibody comprises the heavy chain amino acid sequence of SEQ ID NO:57 or a sequence that is at least 95% identical to SEQ ID NO:57, and the light chain amino acid sequence of SEQ ID NO:58 or a sequence that is at least 95% identical to SEQ ID NO:58. In some embodiments, the anti-BCMA antibody comprises the heavy chain amino acid sequence of SEQ ID NO:57 and the light chain amino acid sequence of SEQ ID NO:58, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-BCMA antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:57 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:58. In some embodiments, the anti-BCMA antibody is J6M0 (WO 2012/163805), or an antigen-binding fragment thereof.

[231] In some embodiments, the anti-BCMA antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of J6M0 or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:15), HCDR2 (SEQ ID NO:16), HCDR3 (SEQ ID NO:17); LCDR1 (SEQ ID NO:18), LCDR2 (SEQ ID NO:19), and LCDR3 (SEQ ID NO:20).

[232] In some embodiments, the target cancer antigen for an ADC is CD33.

[233] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:21, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:22, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:23; light chain CDR1 (LCDR1) consisting of SEQ ID NO:24, light chain CDR2 (LCDR2) consisting of SEQ ID NO:25, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:26.

[234] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:3 and the light chain variable region amino acid sequence of SEQ ID NO:4, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:3 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:4.

[235] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-CD33 antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a glutamine residue (Q) at the amino acid position corresponding to 297 in a wild-type (unmodified) IgG1 heavy chain constant domain. In some embodiments, the anti-CD33 antibody comprises a human Ig kappa light chain constant domain or a modified Ig kappa light chain constant domain.

[236] In some embodiments, the anti-CD33 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:59 or a sequence that is at least 95% identical to SEQ ID NO:59, and the light chain amino acid sequence of SEQ ID NO:60 or a sequence that is at least 95% identical to SEQ ID NO:60. In some embodiments, the anti-CD33 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:59 and the light chain amino acid sequence of SEQ ID NO:60, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD33 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:59 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:60. In some embodiments, the anti-CD33 antibody is MuMy9-6ch (US 2013/0078241), or an antigen-binding fragment thereof.

[237] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of MuMy9-6ch or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:21), HCDR2 (SEQ ID NO:22), HCDR3 (SEQ ID NO:23); LCDR1 (SEQ ID NO:24), LCDR2 (SEQ ID NO:25), and LCDR3 (SEQ ID NO:26).

[238] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:27, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:28, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:29; light chain CDR1 (LCDR1) consisting of SEQ ID NO:30, light chain CDR2 (LCDR2) consisting of SEQ ID NO:31, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:32.

[239] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:5 and the light chain variable region amino acid sequence of SEQ ID NO:6, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:5 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:6.

[240] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-CD33 antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system.

[241] In some embodiments, the anti-CD33 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:61 or a sequence that is at least 95% identical to SEQ ID NO:61, and the light chain amino acid sequence of SEQ ID NO:62 or a sequence that is at least 95% identical to SEQ ID NO:62. In some embodiments, the anti-CD33 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:61 and the light chain amino acid sequence of SEQ ID NO:62, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD33 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:61 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:62. In some embodiments, the anti-CD33 antibody is gemtuzumab, or an antigen-binding fragment thereof.

[242] In some embodiments, the anti-CD33 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of gemtuzumab or

wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:27), HCDR2 (SEQ ID NO:28), HCDR3 (SEQ ID NO:29); LCDR1 (SEQ ID NO:30), LCDR2 (SEQ ID NO:31), and LCDR3 (SEQ ID NO:32).

[243] In some embodiments, the target cancer antigen for an ADC is PCAD.

[244] In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:33, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:34, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:35; light chain CDR1 (LCDR1) consisting of SEQ ID NO:36, light chain CDR2 (LCDR2) consisting of SEQ ID NO:37, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:38.

[245] In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:7 and the light chain variable region amino acid sequence of SEQ ID NO:8, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:7 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:8.

[246] In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-PCAD antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system.

[247] In some embodiments, the anti-PCAD antibody comprises the heavy chain amino acid sequence of SEQ ID NO:63 or a sequence that is at least 95% identical to SEQ ID NO:63, and the light chain amino acid sequence of SEQ ID NO:64 or a sequence that is at least 95% identical to SEQ ID NO:64. In some embodiments, the anti-PCAD antibody comprises the heavy chain amino acid sequence of SEQ ID NO:63 and the light chain amino acid sequence of SEQ ID NO:64, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-PCAD antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99%

identical to SEQ ID NO:63 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:64. In some embodiments, the anti-PCAD antibody is NOV169N31Q (WO 2016/203432), or an antigen-binding fragment thereof.

[248] In some embodiments, the anti-PCAD antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of NOV169N31Q or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:33), HCDR2 (SEQ ID NO:34), HCDR3 (SEQ ID NO:35); LCDR1 (SEQ ID NO:36), LCDR2 (SEQ ID NO:37), and LCDR3 (SEQ ID NO:38).

[249] In some embodiments, the target cancer antigen for an ADC is HER2.

[250] In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:39, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:40, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:41; light chain CDR1 (LCDR1) consisting of SEQ ID NO:42, light chain CDR2 (LCDR2) consisting of SEQ ID NO:43, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:44.

[251] In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10. In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:9 and the light chain variable region amino acid sequence of SEQ ID NO:10, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:9 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:10.

[252] In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-HER2 antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a glutamine residue (Q) at the amino acid position corresponding to 297 in a wild-type (unmodified) IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a serine residue (S) at the amino acid position corresponding to 297 in a wild-type (unmodified) IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises

a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system. In some embodiments, the anti-HER2 antibody comprises a human Ig kappa light chain constant domain or a modified Ig kappa light chain constant domain.

[253] In some embodiments, the anti-HER2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:65 or a sequence that is at least 95% identical to SEQ ID NO:65, and the light chain amino acid sequence of SEQ ID NO:66 or a sequence that is at least 95% identical to SEQ ID NO:66. In some embodiments, the anti-HER2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:65 and the light chain amino acid sequence of SEQ ID NO:66, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-HER2 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:65 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:66. In some embodiments, the anti-HER2 antibody is trastuzumab (US Patent Nos. 5,821,337 and 6,870,034; see also Molina et al. (2001) Cancer Res. 61(12):4744-9), or an antigen-binding fragment thereof.

[254] In some embodiments, the anti-HER2 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of trastuzumab or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:39), HCDR2 (SEQ ID NO:40), HCDR3 (SEQ ID NO:41); LCDR1 (SEQ ID NO:42), LCDR2 (SEQ ID NO:43), and LCDR3 (SEQ ID NO:44).

[255] In some embodiments, the target cancer antigen for an ADC is CD38.

[256] In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:45, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:46, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:47; light chain CDR1 (LCDR1) consisting of SEQ ID NO:48, light chain CDR2 (LCDR2) consisting of SEQ ID NO:49, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:50.

[257] In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:12. In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:11 and the light chain variable region amino acid sequence of SEQ ID NO:12, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid

sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:11 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:12.

[258] In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-CD38 antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system.

[259] In some embodiments, the anti-CD38 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:67 or a sequence that is at least 95% identical to SEQ ID NO:67, and the light chain amino acid sequence of SEQ ID NO:68 or a sequence that is at least 95% identical to SEQ ID NO:68. In some embodiments, the anti-CD33 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:67 and the light chain amino acid sequence of SEQ ID NO:68, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD38 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:67 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:68. In some embodiments, the anti-CD38 antibody is daratumumab, or an antigen-binding fragment thereof.

[260] In some embodiments, the anti-CD38 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of gemtuzumab or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:45), HCDR2 (SEQ ID NO:46), HCDR3 (SEQ ID NO:47); LCDR1 (SEQ ID NO:48), LCDR2 (SEQ ID NO:49), and LCDR3 (SEQ ID NO:50).

[261] In some embodiment, the target cancer antigen for an ADC is CD46.

[262] In some embodiments, the anti-CD46 antibody or antigen-binding fragment are those described in WO2018/089807, incorporated herein by reference. In some embodiments, the anti-CD46 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:90, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:91. In some embodiments, the anti-CD46 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:90 and the light chain variable region amino acid sequence of SEQ ID NO:91, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD46 antibody or

antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:90 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:91.

[263] In some embodiments, the target cancer antigen for an ADC is CD48.

[264] In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:51, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:52, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:53; light chain CDR1 (LCDR1) consisting of SEQ ID NO:54, light chain CDR2 (LCDR2) consisting of SEQ ID NO:55, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:56.

[265] In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:14. In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:13 and the light chain variable region amino acid sequence of SEQ ID NO:14, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:13 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:14.

[266] In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-CD48 antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system.

[267] In some embodiments, the anti-CD48 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:69 or a sequence that is at least 95% identical to SEQ ID NO:69, and the light chain amino acid sequence of SEQ ID NO:70 or a sequence that is at least 95% identical to SEQ ID NO:70. In some embodiments, the anti-CD48 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:69 and the light chain amino acid sequence of SEQ ID NO:70, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD48 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99%

identical to SEQ ID NO:69 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:70. In some embodiments, the anti-CD48 antibody is SGN-48A, or an antigen-binding fragment thereof.

[268] In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of gemtuzumab or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:51), HCDR2 (SEQ ID NO:52), HCDR3 (SEQ ID NO:53); LCDR1 (SEQ ID NO:54), LCDR2 (SEQ ID NO:55), and LCDR3 (SEQ ID NO:56).

[269] In some embodiments, the target cancer antigen for an ADC is CD79B.

[270] In some embodiments, the anti-CD48 antibody or antigen-binding fragment thereof comprises three heavy chain CDRs and three light chain CDRs as follows: heavy chain CDR1 (HCDR1) consisting of SEQ ID NO:82, heavy chain CDR2 (HCDR2) consisting of SEQ ID NO:83, heavy chain CDR3 (HCDR3) consisting of SEQ ID NO:84; light chain CDR1 (LCDR1) consisting of SEQ ID NO:85, light chain CDR2 (LCDR2) consisting of SEQ ID NO:86, and light chain CDR3 (LCDR3) consisting of SEQ ID NO:87.

[271] In some embodiments, the anti-CD79B antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:80, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:81. In some embodiments, the anti-CD79B antibody or antigen-binding fragment thereof comprises the heavy chain variable region amino acid sequence of SEQ ID NO:80 and the light chain variable region amino acid sequence of SEQ ID NO:81, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD79B antibody or antigen-binding fragment thereof has a heavy chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:80 and/or a light chain variable region amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:81.

[272] In some embodiments, the anti-CD79B antibody or antigen-binding fragment thereof is an internalizing antibody or internalizing antigen-binding fragment. In some embodiments, the anti-CD79B antibody comprises a human IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain. In some embodiments, the IgG1 heavy chain constant domain comprises a cysteine residue (C) at the amino acid positions corresponding to 152 and 375 in a wild-type (unmodified) IgG1 heavy chain constant domain numbered according to EU numbering system.

[273] In some embodiments, the anti-CD79B antibody comprises the heavy chain amino acid sequence of SEQ ID NO:88 or a sequence that is at least 95% identical to SEQ ID NO:88, and the light chain amino acid sequence of SEQ ID NO:89 or a sequence that is at

least 95% identical to SEQ ID NO:89. In some embodiments, the anti-CD79B antibody comprises the heavy chain amino acid sequence of SEQ ID NO:88 and the light chain amino acid sequence of SEQ ID NO:89, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD79B antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:88 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:89. In some embodiments, the anti-CD79B antibody is polatizumab, or an antigen-binding fragment thereof.

[274] In some embodiments, the anti-CD79B antibody or antigen-binding fragment thereof comprises the three heavy chain CDRs and three light chain CDRs of gemtuzumab or wherein the CDRs include no more than one, two, three, four, five, or six amino acid additions, deletions or substitutions of HCDR1 (SEQ ID NO:82), HCDR2 (SEQ ID NO:83), HCDR3 (SEQ ID NO:84); LCDR1 (SEQ ID NO:85), LCDR2 (SEQ ID NO:86), and LCDR3 (SEQ ID NO:87).

[275] In some embodiments, the anti-EGFR antibody comprises the heavy chain amino acid sequence of SEQ ID NO:92 or a sequence that is at least 95% identical to SEQ ID NO:92, and the light chain amino acid sequence of SEQ ID NO:93 or a sequence that is at least 95% identical to SEQ ID NO:93. In some embodiments, the anti-EGFR antibody comprises the heavy chain amino acid sequence of SEQ ID NO:92 and the light chain amino acid sequence of SEQ ID NO:93, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-EGFR antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:92 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:93. In some embodiments, the anti-EGFR antibody is cetuximab, or an antigen-binding fragment thereof.

[276] In some embodiments, the anti-EGFR antibody comprises the heavy chain amino acid sequence of SEQ ID NO:124 or a sequence that is at least 95% identical to SEQ ID NO:124, and the light chain amino acid sequence of SEQ ID NO:125 or a sequence that is at least 95% identical to SEQ ID NO:125. In some embodiments, the anti-EGFR antibody comprises the heavy chain amino acid sequence of SEQ ID NO:124 and the light chain amino acid sequence of SEQ ID NO:125, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-EGFR antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:124 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:125.

[277] In some embodiments, the anti-TFRC antibody comprises the heavy chain amino acid sequence of SEQ ID NO:94 or a sequence that is at least 95% identical to SEQ ID

NO:94, and the light chain amino acid sequence of SEQ ID NO:95 or a sequence that is at least 95% identical to SEQ ID NO:95. In some embodiments, the anti-TFRC antibody comprises the heavy chain amino acid sequence of SEQ ID NO:94 and the light chain amino acid sequence of SEQ ID NO:95, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-TFRC antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:94 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:95.

[278] In some embodiments, the anti-EPCAM antibody comprises the heavy chain amino acid sequence of SEQ ID NO:96 or a sequence that is at least 95% identical to SEQ ID NO:96, and the light chain amino acid sequence of SEQ ID NO:97 or a sequence that is at least 95% identical to SEQ ID NO:97. In some embodiments, the anti-EPCAM antibody comprises the heavy chain amino acid sequence of SEQ ID NO:96 and the light chain amino acid sequence of SEQ ID NO:97, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-TFRC antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:96 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:97. In some embodiments, the anti-EPCAM antibody is oportuzumab, or an antigen-binding fragment thereof.

[279] In some embodiments, the anti-FOLR1 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:98 or a sequence that is at least 95% identical to SEQ ID NO:98, and the light chain amino acid sequence of SEQ ID NO:99 or a sequence that is at least 95% identical to SEQ ID NO:99. In some embodiments, the anti- FOLR1 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:98 and the light chain amino acid sequence of SEQ ID NO:99, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-FOLR1 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:98 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:99. In some embodiments, the anti-FOLR1 antibody is mirvetuximab, or an antigen-binding fragment thereof.

[280] In some embodiments, the anti-ENPP3 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:100 or a sequence that is at least 95% identical to SEQ ID NO:100, and the light chain amino acid sequence of SEQ ID NO:101 or a sequence that is at least 95% identical to SEQ ID NO:101. In some embodiments, the anti-ENPP3 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:100 and the light chain amino acid sequence of SEQ ID NO:101, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-ENPP3 antibody has a heavy chain

amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:100 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:101.

[281] In some embodiments, the anti-MET antibody comprises the heavy chain amino acid sequence of SEQ ID NO:102 or a sequence that is at least 95% identical to SEQ ID NO:102, and the light chain amino acid sequence of SEQ ID NO:103 or a sequence that is at least 95% identical to SEQ ID NO:103. In some embodiments, the anti-MET antibody comprises the heavy chain amino acid sequence of SEQ ID NO:102 and the light chain amino acid sequence of SEQ ID NO:103, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-MET antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:102 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:103. In some embodiments, the anti-MET antibody is telisotuzumab, or an antigen-binding fragment thereof.

[282] In some embodiments, the anti-AXL antibody comprises the heavy chain amino acid sequence of SEQ ID NO:104 or a sequence that is at least 95% identical to SEQ ID NO:104, and the light chain amino acid sequence of SEQ ID NO:105 or a sequence that is at least 95% identical to SEQ ID NO:105. In some embodiments, the anti-AXL antibody comprises the heavy chain amino acid sequence of SEQ ID NO:104 and the light chain amino acid sequence of SEQ ID NO:105, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-AXL antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:104 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:105. In some embodiments, the anti-AXL antibody is enapotamab, or an antigen-binding fragment thereof.

[283] In some embodiments, the anti-SLC34A2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:106 or a sequence that is at least 95% identical to SEQ ID NO:106, and the light chain amino acid sequence of SEQ ID NO:107 or a sequence that is at least 95% identical to SEQ ID NO:107. In some embodiments, the anti-SLC34A2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:106 and the light chain amino acid sequence of SEQ ID NO:107, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-SLC34A2 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:106 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:107. In some embodiments, the anti-SLC34A2 antibody is lifastuzumab, or an antigen-binding fragment thereof.

[284] In some embodiments, the anti-NECTIN4 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:108 or a sequence that is at least 95% identical to SEQ ID NO:108, and the light chain amino acid sequence of SEQ ID NO:109 or a sequence that is at least 95% identical to SEQ ID NO:109. In some embodiments, the anti-NECTIN4 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:108 and the light chain amino acid sequence of SEQ ID NO:109, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-NECTIN4 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:108 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:109. In some embodiments, the anti-NECTIN4 antibody is enfortumab, or an antigen-binding fragment thereof.

[285] In some embodiments, the anti-TACSTD2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:110 or a sequence that is at least 95% identical to SEQ ID NO:110, and the light chain amino acid sequence of SEQ ID NO:111 or a sequence that is at least 95% identical to SEQ ID NO:111. In some embodiments, the anti-TACSTD2 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:110 and the light chain amino acid sequence of SEQ ID NO:111, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-TACSTD2 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:110 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:111. In some embodiments, the anti-TACSTD2 antibody is sacituzumab, or an antigen-binding fragment thereof.

[286] In some embodiments, the anti-SLC39A6 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:112 or a sequence that is at least 95% identical to SEQ ID NO:112, and the light chain amino acid sequence of SEQ ID NO:113 or a sequence that is at least 95% identical to SEQ ID NO:113. In some embodiments, the anti-SLC39A6 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:112 and the light chain amino acid sequence of SEQ ID NO:113, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-SLC39A6 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:112 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:113. In some embodiments, the anti-SLC39A6 antibody is ladiratuzumab, or an antigen-binding fragment thereof.

[287] In some embodiments, the anti-GPNMB antibody comprises the heavy chain amino acid sequence of SEQ ID NO:114 or a sequence that is at least 95% identical to SEQ ID NO:114, and the light chain amino acid sequence of SEQ ID NO:115 or a sequence that is at least 95% identical to SEQ ID NO:115. In some embodiments, the anti-GPNMB antibody comprises the heavy chain amino acid sequence of SEQ ID NO:114 and the light chain amino acid sequence of SEQ ID NO:115, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-GPNMB antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:114 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:115. In some embodiments, the anti-GPNMB antibody is glembatumumab, or an antigen-binding fragment thereof.

[288] In some embodiments, the anti-MSLN antibody comprises the heavy chain amino acid sequence of SEQ ID NO:116 or a sequence that is at least 95% identical to SEQ ID NO:116, and the light chain amino acid sequence of SEQ ID NO:117 or a sequence that is at least 95% identical to SEQ ID NO:117. In some embodiments, the anti-MSLN antibody comprises the heavy chain amino acid sequence of SEQ ID NO:116 and the light chain amino acid sequence of SEQ ID NO:117, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-MSLN antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:116 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:117. In some embodiments, the anti-MSLN antibody is anetumab, or an antigen-binding fragment thereof.

[289] In some embodiments, the anti-CD74 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:118 or a sequence that is at least 95% identical to SEQ ID NO:118, and the light chain amino acid sequence of SEQ ID NO:119 or a sequence that is at least 95% identical to SEQ ID NO:119. In some embodiments, the anti-CD74 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:118 and the light chain amino acid sequence of SEQ ID NO:119, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD74 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:118 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:119. In some embodiments, the anti-CD74 antibody is milatuzumab, or an antigen-binding fragment thereof.

[290] In some embodiments, the anti-F3 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:120 or a sequence that is at least 95% identical to SEQ ID NO:120,

and the light chain amino acid sequence of SEQ ID NO:121 or a sequence that is at least 95% identical to SEQ ID NO:121. In some embodiments, the anti-F3 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:120 and the light chain amino acid sequence of SEQ ID NO:121, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-F3 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:120 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:121. In some embodiments, the anti-F3 antibody is tisotumab, or an antigen-binding fragment thereof.

[291] In some embodiments, the anti-MUC16 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:122 or a sequence that is at least 95% identical to SEQ ID NO:122, and the light chain amino acid sequence of SEQ ID NO:123 or a sequence that is at least 95% identical to SEQ ID NO:123. In some embodiments, the anti-MUC16 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:122 and the light chain amino acid sequence of SEQ ID NO:123, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-MUC16 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:122 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:123. In some embodiments, the anti-MUC16 antibody is tisotumab, or an antigen-binding fragment thereof.

[292] In some embodiments, the anti-CD7 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:143 or a sequence that is at least 95% identical to SEQ ID NO:143, and the light chain amino acid sequence of SEQ ID NO:144 or a sequence that is at least 95% identical to SEQ ID NO:144. In some embodiments, the anti-CD7 antibody comprises the heavy chain amino acid sequence of SEQ ID NO:143 and the light chain amino acid sequence of SEQ ID NO:144, or sequences that are at least 95% identical to the disclosed sequences. In some embodiments, the anti-CD7 antibody has a heavy chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:143 and a light chain amino acid sequence that is at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:144.

[293] Residues in two or more polypeptides are said to "correspond" if the residues occupy an analogous position in the polypeptide structures. Analogous positions in two or more polypeptides can be determined by aligning the polypeptide sequences based on amino acid sequence or structural similarities. Those skilled in the art understand that it may be necessary to introduce gaps in either sequence to produce a satisfactory alignment.

[294] In some embodiments, amino acid substitutions are of single residues. Insertions usually will be on the order of from about 1 to about 20 amino acid residues, although considerably larger insertions may be tolerated as long as biological function is retained (e.g., binding to a target antigen). Deletions usually range from about 1 to about 20 amino acid residues, although in some cases deletions may be much larger. Substitutions, deletions, insertions, or any combination thereof may be used to arrive at a final derivative or variant. Generally, these changes are done on a few amino acids to minimize the alteration of the molecule, particularly the immunogenicity and specificity of the antigen binding protein. However, larger changes may be tolerated in certain circumstances. Conservative substitutions can be made in accordance with the following chart depicted as Table 7.

Table 7

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

[295] In some embodiments where variant antibody sequences are used in an ADC, the variants typically exhibit the same qualitative biological activity and will elicit the same immune response, although variants may also be selected to modify the characteristics of the antigen binding proteins as needed. Alternatively, the variant may be designed such that

the biological activity of the antigen binding protein is altered. For example, glycosylation sites may be altered or removed.

[296] Various antibodies may be used with the ADCs used herein to target cancer cells. As shown below, the linker-payloads in the ADCs disclosed herein are surprisingly effective with different tumor antigen-targeting antibodies. Suitable antigens expressed on cancer cells but not healthy cells, or expressed on cancer cells at a higher level than on healthy cells, are known in the art, as are antibodies directed against them. Further antibodies against those antigen targets may be prepared by those of skill in the art. These antibodies may be used with the linkers and Bcl-xL inhibitor payloads disclosed herein. In some embodiments, the antibody or antigen-binding fragment targets BCMA, and the BCMA-targeting antibody or antigen-binding fragment is J6M0. In some embodiments, the antibody or antigen-binding fragment targets CD33, and in some embodiments the CD33-targeting antibody or antigen-binding fragment is MuMy9-6ch. In some embodiments, the antibody or antigen-binding fragment targets PCAD, and in some embodiments the PCAD-targeting antibody or antigen-binding fragment is NOV169N31Q. In some embodiments, the antibody or antigen-binding fragment targets HER2, and in some embodiments the HER2-targeting antibody or antigen-binding fragment is trastuzumab. In some embodiments, while the disclosed linkers and Bcl-xL inhibitor payloads are surprisingly effective with several different tumor-targeting antibodies, BCMA-targeting antibodies such as J6M0, CD33-targeting antibodies such as MuMy9-6ch, PCAD-targeting antibodies such as NOV169N31Q, and HER2-targeting antibodies such as trastuzumab, provided particularly improved drug:antibody ratio, aggregation level, stability (i.e., *in vitro* and *in vivo* stability), tumor targeting (i.e., cytotoxicity, potency), minimized off-target killing, and/or treatment efficacy. Improved treatment efficacy can be measured *in vitro* or *in vivo*, and may include reduced tumor growth rate and/or reduced tumor volume.

[297] In some embodiments, alternate antibodies to the same targets or antibodies to different antigen targets are used and provide at least some of the favorable functional properties described above (e.g., improved stability, improved tumor targeting, improved treatment efficacy, etc.). In some embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and Bcl-xL inhibitor payloads are conjugated to an alternate EGFR, CD7, or HER2-targeting antibody or antigen-binding fragment. In some other embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and Bcl-xL inhibitor payloads are conjugated to a EGFR-targeting antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment targets EGFR. In some embodiments, the EGFR-targeting antibody or antigen-binding fragment is J6M0. In other embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and Bcl-xL inhibitor

payloads are conjugated to a CD7-targeting antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment targets CD7. In some embodiments, the CD7-targeting antibody or antigen-binding fragment is MuMy9-6ch. In other embodiments, some or all of these favorable functional properties are observed when the disclosed linkers and Bcl-xL inhibitor payloads are conjugated to an HER2-targeting antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment targets HER2. In some embodiments, the HER2-targeting antibody or antigen-binding fragment is trastuzumab.

Linkers

[298] In some embodiments, the linker in an ADC is stable extracellularly in a sufficient manner to be therapeutically effective. In some embodiments, the linker is stable outside a cell, such that the ADC remains intact when present in extracellular conditions (e.g., prior to transport or delivery into a cell). The term "intact," used in the context of an ADC, means that the antibody or antigen-binding fragment remains attached to the drug moiety (e.g., the Bcl-xL inhibitor).

[299] As used herein, "stable," in the context of a linker or ADC comprising a linker, means that no more than 20%, no more than about 15%, no more than about 10%, no more than about 5%, no more than about 3%, or no more than about 1% of the linkers (or any percentage in between) in a sample of ADC are cleaved (or in the case of an overall ADC are otherwise not intact) when the ADC is present in extracellular conditions. In some embodiments, the linkers and/or ADCs disclosed herein are stable compared to alternate linkers and/or ADCs with alternate linkers and/or Bcl-xL inhibitor payloads. In some embodiments, the ADCs disclosed herein can remain intact for more than about 48 hours, more than 60 hours, more than about 72 hours, more than about 84 hours, or more than about 96 hours.

[300] Whether a linker is stable extracellularly can be determined, for example, by including an ADC in plasma for a predetermined time period (e.g., 2, 4, 6, 8, 16, 24, 48, or 72 hours) and then quantifying the amount of free drug moiety present in the plasma. Stability may allow the ADC time to localize to target cancer cells and prevent the premature release of the drug moiety, which could lower the therapeutic index of the ADC by indiscriminately damaging both normal and cancer tissues. In some embodiments, the linker is stable outside of a target cell and releases the drug moiety from the ADC once inside of the cell, such that the drug can bind to its target. Thus, an effective linker will: (i) maintain the specific binding properties of the antibody or antigen-binding fragment; (ii) allow delivery, e.g., intracellular delivery, of the drug moiety via stable attachment to the antibody or antigen-binding fragment; (iii) remain stable and intact until the ADC has been transported or

delivered to its target site; and (iv) allow for the therapeutic effect, e.g., cytotoxic effect, of the drug moiety after cleavage or alternate release mechanism.

[301] Linkers may impact the physico-chemical properties of an ADC. As many cytotoxic agents are hydrophobic in nature, linking them to the antibody with an additional hydrophobic moiety may lead to aggregation. ADC aggregates are insoluble and often limit achievable drug loading onto the antibody, which can negatively affect the potency of the ADC. Protein aggregates of biologics, in general, have also been linked to increased immunogenicity. As shown below, linkers disclosed herein result in ADCs with low aggregation levels and desirable levels of drug loading.

[302] A linker may be "cleavable" or "non-cleavable" (Ducry and Stump (2010) Bioconjugate Chem. 21:5-13). Cleavable linkers are designed to release the drug moiety (e.g., a Bcl-xL inhibitor) when subjected to certain environment factors, e.g., when internalized into the target cell, whereas non-cleavable linkers generally rely on the degradation of the antibody or antigen-binding fragment itself.

[303] The term "alkyl", as used herein, refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation. The term "C_1-C_6alkyl", as used herein, refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to six carbon atoms, and which is attached to the rest of the molecule by a single bond. Non-limiting examples of "C_1-C_6alkyl" groups include methyl (a C_1alkyl), ethyl (a C_2alkyl), 1-methylethyl (a C_3alkyl), n-propyl (a C_3alkyl), isopropyl (a C_3alkyl), n-butyl (a C_4alkyl), isobutyl (a C_4alkyl), sec-butyl (a C_4alkyl), tert-butyl (a C_4alkyl), n-pentyl (a C_5alkyl), isopentyl (a C_5alkyl), neopentyl (a C_5alkyl) and hexyl (a C_6alkyl).

[304] The term “alkenyl”, as used herein, refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond. The term “C_2-C_6alkenyl”, as used herein, refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, having from two to six carbon atoms, which is attached to the rest of the molecule by a single bond. Non-limiting examples of "C_2-C_6alkenyl" groups include ethenyl (a C_2alkenyl), prop-1-enyl (a C_3alkenyl), but-1-enyl (a C_4alkenyl), pent-1-enyl (a C_5alkenyl), pent-4-enyl (a C_5alkenyl), penta-1,4-dienyl (a C_5alkenyl), hexa-1-enyl (a C_6alkenyl), hexa-2-enyl (a C_6alkenyl), hexa-3-enyl (a C_6alkenyl), hexa-1-,4-dienyl (a C_6alkenyl), hexa-1-,5-dienyl (a C_6alkenyl) and hexa-2-,4-dienyl (a C_6alkenyl). The term “C_2-C_3alkenyl”, as used herein, refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, having from two to three carbon atoms, which is attached to the rest of the molecule by a single

bond. Non-limiting examples of "C_2-C_3alkenyl" groups include ethenyl (a C_2alkenyl) and prop-1-enyl (a C_3alkenyl).

[305] The term "alkylene", as used herein, refers to a bivalent straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms and containing no unsaturation. The term "C_1-C_6alkylene", as used herein, refers to a bivalent straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to six carbon atoms. Non-limiting examples of "C_1-C_6alkylene" groups include methylene (a C_1alkylene), ethylene (a C_2alkylene), 1-methylethylene (a C_3alkylene), n-propylene (a C_3alkylene), isopropylene (a C_3alkylene), n-butylene (a C_4alkylene), isobutylene (a C_4alkylene), sec-butylene (a C_4alkylene), tert-butylene (a C_4alkylene), n-pentylene (a C_5alkylene), isopentylene (a C_5alkylene), neopentylene (a C_5alkylene), and hexylene (a C_6alkylene).

[306] The term “alkenylene”, as used herein, refers to a bivalent straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms and containing at least one double bond. The term “C_2-C_6alkenylene”, as used herein, refers to a bivalent straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, and having from two to six carbon atoms. Non-limiting examples of "C_2-C_6alkenylene" groups include ethenylene (a C_2alkenylene), prop-1-enylene (a C_3alkenylene), but-1-enylene (a C_4alkenylene), pent-1-enylene (a C_5alkenylene), pent-4-enylene (a C_5alkenylene), penta-1,4-dienylene (a C_5alkenylene), hexa-1-enylene (a C_6alkenylene), hexa-2-enylene (a C_6alkenylene), hexa-3-enylene (a C_6alkenylene), hexa-1-,4-dienylene (a C_6alkenylene), hexa-1-,5-dienylene (a C_6alkenylene) and hexa-2-,4-dienylene (a C_6alkenylene). The term “C_2-C_6alkenylene”, as used herein, refers to a bivalent straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, and having from two to thee carbon atoms. Non-limiting examples of "C_2-C_3alkenylene" groups include ethenylene (a C_2alkenylene) and prop-1-enylene (a C_3alkenylene).

[307] The term “cycloalkyl,” or “C_3-C_8cycloalkyl,” as used herein, refers to a saturated, monocyclic, fused bicyclic, fused tricyclic or bridged polycyclic ring system. Non-limiting examples of fused bicyclic or bridged polycyclic ring systems include bicyclo[1.1.1]pentane, bicyclo[2.1.1]hexane, bicyclo[2.2.1]heptane, bicyclo[3.1.1]heptane, bicyclo[3.2.1]octane, bicyclo[2.2.2]octane and adamantanyl. Non-limiting examples monocyclic C_3-C_8cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl groups.

[308] The term "aryl" as used herein, refers to a phenyl, naphthyl, biphenyl or indenyl group.

[309] The term "heteroaryl" as used herein, refers any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 4 hetero atoms selected from oxygen, sulphur and nitrogen (including quaternary nitrogens).

[310] The term "cycloalkyl" as used herein, refers to any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members, which may include fused, bridged or spiro ring systems. Non-limiting examples of fused bicyclic or bridged ring systems include bicyclo[1.1.1]pentane, bicyclo[2.1.1]hexane, bicyclo[2.2.1]heptane, bicyclo[3.1.1]heptane, bicyclo[3.2.1]octane, and bicyclo[2.2.2]octane. Non-limiting examples monocyclic C_3-C_8cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl groups.

[311] The term "heterocycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group, composed of from 3 to 10 ring members, and containing from one to 3 hetero atoms selected from oxygen, sulphur, SO, SO_2 and nitrogen, it being understood that bicyclic group may be fused or spiro type. C_3-C_8heterocycloalkyl refers to heterocycloalkyl having 3 to 8 ring carbon atoms. The heterocycloalkyl can have 4 to 10 ring members.

[312] The term heteroarylene, cycloalkylene, heterocycloalkylene mean a divalent heteroaryl, cycloalkyl and heterocycloalkyl.

[313] The term "haloalkyl," as used herein, refers to a linear or branched alkyl chain substituted with one or more halogen groups in place of hydrogens along the hydrocarbon chain. Examples of halogen groups suitable for substitution in the haloalkyl group include Fluorine, Bromine, Chlorine, and Iodine. Haloalkyl groups may include substitution with multiple halogen groups in place of hydrogens in an alkyl chain, wherein said halogen groups can be attached to the same carbon or to another carbon in the alkyl chain.

[314] As used herein, the alkyl, alkenyl, alkynyl, alkoxy, amino, aryl, heteroaryl, cycloalkyl, and heterocycloalkyl groups may be optionally substituted by 1 to 4 groups selected from optionally substituted linear or branched (C_1-C_6)alkyl, optionally substituted linear or branched (C_2-C_6)alkenyl group, optionally substituted linear or branched (C_2-C_6)alkynyl group, optionally substituted linear or branched (C_1-C_6)alkoxy, optionally substituted (C_1-C_6)alkyl-S-, hydroxy, oxo (or N-oxide where appropriate), nitro, cyano, -C(O)-OR_0', -O-C(O)-R_0', -C(O)-NR_0'R_0'', -NR_0'R_0'', -(C=NR_0')-OR_0'', linear or branched (C_1-C_6) haloalkyl, trifluoromethoxy, or halogen, wherein R_0' and R_0'' are each independently a hydrogen atom or an optionally substituted linear or branched (C_1-C_6)alkyl group, and wherein one or more of the carbon atoms of linear or branched (C_1-C_6)alkyl group is optionally deuterated.

[315] The term "polyoxyethylene", "polyethylene glycol" or "PEG", as used herein, refers to a linear chain, a branched chain or a star shaped configuration comprised of (OCH_2CH_2) groups. In certain embodiments a polyethylene or PEG group is -$(OCH_2CH_2)_t$*-, where t is 1-40 or 4-40, and where the "-" indicates the end directed toward the self-immolative spacer

and the "*-" indicates the point of attachment to a terminal end group R' where R' is OH, OCH_3 or $OCH_2CH_2C(=O)OH$. In other embodiments a polyethylene or PEG group is -$(CH_2CH_2O)_t$*-, where t is 1-40 or 4-40, and where the "-" indicates the end directed toward the self-immolative spacer and the "*-" indicates the point of attachment to a terminal end group R" where R" is H, CH_3 or $CH_2CH_2C(=O)OH$. For example, the term "PEG12" as used herein means that t is 12.

[316] The term "polyalkylene glycol", as used herein, refers to a linear chain, a branched chain or a star shaped configuration comprised of $(O(CH_2)_m)_n$ groups. In certain embodiments a polyethylene or PEG group is -$(O(CH_2)_m)_t$*-, where m is 1-10, t is 1-40 or 4-40, and where the "-" indicates the end directed toward the self-immolative spacer and the "*-" indicates the point of attachment to a terminal end group R' where R' is OH, OCH_3 or $OCH_2CH_2C(=O)OH$. In other embodiments a polyethylene or PEG group is -$((CH_2)_mO)_t$*-, where m is 1-10, t is 1-40 or 4-40, and where the "-" indicates the end directed toward the self-immolative spacer and the "*-" indicates the point of attachment to a terminal end group R" where R" is H, CH_3 or $CH_2CH_2C(=O)OH$.

[317] The term "reactive group", as used herein, is a functional group capable of forming a covalent bond with a functional group of an antibody, an antibody fragment, or another reactive group attached to an antibody or antibody fragment. Non limiting examples of such functional groups include reactive groups of Table 8 provided herein.

[318] The term "attachment group" or "coupling group", as used herein, refers to a bivalent moiety which links the bridging spacer to the antibody or fragment thereof. The attachment or coupling group is a bivalent moiety formed by the reaction between a reaction group and a functional group on the antibody or fragment thereof. Non limiting examples of such bivalent moieties include the bivalent chemical moieties given in Table 8 and Table 9 provided herein.

[319] The term "bridging spacer", as used herein, refers to one or more linker components which are covalently attached together to form a bivalent moiety which links the bivalent peptide spacer to the reactive group, links the bivalent peptide space to the coupling group, or links the attachment group to the at least one cleavable group. In certain embodiments the "bridging spacer" comprises a carboxyl group attached to the N-terminus of the bivalent peptide spacer via an amide bond.

[320] The term "spacer moiety", as used herein, refers to one or more linker components which are covalently attached together to form a moiety which links the self-immolative spacer to the hydrophilic moiety.

[321] The term "bivalent peptide spacer", as used herein, refers to bivalent linker comprising one or more amino acid residues covalently attached together to form a moiety which links the bridging spacer to the self immolative spacer. The one or more amino acid

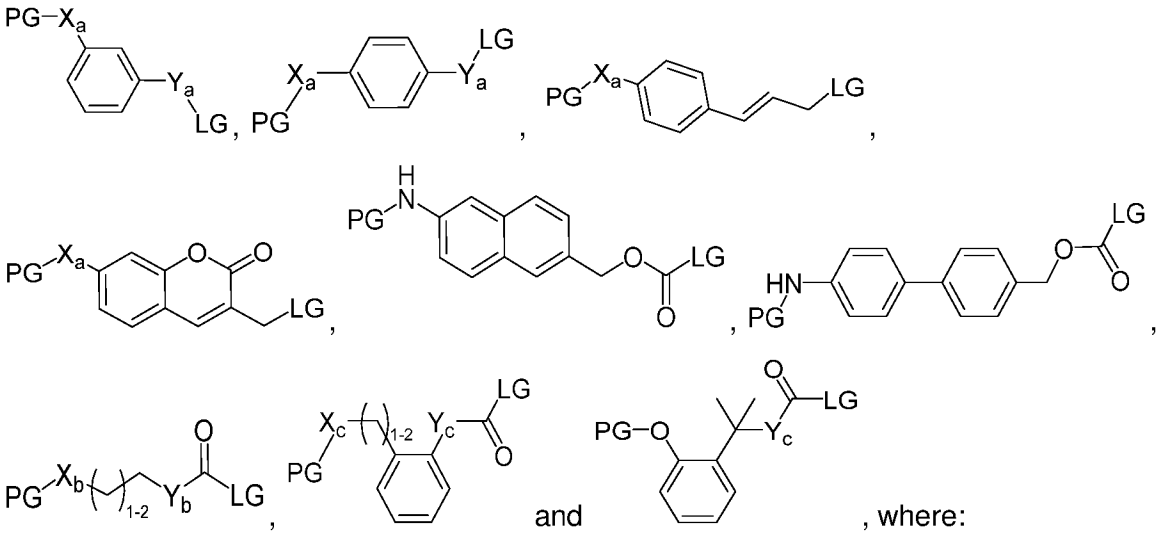
residues can be an residue of amino acids selected from alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), citrulline (Cit), norvaline (Nva), norleucune (Nle), selenocysteine (Sec), pyrrolysine (Pyl), homoserine, homocysteine, and desmethyl pyrrolysine.

[322] In certain embodiments a "bivalent peptide spacer" is a combination of 2 to four amino acid residues where each residue is independently selected from a residue of an amino acid selected from alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu),methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), citrulline (Cit), norvaline (Nva), norleucune (Nle), selenocysteine (Sec), pyrrolysine (Pyl), homoserine, homocysteine, and desmethyl pyrrolysine, for example -ValCit*; -CitVal*; -AlaAla*; -AlaCit*; -CitAla*; -AsnCit*; -CitAsn*; -CitCit*; -ValGlu*; -GluVal*; -SerCit*; -CitSer*; -LysCit*; -CitLys*; -AspCit*; -CitAsp*; -AlaVal*; -ValAla*; -PheAla*; -AlaPhe*; -PheLys*; -LysPhe*; -ValLys*; -LysVal*; -AlaLys*; -LysAla*; -PheCit*; -CitPhe*; -LeuCit*; -CitLeu*; -IleCit*; -CitIle*; -PheArg*; -ArgPhe*; -CitTrp*; -TrpCit*; -PhePheLys*; -LysPhePhe*; -DPhePheLys*; -DLysPhePhe*; -GlyPheLys*; -LysPheGly*; -GlyPheLeuGly- [SEQ ID NO:145]; -GlyLeuPheGly- [SEQ ID NO:146]; -AlaLeuAlaLeu- [SEQ ID NO:147], -GlyGlyGly*; -GlyGlyGlyGly- [SEQ ID NO:148]; -GlyPheValGly- [SEQ ID NO:149]; and –GlyValPheGly- [SEQ ID NO:150], wher the "-" indicates the point of attachment to the bridging spacer and the "*" indicates the point of attachment to the self-immolative spacer.

[323] The term "linker component", as used herein, refers to a chemical moiety that is a part of the linker. Examples of linker components include: an alkylene group: $-(CH_2)_n-$ which can either be linear or branched (where in this instance n is 1-18); an alkenylene group; an alkynylene group; an alkenyl group; an alkynyl group; an ethylene glycol unit: $-OCH_2CH_2-$ or $-CH_2CH_2O-$; an polyethylene glycol unit: $(-CH_2CH_2O-)_x$ (where x in this instance is 2-20); -O-; -S-; a carbonyl: -C(=O); an ester: C(=O)-O or O-C(=O); a carbonate: -OC(=O)O-; an amine: -NH-; an tertiary amine; an amide: -C(=O)-NH-, -NH-C(=O)- or –C(=O)N(C_{1-6}alkyl); a carbamate: -OC(=O)NH- or –NHC(=O)O; a urea: -NHC(=O)NH; a sulfonamide: $-S(O)_2NH-$ or $-NHS(O)_2$;an ether: $-CH_2O-$ or $–OCH_2-$; an alkylene substituted with one or more groups independently selected from carboxy, sulfonate, hydroxyl, amine, amino acid, saccharide, phosphate and phosphonate); an alkenylene substituted with one or more groups independently selected from carboxy, sulfonate, hydroxyl, amine, amino acid, saccharide, phosphate and phosphonate); an alkynylene substituted with one or more groups independently selected from carboxy, sulfonate, hydroxyl, amine, amino acid, saccharide,

phosphate and phosphonate); a C_1-C_{10}alkylene in which one or more methylene groups is replace by one or more –S-, -NH- or -O- moieties; a ring systems having two available points of attachment such as a divalent ring selected from phenyl (including 1,2- 1,3- and 1,4- di-substituted phenyls), a C_5-C_6 heteroaryl, a C_3-C_8 cycloalkyl (including 1,1-disubstituted cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl, and 1,4-disubstituted cyclohexyl), and a C_4-C_8 heterocycloalkyl; a residue of an amino acid selected from alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu),methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), citrulline (Cit), norvaline (Nva), norleucune (Nle), selenocysteine (Sec), pyrrolysine (Pyl), homoserine, homocysteine, and desmethyl pyrrolysine; a combination of 2 or more amino acid residues where each residue is independently selected from a residue of an amino acid selected from alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu),methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), citrulline (Cit), norvaline (Nva), norleucune (Nle), selenocysteine (Sec), pyrrolysine (Pyl), homoserine, homocysteine, and desmethyl pyrrolysine, for example Val-Cit; Cit-Val; Ala-Ala; Ala-Cit; Cit-Ala; Asn-Cit; Cit-Asn; Cit-Cit; Val-Glu; Glu-Val; Ser-Cit; Cit-Ser; Lys-Cit; Cit-Lys; Asp-Cit; Cit-Asp; Ala-Val; Val-Ala; Phe-Lys; Lys-Phe; Val-Lys; Lys-Val; Ala-Lys; Lys-Ala; Phe-Cit; Cit-Phe; Leu-Cit; Cit-Leu; Ile-Cit; Cit-Ile; Phe-Arg; Arg-Phe; Cit-Trp; and Trp-Cit; and a self-immolative spacer, wherein the self-immolative spacer comprises one or more protecting (triggering) groups which are susceptible to acid-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, glycosidase induced cleavage, phosphodiesterase induced cleavage, phosphatase induced cleavage, protease induced cleavage, lipase induced cleavage or disulfide bond cleavage.

[324] Non-limiting examples of such self-immolative spacers include:



and , where:

PG is a protecting (triggering) group;

X_a is O, NH or S;

X_b is O, NH, NCH_3 or S;

X_c is O or NH;

Y_a is CH_2, CH_2O or CH_2NH;

Y_b is CH_2, O or NH;

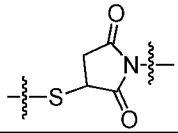
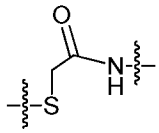
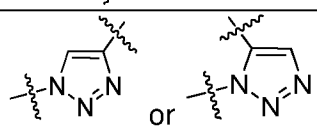
Y_c is a bond, CH_2, O or NH, and

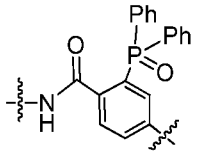
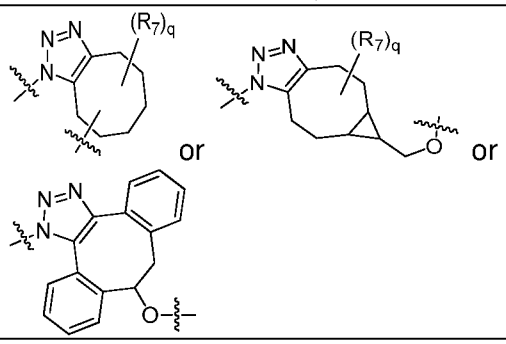
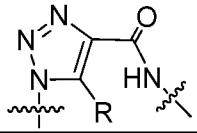
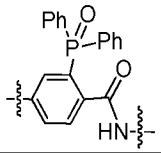
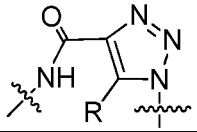
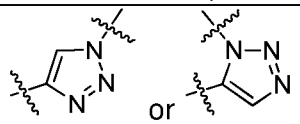
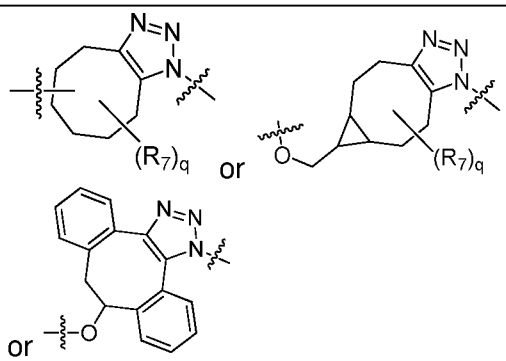
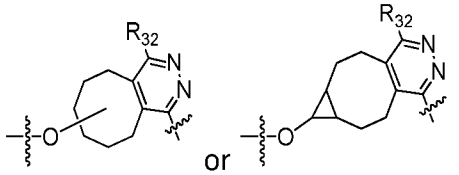
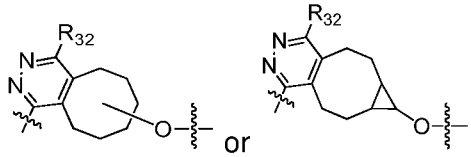
LG is a leaving group such as a Drug moiety (D) of the Linker-Drug group of the invention.

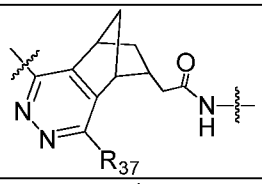
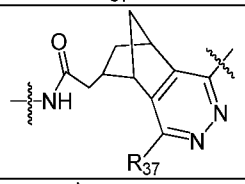
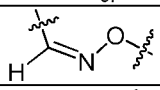
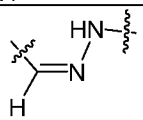
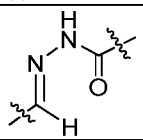
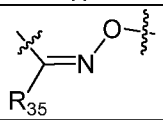
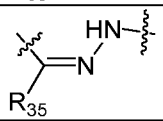
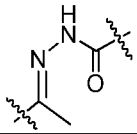
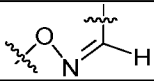
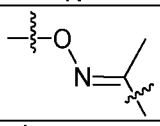
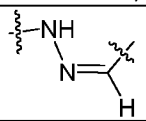
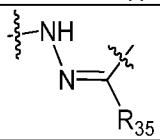
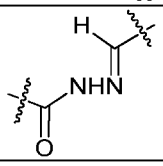
Additional non-limiting examples of such self-immolative spacers are described in Angew. Chem. Int. Ed. 2015, 54, 7492 – 7509.

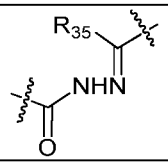
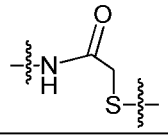
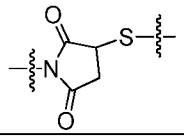
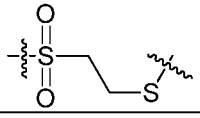
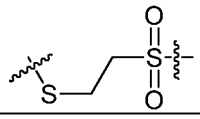
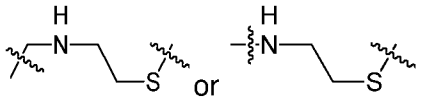
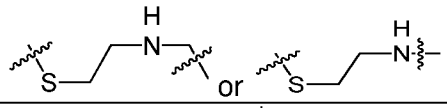
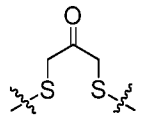
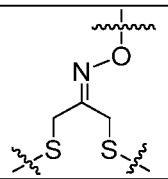
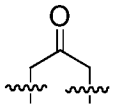
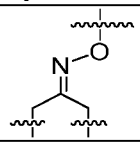
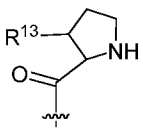
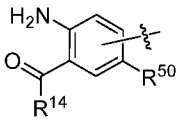
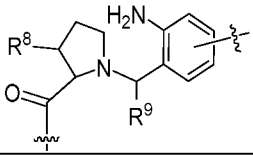
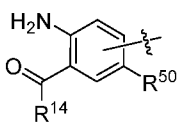
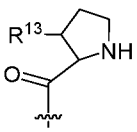
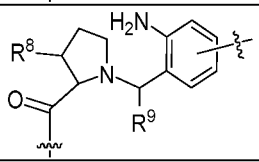
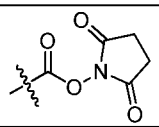
[325] In addition, a linker component can be a chemical moiety which is readily formed by reaction between two reactive groups. Non-limiting examples of such chemical moieties are given in Table 8.

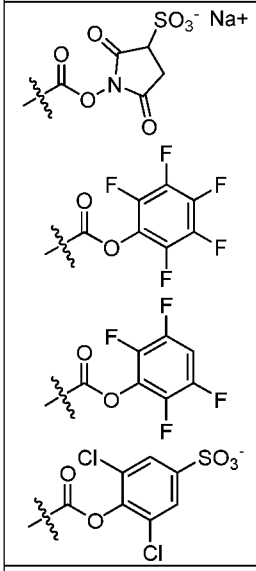
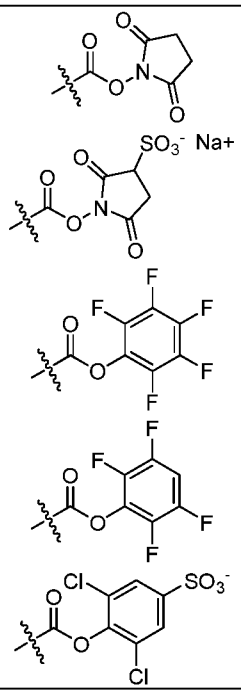
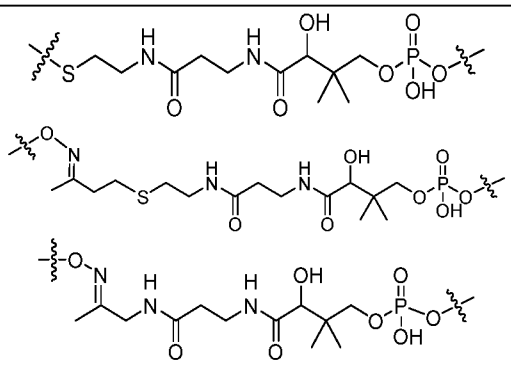
Table 8

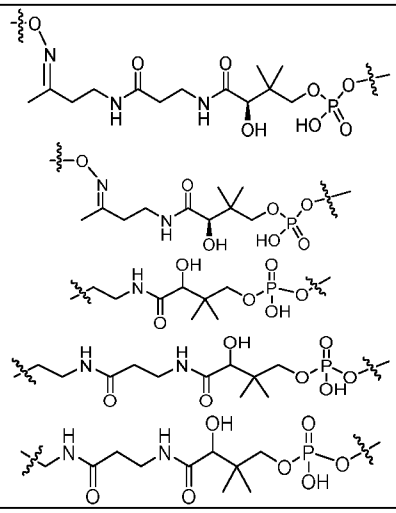
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
a thiol	a thiol	-S-S-
a thiol	a maleimide	
a thiol	a haloacetamide	
an azide	an alkyne	

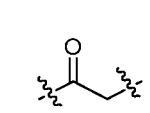
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
an azide	a triaryl phosphine	
an azide	a cyclooctyne	
an azide	an oxanobornadiene	
a triaryl phosphine	an azide	
an oxanobornadiene	an azide	
an alkyne	an azide	
a cyclooctyne	azide	
a cyclooctene	a diaryl tetrazine	
a diaryl tetrazine	a cyclooctene	

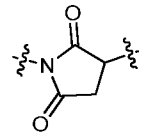
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
a monoaryl tetrazine	a norbornene	
a norbornene	a monoaryl tetrazine	
an aldehyde	a hydroxylamine	
an aldehyde	a hydrazine	
an aldehyde	NH_2-NH-C(=O)-	
a ketone	a hydroxylamine	
a ketone	a hydrazine	
a ketone	NH_2-NH-C(=O)-	
a hydroxylamine	an aldehyde	
a hydroxylamine	a ketone	
a hydrazine	an aldehyde	
a hydrazine	a ketone	
NH_2-NH-C(=O)-	an aldehyde	

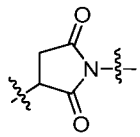
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
NH_2-NH-C(=O)-	a ketone	
a haloacetamide	a thiol	
a maleimide	a thiol	
a vinyl sulfone	a thiol	
a thiol	a vinyl sulfone	
an aziridine	a thiol	
a thiol	an aziridine	
	hydroxylamine	
	hydroxylamine	
		
		
	-NH_2,	amide

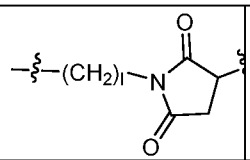
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
		
-NH_2,		amide
CoA or CoA analogue	Serine residue	

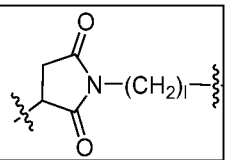


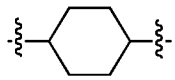


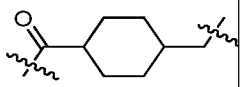


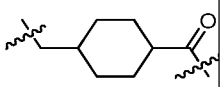


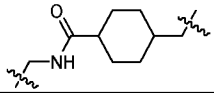


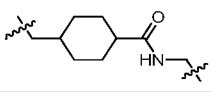


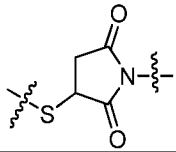


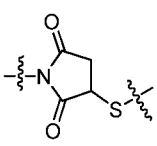


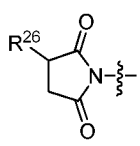


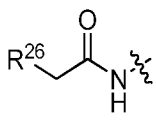


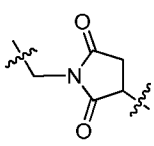


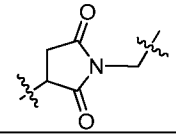


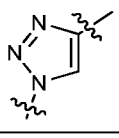


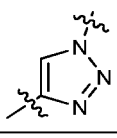


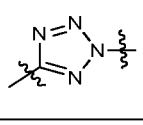


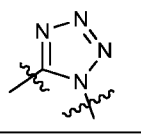










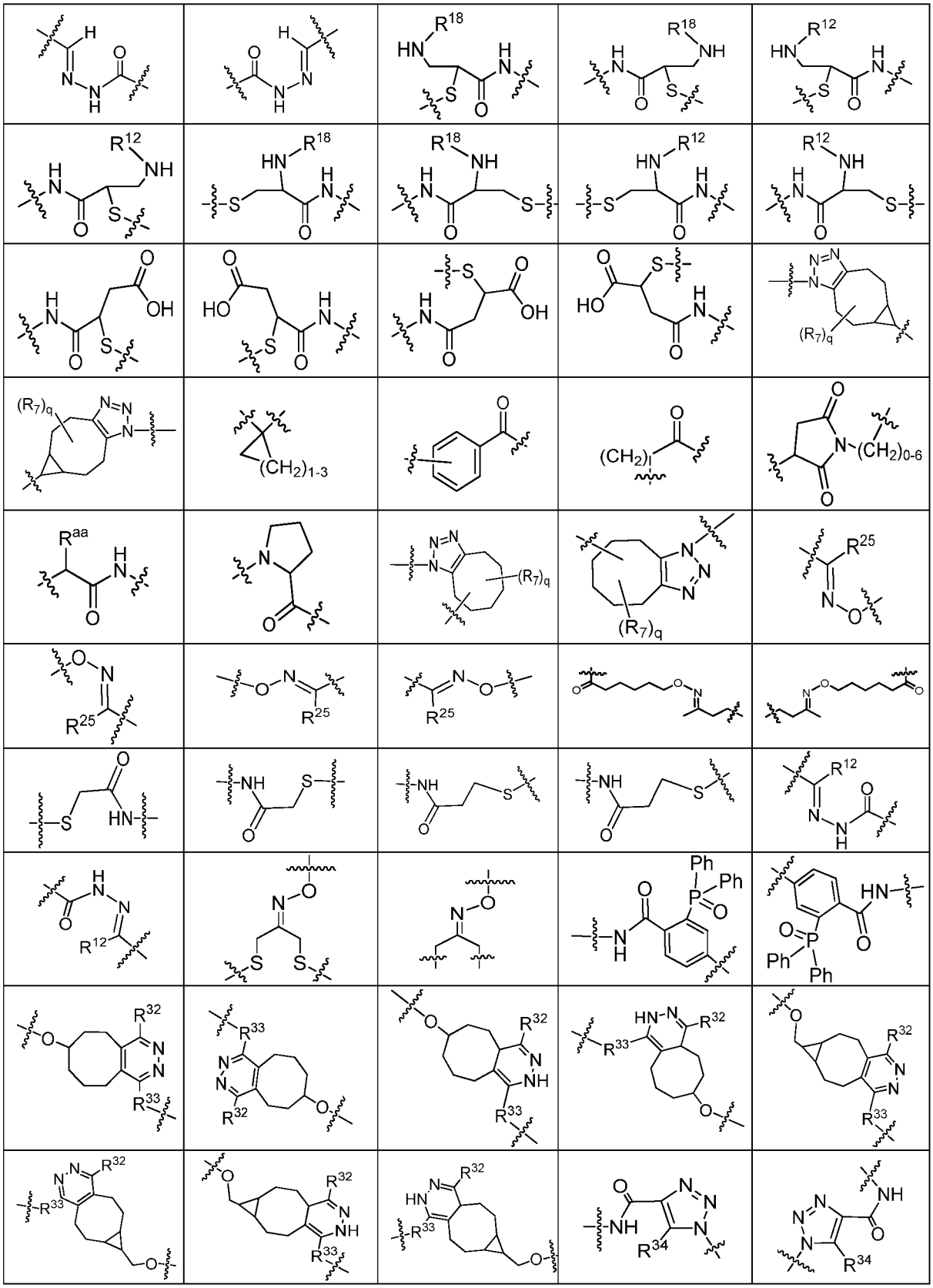


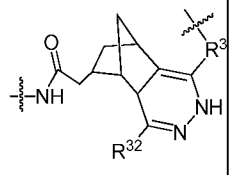
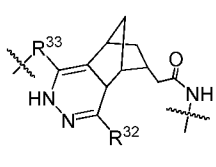
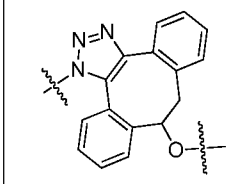
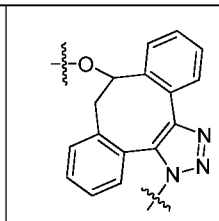
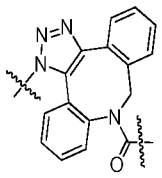
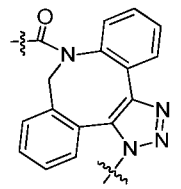
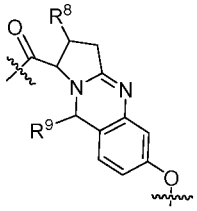
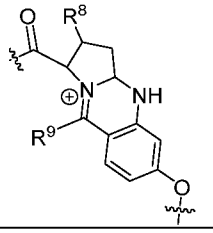
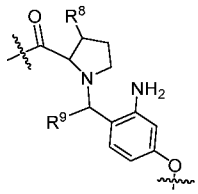
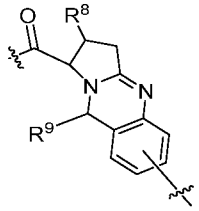
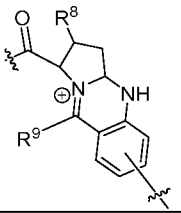
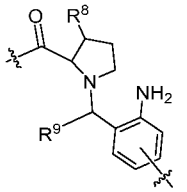
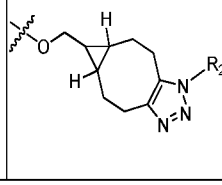
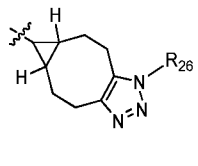
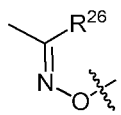
Reactive Group 1 (RG1)	Reactive Group 2 (RG2)	Chemical Moiety
pyridyldithiol	thiol	disulfide

where: R^{32} in Table 8 is H, C_{1-4} alkyl, phenyl, pyrimidine or pyridine; R^{35} in Table 8 is H, C_{1-6}alkyl, phenyl or C_{1-4}alkyl substituted with 1 to 3 –OH groups; each R^7 in Table 8 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH; R^{37} in Table 8 is independently selected from H, phenyl and pyridine; q in Table 8 is 0, 1, 2 or 3; R^8 and R^{13} in Table 8 is H or methyl; and R^9 and R^{14} in Table 8 is H, $-CH_3$ or phenyl; R in Table 8 is H or any suitable substituent; and R^{50} in Table 8 is H.

[326] In addition, a linker component can be a group listed in Table 9 below.

Table 9.



each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH;

each R^{12} is independently selected from H and C_1-C_6alkyl

R^8 is H or methyl;

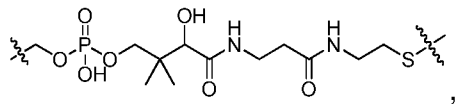
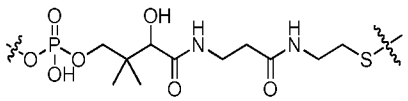
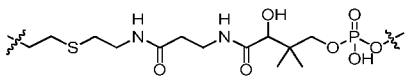
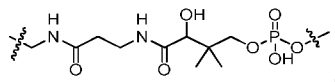
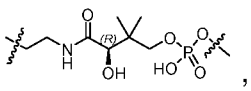
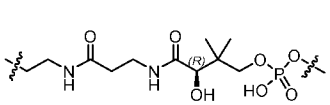
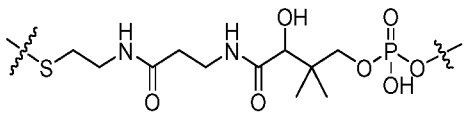
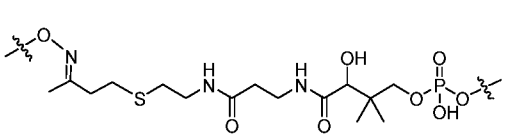
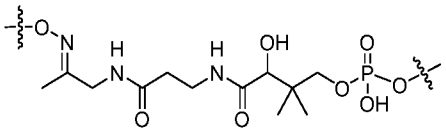
R^9 is H, -CH_3 or phenyl;

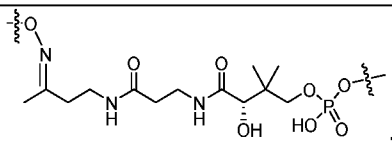
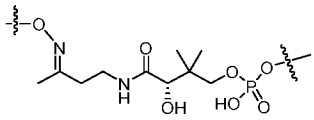
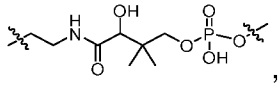
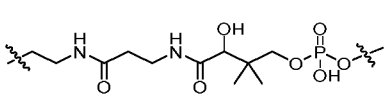
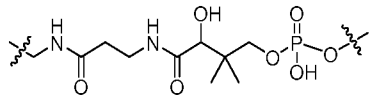
each R^{25} is independently selected from H or C_{1-4} alkyl;

each R^{18} is independently selected from a C_1-C_6alkyl, a C_1-C_6alkyl which is substituted with azido and a C_1-C_6alkyl which is substituted with 1 to 5 hydroxyl;

q is 0, 1, 2 or 3;

l is 1, 2, 3, 4, 5 or 6;

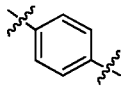
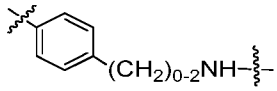
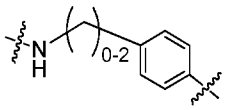
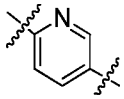
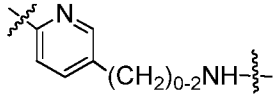
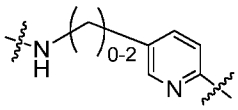
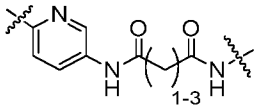
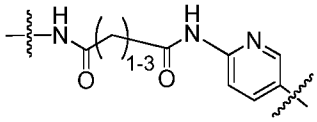
R^{26} is  ,  ,  ,  ,  ,  ,  ,  , 

, or

R^{32} is independently selected from H, C_{1-4} alkyl, phenyl, pyrimidine and pyridine;

R^{33} is independently selected from

, and ;

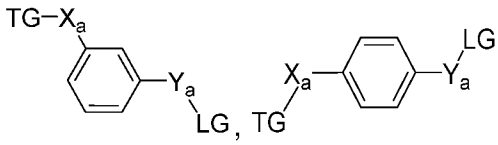
R^{34} is independently selected from H, C_{1-4} alkyl, and C_{1-6} haloalkyl, and

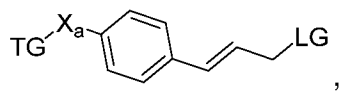
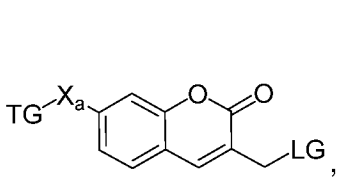
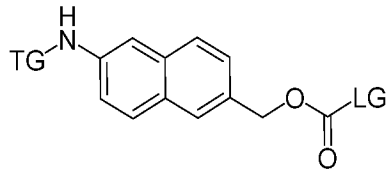
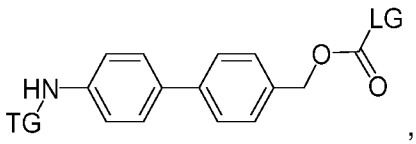
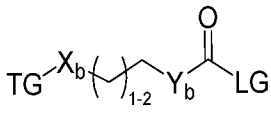
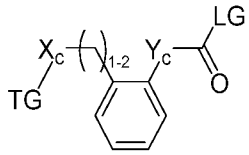
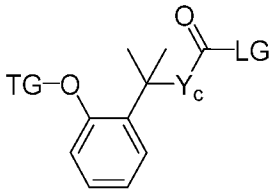
R^{aa} is an amino acid side chain.

[327] As used herein, when a partial structure of a compound is illustrated, a wavy line ( ) indicates the point of attachment of the partial structure to the rest of the molecule.

[328] The terms “self-immolative spacer” and “self-immolative group”, as used herein, refer a moiety comprising one or more triggering groups (TG) which are activated by acid-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, glycosidase induced cleavage, phosphodiesterase induced cleavage, phosphatase induced cleavage, protease induced cleavage, lipase induced cleavage or disulfide bond cleavage, and after activation the protecting group is removed, which generates a cascade of disassembling reactions leading to the temporally sequential release of a leaving group. Such cascade of reactions can be, but not limited to, 1,4-, 1,6- or 1,8- elimination reactions.

[329] Non-limiting examples of self-immolative spacer or group include:









and

, wherein such groups can be optionally substituted, and wherein:

TG is a triggering group;

X_a is O, NH or S;

X_b is O, NH, NCH_3 or S;

X_c is O or NH;

Y_a is CH_2, CH_2O or CH_2NH;

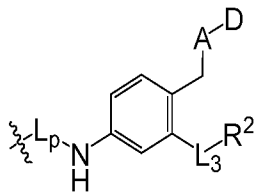
Y_b is CH_2, O or NH;

Y_c is a bond, CH_2, O or NH, and

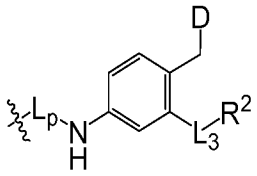
LG is a leaving group such as a Drug moiety (D) of the Linker-Drug group of the invention.

Additional non-limiting examples of self-immolative spacers are described in Angew. Chem. Int. Ed. 2015, 54, 7492 – 7509.

In certain embodiment the self-immolative spacer is moiety having the structure

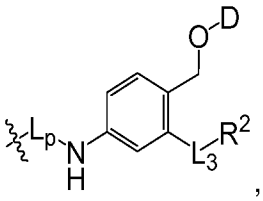

, where Lp is an enzymatically cleavable bivalent peptide spacer and A, D, L_3 and R^2 are as defined herein.

In preferred embodiments, the self-immolative spacer is moiety having the structure

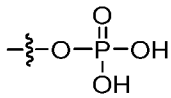


, where Lp is an enzymatically cleavable bivalent peptide spacer and D, L_3 and R^2 are as defined herein. In some embodiments, D is a quaternized tertiary amine-containing Bcl-xL inhibitor.

In other preferred embodiments, the self-immolative spacer is moiety having the structure



, where Lp is an enzymatically cleavable bivalent peptide spacer and D, L_3 and R^2 are as defined herein.

The term "hydrophilic moiety", as used herein, refers to moiety that is has hydrophilic properties which increases the aqueous solubility of the Drug moiety (D) when the Drug moiety (D) is attached to the linker group of the invention. Examples of such hydrophilic groups include, but are not limited to, polyethylene glycols, polyalkylene glycols, sugars, oligosaccharides, polypeptides a C_2-C_6alkyl substituted with 1 to 3  groups.

Drug Moieties

[330] In some embodiments, an intermediate, which is the precursor of the linker moiety, is reacted with the drug moiety (e.g., the Bcl-xL inhibitor) under appropriate conditions. In some embodiments, reactive groups are used on the drug and/or the intermediate or linker. The product of the reaction between the drug and the intermediate, or the derivatized drug (drug plus linker), is subsequently reacted with the antibody or antigen-binding fragment under conditions that facilitate conjugation of the drug and intermediate or derivatized drug and antibody or antigen-binding fragment. Alternatively, the intermediate or linker may first be reacted with the antibody or antigen-binding fragment, or a derivatized antibody or antigen-binding fragment, and then reacted with the drug or derivatized drug.

[331] A number of different reactions are available for covalent attachment of the drug moiety and/or linker moiety to the antibody or antigen-binding fragment. This is often accomplished by reaction of one or more amino acid residues of the antibody or antigen-binding fragment, including the amine groups of lysine, the free carboxylic acid groups of glutamic acid and aspartic acid, the sulfhydryl groups of cysteine, and the various moieties of the aromatic amino acids. For instance, non-specific covalent attachment may be

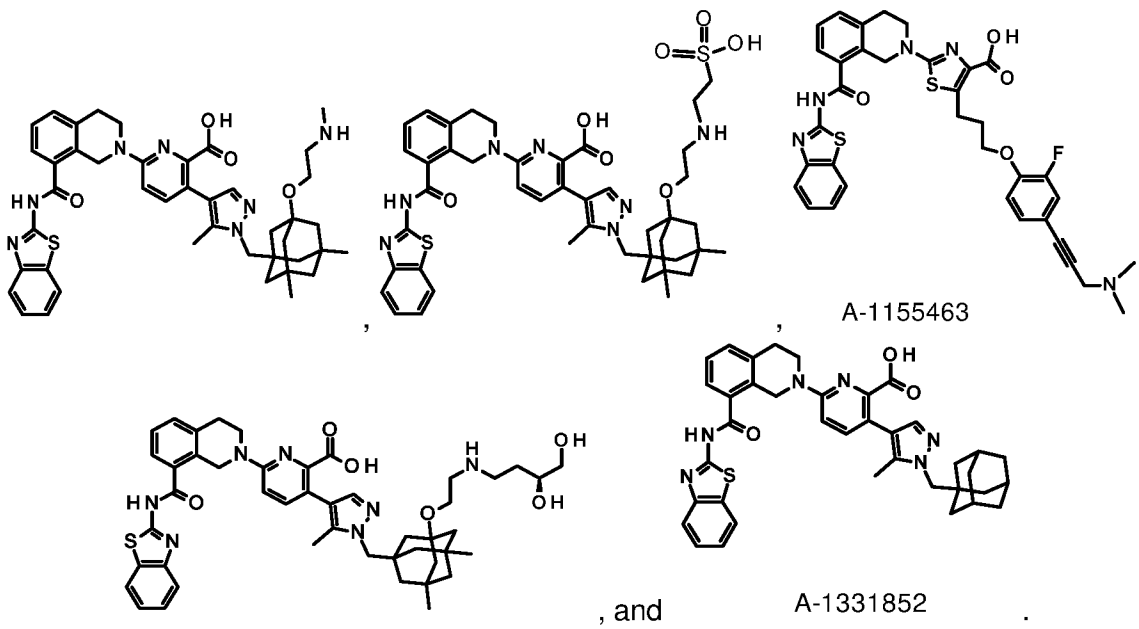
undertaken using a carbodiimide reaction to link a carboxy (or amino) group on a drug moiety to an amino (or carboxy) group on an antibody or antigen-binding fragment. Additionally, bifunctional agents such as dialdehydes or imidoesters may also be used to link the amino group on a drug moiety to an amino group on an antibody or antigen-binding fragment. Also available for attachment of drugs (e.g., a Bcl-xL inhibitor) to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates may also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present disclosure. Examples of drug moieties that can be generated and linked to an antibody or antigen-binding fragment using various chemistries known to in the art include Bcl-xL inhibitors, e.g., the Bcl-xL inhibitors described and exemplified herein.

[332] Suitable drug moieties may comprise a compound of the formulas (I), (IA), (IB), (IC), (II), (IIA), (IIB) or (IIC) or an enantiomer, diastereoisomer, and/or addition salt thereof with a pharmaceutically acceptable acid or base. Additionally, the drug moiety may comprise any compounds of the Bcl-xL inhibitor (D) described herein.

[333] In some embodiments, the drug moiety (D) comprises a formula selected from Table A2.

[334] In some embodiments, the drug moiety (D) comprises a Bcl-xL inhibitor known in the art, for example, ABT-737 and ABT-263.

[335] In some embodiments, the drug moiety (D) comprises a Bcl-xL inhibitor selected from:



, , A-1155463 , , and A-1331852 .

[336] In some embodiments, the linker-drug (or "linker-payload") moiety -(L-D) may comprise a compounds in Table B or an enantiomer, diastereoisomer, deuterated derivative, and/or a pharmaceutically acceptable salt of any of the foregoing.

Drug Loading

[337] Drug loading is represented by *p*, and is also referred to herein as the drug-to-antibody ratio (DAR). Drug loading may range from 1 to 16 drug moieties per antibody or antigen-binding fragment. In some embodiments, *p* is an integer from 1 to 16. In some embodiments, *p* is an integer from 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, *p* is an integer from 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4, or 2 to 3. In some embodiments, *p* is an integer from 1 to 16. In some embodiments, *p* is an integer from 1 to 8. In some embodiments, *p* is an integer from 1 to 5. In some embodiments, *p* is an integer from 2 to 4. In some embodiments, *p* is 1, 2, 3, 4, 5, 6, 7, or 8. In some embodiments, *p* is 2. In some embodiments, *p* is 4.

[338] Drug loading may be limited by the number of attachment sites on the antibody or antigen-binding fragment. In some embodiments, the linker moiety (L) of the ADC attaches to the antibody or antigen-binding fragment through a chemically active group on one or more amino acid residues on the antibody or antigen-binding fragment. For example, the linker may be attached to the antibody or antigen-binding fragment via a free amino, imino, hydroxyl, thiol, or carboxyl group (e.g., to the N- or C-terminus, to the epsilon amino group of one or more lysine residues, to the free carboxylic acid group of one or more glutamic acid or aspartic acid residues, or to the sulfhydryl group of one or more cysteine residues). The site to which the linker is attached can be a natural residue in the amino acid sequence of the antibody or antigen-binding fragment, or it can be introduced into the antibody or antigen-binding fragment, e.g., by DNA recombinant technology (e.g., by introducing a cysteine residue into the amino acid sequence) or by protein biochemistry (e.g., by reduction, pH adjustment, or hydrolysis).

[339] In some embodiments, the number of drug moieties that can be conjugated to an antibody or antigen-binding fragment is limited by the number of free cysteine residues. For example, where the attachment is a cysteine thiol group, an antibody may have only one or a few cysteine thiol groups, or may have only one or a few sufficiently reactive thiol groups through which a linker may be attached. Generally, antibodies do not contain many free and reactive cysteine thiol groups that may be linked to a drug moiety. Indeed, most cysteine thiol residues in antibodies are involved in either interchain or intrachain disulfide bonds. Conjugation to cysteines can therefore, in some embodiments, require at least partial reduction of the antibody. Over-attachment of linker-toxin to an antibody may destabilize the

antibody by reducing the cysteine residues available to form disulfide bonds. Therefore, an optimal drug:antibody ratio should increase potency of the ADC (by increasing the number of attached drug moieties per antibody) without destabilizing the antibody or antigen-binding fragment. In some embodiments, an optimal ratio may be 2, 4, 6, or 8. In some embodiments, an optimal ratio may be 2 or 4.

[340] In some embodiments, an antibody or antigen-binding fragment is exposed to reducing conditions prior to conjugation in order to generate one or more free cysteine residues. An antibody, in some embodiments, may be reduced with a reducing agent such as dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. Unpaired cysteines may be generated through partial reduction with limited molar equivalents of TCEP, which can reduce the interchain disulfide bonds which link the light chain and heavy chain (one pair per H-L pairing) and the two heavy chains in the hinge region (two pairs per H-H pairing in the case of human IgG1) while leaving the intrachain disulfide bonds intact (Stefano et al. (2013) Methods Mol Biol. 1045:145-71). In embodiments, disulfide bonds within the antibodies are reduced electrochemically, e.g., by employing a working electrode that applies an alternating reducing and oxidizing voltage. This approach can allow for on-line coupling of disulfide bond reduction to an analytical device (e.g., an electrochemical detection device, an NMR spectrometer, or a mass spectrometer) or a chemical separation device (e.g., a liquid chromatograph (e.g., an HPLC) or an electrophoresis device (see, e.g., US 2014/0069822)). In some embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups on amino acid residues, such as cysteine.

[341] The drug loading of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody; (ii) limiting the conjugation reaction time or temperature; (iii) partial or limiting reductive conditions for cysteine thiol modification; and/or (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachments.

[342] In some embodiments, free cysteine residues are introduced into the amino acid sequence of the antibody or antigen-binding fragment. For example, cysteine engineered antibodies can be prepared wherein one or more amino acids of a parent antibody are replaced with a cysteine amino acid. Any form of antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab referred to as a "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." A single site mutation yields a single engineered cysteine residue in a ThioFab, whereas a single site mutation yields two engineered cysteine residues in a ThioMab, due to the dimeric nature of the IgG antibody. DNA encoding an

amino acid sequence variant of the parent polypeptide can be prepared by a variety of methods known in the art (see, e.g., the methods described in WO 2006/034488). These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the polypeptide. Variants of recombinant antibodies may also be constructed by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. ADCs of Formula (1) include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon et al. (2012) Methods Enzymol. 502:123-38). In some embodiments, one or more free cysteine residues are already present in an antibody or antigen-binding fragment, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody or antigen-binding fragment to a drug moiety.

[343] Where more than one nucleophilic group reacts with a drug-linker intermediate or a linker moiety reagent followed by drug moiety reagent, in a reaction mixture comprising multiple copies of the antibody or antigen-binding fragment and linker moiety, then the resulting product can be a mixture of ADC compounds with a distribution of one or more drug moieties attached to each copy of the antibody or antigen-binding fragment in the mixture. In some embodiments, the drug loading in a mixture of ADCs resulting from a conjugation reaction ranges from 1 to 16 drug moieties attached per antibody or antigen-binding fragment. The average number of drug moieties per antibody or antigen-binding fragment (i.e., the average drug loading, or average *p*) may be calculated by any conventional method known in the art, e.g., by mass spectrometry (e.g., liquid chromatography-mass spectrometry (LC-MS)) and/or high-performance liquid chromatography (e.g., HIC-HPLC). In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is determined by liquid chromatography-mass spectrometry (LC-MS). In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is from about 1.5 to about 3.5, about 2.5 to about 4.5, about 3.5 to about 5.5, about 4.5 to about 6.5, about 5.5 to about 7.5, about 6.5 to about 8.5, or about 7.5 to about 9.5. In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is from about 2 to about 4, about 3 to about 5, about 4 to about 6, about 5 to about 7, about 6 to about 8, about 7 to about 9, about 2 to about 8, or about 4 to about 8.

[344] In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is about 2. In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.1, about 2.2, about 2.3, about 2.4, or about 2.5. In some

embodiments, the average number of drug moieties per antibody or antigen-binding fragment is 2.

[345] In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is about 4. In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4, about 4.1, about 4.2, about 4.3, about 4.4, or about 4.5. In some embodiments, the average number of drug moieties per antibody or antigen-binding fragment is 4.

[346] In some embodiments, the term "about," as used with respect to the average number of drug moieties per antibody or antigen-binding fragment, means plus or minus 20%, 15%, 10%, 5%, or 1%. In one embodiment, the term "about" refers to a range of values which are 10% more or less than the specified value. In another embodiment, the term "about" refers to a range of values which are 5% more or less than the specified value. In another embodiment, the term "about" refers to a range of values which are 1% more or less than the specified value.

[347] Individual ADC compounds, or "species," may be identified in the mixture by mass spectroscopy and separated by, e.g., UPLC or HPLC, e.g. hydrophobic interaction chromatography (HIC-HPLC). In some embodiments, a homogeneous or nearly homogenous ADC product with a single loading value may be isolated from the conjugation mixture, e.g., by electrophoresis or chromatography.

[348] In some embodiments, higher drug loading (e.g., $p > 16$) may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. Higher drug loading may also negatively affect the pharmacokinetics (e.g., clearance) of certain ADCs. In some embodiments, lower drug loading (e.g., $p < 2$) may reduce the potency of certain ADCs against target-expressing cells. In some embodiments, the drug loading for an ADC of the present disclosure ranges from about 2 to about 16, about 2 to about 10, about 2 to about 8; from about 2 to about 6; from about 2 to about 5; from about 3 to about 5; from about 2 to about 4; or from about 4 to about 8.

[349] In some embodiments, a drug loading and/or an average drug loading of about 2 is achieved, e.g., using partial reduction of intrachain disulfides on the antibody or antigen-binding fragment, and provides beneficial properties. In some embodiments, a drug loading and/or an average drug loading of about 4 or about 6 or about 8 is achieved, e.g., using partial reduction of intrachain disulfides on the antibody or antigen-binding fragment, and provides beneficial properties. In some embodiments, a drug loading and/or an average drug loading of less than about 2 may result in an unacceptably high level of unconjugated antibody species, which can compete with the ADC for binding to a target antigen and/or provide for reduced treatment efficacy. In some embodiments, a drug loading and/or

average drug loading of more than about 16 may result in an unacceptably high level of product heterogeneity and/or ADC aggregation. A drug loading and/or an average drug loading of more than about 16 may also affect stability of the ADC, due to loss of one or more chemical bonds required to stabilize the antibody or antigen-binding fragment.

[350] The present disclosure includes methods of producing the described ADCs. Briefly, the ADCs comprise an antibody or antigen-binding fragment as the antibody or antigen-binding fragment, a drug moiety (e.g., a Bcl-xL inhibitor), and a linker that joins the drug moiety and the antibody or antigen-binding fragment. In some embodiments, the ADCs can be prepared using a linker having reactive functionalities for covalently attaching to the drug moiety and to the antibody or antigen-binding fragment. In some embodiments, the antibody or antigen-binding fragment is functionalized to prepare a functional group that is reactive with a linker or a drug-linker intermediate. For example, in some embodiments, a cysteine thiol of an antibody or antigen-binding fragment can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC. In some embodiments, an antibody or antigen-binding fragment is prepared with bacterial transglutaminase (BTG) - reactive glutamines specifically functionalized with an amine containing cyclooctyne BCN (*N*-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane) moiety. In some embodiments, site-specific conjugation of a linker or a drug-linker intermediate to a BCN moiety of an antibody or antigen-binding fragment is performed, e.g., as described and exemplified herein. The generation of the ADCs can be accomplished by techniques known to the skilled artisan.

[351] In some embodiments, an ADC is produced by contacting an antibody or antigen-binding fragment with a linker and a drug moiety (e.g., a Bcl-xL inhibitor) in a sequential manner, such that the antibody or antigen-binding fragment is covalently linked to the linker first, and then the pre-formed antibody-linker intermediate reacts with the drug moiety. The antibody-linker intermediate may or may not be subjected to a purification step prior to contacting the drug moiety. In other embodiments, an ADC is produced by contacting an antibody or antigen-binding fragment with a linker-drug compound pre-formed by reacting a linker with a drug moiety. The pre-formed linker-drug compound may or may not be subjected to a purification step prior to contacting the antibody or antigen-binding fragment. In other embodiments, the antibody or antigen-binding fragment contacts the linker and the drug moiety in one reaction mixture, allowing simultaneous formation of the covalent bonds between the antibody or antigen-binding fragment and the linker, and between the linker and the drug moiety. This method of producing ADCs may include a reaction, wherein the antibody or antigen-binding fragment contacts the antibody or antigen-binding fragment prior to the addition of the linker to the reaction mixture, and vice versa. In some embodiments,

an ADC is produced by reacting an antibody or antigen-binding fragment with a linker joined to a drug moiety, such as a Bcl-xL inhibitor, under conditions that allow conjugation.

[352] The ADCs prepared according to the methods described above may be subjected to a purification step. The purification step may involve any biochemical methods known in the art for purifying proteins, or any combination of methods thereof. These include, but are not limited to, tangential flow filtration (TFF), affinity chromatography, ion exchange chromatography, any charge or isoelectric point-based chromatography, mixed mode chromatography, e.g., CHT (ceramic hydroxyapatite), hydrophobic interaction chromatography, size exclusion chromatography, dialysis, filtration, selective precipitation, or any combination thereof.

Therapeutic Uses and Compositions

[353] Disclosed herein are methods of using the compositions described herein, e.g., the disclosed ADC compounds and compositions, in treating a subject for a disorder, e.g., a cancer. Compositions, e.g., ADCs, may be administered alone or in combination with at least one additional inactive and/or active agent, e.g., at least one additional therapeutic agent, and may be administered in any pharmaceutically acceptable formulation, dosage, and dosing regimen. Treatment efficacy may be evaluated for toxicity as well as indicators of efficacy and adjusted accordingly. Efficacy measures include, but are not limited to, a cytostatic and/or cytotoxic effect observed *in vitro* or *in vivo*, reduced tumor volume, tumor growth inhibition, and/or prolonged survival.

[354] Methods of determining whether an ADC exerts a cytostatic and/or cytotoxic effect on a cell are known. For example, the cytotoxic or cytostatic activity of an ADC can be measured by, e.g., exposing mammalian cells expressing a target antigen of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 6 days; and measuring cell viability (e.g., using a CellTiter-Glo® (CTG) or MTT cell viability assay). Cell-based *in vitro* assays may also be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the ADC.

[355] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) may be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane, swelling of the cell, and rupture of the plasma membrane. Apoptosis can be quantitated, for example, by measuring DNA fragmentation. Commercial photometric methods for the quantitative *in vitro* determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica (1999) 2:34-7 (Roche Molecular Biochemicals).

[356] Apoptosis may also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by

measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al., eds. (1992) pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Apoptosis may also be determined, in some embodiments, by screening for caspase activity. In some embodiments, a Caspase-Glo® Assay can be used to measure activity of caspase-3 and caspase-7. In some embodiments, the assay provides a luminogenic caspase-3/7 substrate in a reagent optimized for caspase activity, luciferase activity, and cell lysis. In some embodiments, adding Caspase-Glo® 3/7 Reagent in an "add-mix-measure" format may result in cell lysis, followed by caspase cleavage of the substrate and generation of a "glow-type" luminescent signal, produced by luciferase. In some embodiments, luminescence may be proportional to the amount of caspase activity present, and can serve as an indicator of apoptosis. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage. Determination of any of these effects on cancer cells indicates that an ADC is useful in the treatment of cancers.

[357] Cell viability may be measured, e.g., by determining in a cell the uptake of a dye such as neutral red, trypan blue, Crystal Violet, or ALAMAR™ blue (see, e.g., Page et al. (1993) Intl J Oncology 3:473-6). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically.

[358] Cell viability may also be measured, e.g., by quantifying ATP, an indicator of metabolically active cells. In some embodiments, *in vitro* potency and/or cell viability of prepared ADCs or Bcl-xL inhibitor compounds may be assessed using a CellTiter-Glo® (CTG) cell viability assay, as described in the examples provided herein. In this assay, in some embodiments, the single reagent (CellTiter-Glo® Reagent) is added directly to cells cultured in serum-supplemented medium. The addition of reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture

[359] Cell viability may also be measured, e.g., by measuring the reduction of tetrazolium salts. In some embodiments, *in vitro* potency and/or cell viability of prepared ADCs or Bcl-xL inhibitor compounds may be assessed using an MTT cell viability assay, as described in the examples provided herein. In this assay, in some embodiments, the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing

equivalents such as NADH and NADPH. The resulting intracellular purple formazan can then be solubilized and quantified by spectrophotometric means.

[360] In certain aspects, the present disclosure features a method of killing, inhibiting or modulating the growth of a cancer cell or tissue by disrupting the expression and/or activity of Bcl-xL and/or one or more upstream modulators or downstream targets thereof. The method may be used with any subject where disruption of Bcl-xL expression and/or activity provides a therapeutic benefit. Subjects that may benefit from disrupting Bcl-xL expression and/or activity include, but are not limited to, those having or at risk of having a cancer such as a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[361] In some embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses BCMA, such as a BCMA-expressing cancer cell or tissue. An exemplary embodiment includes a method of killing a BCMA-expressing cancer cell or tissue. The method may be used with any cell or tissue that expresses BCMA, such as a cancerous cell or a metastatic lesion. Non-limiting examples of BCMA-expressing cancers include multiple myeloma (Cho et al. (2018) Front Immunol. 9:1821). Non-limiting examples of BCMA-expressing cells include NCI-H929 multiple myeloma cells, and cells comprising a recombinant nucleic acid encoding BCMA or a portion thereof.

[362] In some embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses CD33, such as a CD33-expressing cancer cell or tissue. An exemplary embodiment includes a method of killing a CD33-expressing cancer cell or tissue. The method may be used with any cell or tissue that expresses CD33, such as a cancerous cell or a metastatic lesion. Non-limiting examples of CD33-expressing cancers include colorectal cancer, pancreatic cancer, lymphoma, and leukemia (e.g., acute myeloid leukemia) (Human Protein Atlas; Walter (2014) Expert Opin Ther Targets 18(7):715-8). Non-limiting examples of CD33-expressing cells include MOLM-13 leukemia cells, and cells comprising a recombinant nucleic acid encoding CD33 or a portion thereof.

[363] In some embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses PCAD, such as a PCAD-expressing cancer cell or tissue. An exemplary embodiment includes a method of killing a PCAD-expressing cancer cell or tissue. The

method may be used with any cell or tissue that expresses PCAD, such as a cancerous cell or a metastatic lesion. Non-limiting examples of PCAD-expressing cancers include breast cancer, gastric cancer, endometrial cancer, ovarian cancer, pancreatic cancer, bladder cancer, prostate cancer, and melanoma (Vieira and Paredes (2015) Mol Cancer 14:178).

[364] In some embodiments, the disclosed ADCs may be administered in any cell or tissue that expresses HER2, such as a HER2-expressing cancer cell or tissue. An exemplary embodiment includes a method of killing a HER2-expressing cancer cell or tissue. The method may be used with any cell or tissue that expresses HER2, such as a cancerous cell or a metastatic lesion. Non-limiting examples of HER2-expressing cancers include breast cancer, gastric cancer, bladder cancer, urothelial cell carcinoma, esophageal cancer, lung cancer (e.g., lung adenocarcinoma), uterine cancer (e.g., uterine serous endometrial carcinoma), salivary duct carcinoma, cervical cancer, endometrial cancer, and ovarian cancer (English et al. (2013) Mol Diagn Ther. 17:85-99). Non-limiting examples of HER2-expressing cells include HCC1954 and HCC2218 breast cancer cells, and cells comprising a recombinant nucleic acid encoding HER2 or a portion thereof.

[365] Exemplary methods include the steps of contacting a cell with an ADC, as described herein, in an effective amount, i.e., an amount sufficient to kill the cell. The method can be used on cells in culture, e.g., *in vitro*, *in vivo*, *ex vivo*, or *in situ.* For example, cells that express HER2 (e.g., cells collected by biopsy of a tumor or metastatic lesion; cells from an established cancer cell line; or recombinant cells), can be cultured *in vitro* in culture medium and the contacting step can be affected by adding the ADC to the culture medium. The method will result in killing of cells expressing HER2, including in particular cancer cells expressing HER2. Alternatively, the ADC can be administered to a subject by any suitable administration route (e.g., intravenous, subcutaneous, or direct contact with a tumor tissue) to have an effect *in vivo*. This approach can be used for antibodies targeting other cell surface antigens (e.g., EGFR, CD7, HER2).

[366] The *in vivo* effect of a disclosed ADC therapeutic composition can be evaluated in a suitable animal model. For example, xenogeneic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein et al. (1997) Nature Med. 3:402-8). Efficacy may be predicted using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[367] *In vivo* assays that evaluate the promotion of tumor death by mechanisms such as apoptosis may also be used. In some embodiments, xenografts from tumor bearing mice treated with the therapeutic composition can be examined for the presence of apoptotic foci and compared to untreated control xenograft-bearing mice. The extent to which apoptotic

foci are found in the tumors of the treated mice provides an indication of the therapeutic efficacy of the composition.

[368] Further provided herein are methods of treating a disorder, e.g., a cancer. The compositions described herein, e.g., the ADCs disclosed herein, can be administered to a non-human mammal or human subject for therapeutic purposes. The therapeutic methods include administering to a subject having or suspected of having a cancer a therapeutically effective amount of a composition comprising an Bcl-xL inhibitor, e.g., an ADC where the inhibitor is linked to a targeting antibody that binds to an antigen (1) expressed on a cancer cell, (2) is accessible to binding, and/or (3) is localized or predominantly expressed on a cancer cell surface as compared to a non-cancer cell.

[369] An exemplary embodiment is a method of treating a subject having or suspected of having a cancer, comprising administering to the subject a therapeutically effective amount of a composition disclosed herein, e.g., an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the cancer expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2,

tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer is a tumor or a hematological cancer. In some embodiments, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer is a lymphoma or gastric cancer.

[370] Another exemplary embodiment is a method of delivering a Bcl-xL inhibitor to a cell expressing BCMA, comprising conjugating the Bcl-xL inhibitor to an antibody that immunospecifically binds to a BCMA epitope and exposing the cell to the ADC. Exemplary cancer cells that express BCMA for which the ADCs of the present disclosure are indicated include multiple myeloma cells.

[371] Another exemplary embodiment is a method of delivering a Bcl-xL inhibitor to a cell expressing CD33, comprising conjugating the Bcl-xL inhibitor to an antibody that immunospecifically binds to a CD33 epitope and exposing the cell to the ADC. Exemplary cancer cells that express CD33 for which the ADCs of the present disclosure are indicated include leukemia cells.

[372] Another exemplary embodiment is a method of delivering a Bcl-xL inhibitor to a cell expressing PCAD, comprising conjugating the Bcl-xL inhibitor to an antibody that immunospecifically binds to a PCAD epitope and exposing the cell to the ADC. Exemplary cancer cells that express PCAD for which the ADCs of the present disclosure are indicated include breast cancer and gastric cancer cells.

[373] Another exemplary embodiment is a method of delivering a Bcl-xL inhibitor to a cell expressing HER2, comprising conjugating the Bcl-xL inhibitor to an antibody that immunospecifically binds to a HER2 epitope and exposing the cell to the ADC. Exemplary cancer cells that express HER2 for which the ADCs of the present disclosure are indicated include breast cancer cells.

[374] In certain aspects, the present disclosure further provides methods of reducing or inhibiting growth of a tumor (e.g., a BCMA-expressing tumor, a CD33-expressing tumor, a

PCAD-expressing tumor, an HER2-expressing tumor), comprising administering a therapeutically effective amount of an ADC or composition comprising an ADC. In some embodiments, the treatment is sufficient to reduce or inhibit the growth of the patient's tumor, reduce the number or size of metastatic lesions, reduce tumor load, reduce primary tumor load, reduce invasiveness, prolong survival time, and/or maintain or improve the quality of life. In some embodiments, the tumor is resistant or refractory to treatment with the antibody or antigen-binding fragment of the ADC (e.g., an anti-BCMA antibody, an anti-CD33 antibody, an anti-PCAD antibody, an anti-HER2 antibody) when administered alone, and/or the tumor is resistant or refractory to treatment with the Bcl-xL inhibitor drug moiety when administered alone.

[375] An exemplary embodiment is a method of reducing or inhibiting the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the tumor expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-

E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR. CD7, or HER2. In some embodiments, the tumor is a breast cancer, gastric cancer, bladder cancer, brain cancer, cervical cancer, colorectal cancer, esophageal cancer, hepatocellular cancer, melanoma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, or spleen cancer. In some embodiments, the tumor is a gastric cancer. In some embodiments, administration of the ADC, composition, or pharmaceutical composition reduces or inhibits the growth of the tumor by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%, as compared to growth in the absence of treatment.

[376] Another exemplary embodiment is a method of delaying or slowing the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the tumor expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51, CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin,

megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the tumor is a breast cancer, gastric cancer, bladder cancer, brain cancer, cervical cancer, colorectal cancer, esophageal cancer, hepatocellular cancer, melanoma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, or spleen cancer. In some embodiments, the tumor is a gastric cancer. In some embodiments, administration of the ADC, composition, or pharmaceutical composition delays or slows the growth of the tumor by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%, as compared to growth in the absence of treatment.

[377] In certain aspects, the present disclosure further provides methods of reducing or slowing the expansion of a cancer cell population (e.g., a BCMA-expressing cancer cell population, a CD33-expressing cancer cell population, a PCAD-expressing cancer cell population, a HER2-expressing cancer cell population), comprising administering a therapeutically effective amount of an ADC or composition comprising an ADC.

[378] An exemplary embodiment is a method of reducing or slowing the expansion of a cancer cell population in a subject, comprising administering to the subject a therapeutically effective amount of an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein). In some embodiments, the cancer cell population expresses a target antigen. In some embodiments, the target antigen is BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EphA2, CD56, SEZ6, CD25, CCR8,CEACAM5, CEACAM6, 4-1BB, 5AC, 5T4, Alpha-fetoprotein, angiopoietin 2, ASLG659, TCLI, BMPRIB, Brevican BCAN, BEHAB, C242 antigen, C5, CA-125, CA-125 (imitation), CA-IX (Carbonic anhydrase 9), CCR4, CD140a, CD152, CD19, CD20, CD200, CD21 (C3DR) I), CD22 (B-cell receptor CD22-B isoform), CD221, CD23 (gE receptor), CD28, CD30 (TNFRSF8), CD37, CD4, CD40, CD44 v6, CD51,

CD52, CD70, CD72 (Lyb-2, B-cell differentiation antigen CD72), CD79a, CD80, CEA, CEA-related antigen, ch4D5, CLDN18.2, CRIPTO (CR, CRI, CRGF, TDGF1), CTLA-4, CXCR5, DLL4, DR5, E16 (LATI, SLC7A5), EGFL7, EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5), Episialin, ERBB3, ETBR (Endothelin type B receptor), FCRHI (Fc receptor-like protein I), FcRH2 (IFGP4, IRTA4, SPAPI, SPAP IB, SPAP IC), Fibronectin extra domain-B, Frizzled receptor, GD2, GD3 ganglioside, GEDA, HER1, HER2/neu, HER3, HGF, HLA-DOB, HLA-DR, Human scatter factor receptor kinase, IGF-I receptor, IL-13, IL20R (ZCYTOR7), IL-6, ILGF2, ILFRIR, integrin u, IRTA2 (Immunoglobulin superfamily receptor translocation associated 2), Lewis-Y antigen, LY64 (RP105), MCP-I, MDP (DPEPI), MPF, MSLN, SMR, mesothelin, megakaryocyte, PD-I, PDCDI, PDGF-R u, Prostate specific membrane antigen, PSCA (Prostate stem cell antigen precursor), PSCA hlg, RANKL, RON, SDCI, Sema Sb, STEAP I, STEAP2, PCANAP I, STAMP I, STEAP2, STMP, prostate cancer associated gene I, TAG-72, TEMI, Tenascin C, TENB2, (TMEFF2, tomoregulin, TPEF, HPPI, TR), TGF-IJ, TRAIL-E2, TRAIL-RI, TRAIL-R2, T17M4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel subfamily M, member 4), TWEAK-R, TYRP I (glycoprotein 75), VEGF, VEGF-A, EGFR-I, VEGFR-2, or Vimentin. In some embodiments, the target antigen is EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, or GPNMB. In some embodiments, the target antigen is EGFR, CD7, or HER2. In some embodiments, the cancer cell population is from a tumor or a hematological cancer. In some embodiments, the cancer cell population is from a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer. In some embodiments, the cancer cell population is from a lymphoma or gastric cancer. In some embodiments, administration of the ADC, composition, or pharmaceutical composition reduces the cancer cell population by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%, as compared to the population in the absence of treatment. In some embodiments, administration of the ADC, composition, or pharmaceutical composition slows the expansion of the cancer cell population by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least

about 80%, at least about 90%, at least about 95%, or at least about 99%, as compared to expansion in the absence of treatment.

[379] Also provided herein are methods of determining whether a subject having or suspected of having a cancer will be responsive to treatment with the disclosed ADCs and compositions. An exemplary embodiment is a method of determining whether a subject having or suspected of having a cancer will be responsive to treatment with an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein) by providing a biological sample from the subject; contacting the sample with the ADC; and detecting binding of the ADC to cancer cells in the sample. In some embodiments, the sample is a tissue biopsy sample, a blood sample, or a bone marrow sample. In some embodiments, the method comprises providing a biological sample from the subject; contacting the sample with the ADC; and detecting one or more markers of cancer cell death in the sample (e.g., increased expression of one or more apoptotic markers, reduced expansion of a cancer cell population in culture, etc.).

[380] Further provided herein are therapeutic uses of the disclosed ADCs and compositions. An exemplary embodiment is an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein) for use in treating a subject having or suspected of having a cancer (e.g., a BCMA-expressing cancer, a CD33-expressing cancer, a PCAD-expressing cancer, a HER2-expressing cancer). Another exemplary embodiment is a use of an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein) in treating a subject having or suspected of having a cancer (e.g., a BCMA-expressing cancer, a CD33-expressing cancer, a PCAD-expressing cancer, a HER2-expressing cancer). Another exemplary embodiment is a use of an ADC, composition, or pharmaceutical composition (e.g., any of the exemplary ADCs, compositions, or pharmaceutical compositions disclosed herein) in a method of manufacturing a medicament for treating a subject having or suspected of having a cancer (e.g., a BCMA-expressing cancer, a CD33-expressing cancer, a PCAD-expressing cancer, a HER2-expressing cancer). Methods for identifying subjects having cancers that express a target antigen (e.g., EGFR, CD7, or HER2) are known in the art and may be used to identify suitable patients for treatment with a disclosed ADC compound or composition.

[381] Moreover, ADCs of the present disclosure may be administered to a non-human mammal expressing an antigen with which the ADC is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of the disclosed ADCs (e.g., testing of dosages and time courses of administration).

[382] The therapeutic compositions used in the practice of the foregoing methods may be formulated into pharmaceutical compositions comprising a pharmaceutically acceptable carrier suitable for the desired delivery method. An exemplary embodiment is a pharmaceutical composition comprising an ADC of the present disclosure and a pharmaceutically acceptable carrier, e.g., one suitable for a chosen means of administration, e.g., intravenous administration. The pharmaceutical composition may also comprise one or more additional inactive and/or therapeutic agents that are suitable for treating or preventing, for example, a cancer (e.g., a standard-of-care agent, etc.). The pharmaceutical composition may also comprise one or more carrier, excipient, and/or stabilizer components, and the like. Methods of formulating such pharmaceutical compositions and suitable formulations are known in the art (see, e.g., "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA).

[383] Suitable carriers include any material that, when combined with the therapeutic composition, retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, mesylate salt, and the like, as well as combinations thereof. In many cases, isotonic agents are included, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the ADC.

[384] A pharmaceutical composition of the present disclosure can be administered by a variety of methods known in the art. The route and/or mode of administration may vary depending upon the desired results. In some embodiments, the therapeutic formulation is solubilized and administered via any route capable of delivering the therapeutic composition to the cancer site. Potentially effective routes of administration include, but are not limited to, parenteral (e.g., intravenous, subcutaneous), intraperitoneal, intramuscular, intratumor, intradermal, intraorgan, orthotopic, and the like. In some embodiments, the administration is intravenous, subcutaneous, intraperitoneal, or intramuscular. The pharmaceutically acceptable carrier should be suitable for the route of administration, e.g., intravenous or subcutaneous administration (e.g., by injection or infusion). Depending on the route of administration, the active compound(s), i.e., the ADC and/or any additional therapeutic agent, may be coated in a material to protect the compound(s) from the action of acids and

other natural conditions that may inactivate the compound(s). Administration can be either systemic or local.

[385] The therapeutic compositions disclosed herein may be sterile and stable under the conditions of manufacture and storage, and may be in a variety of forms. These include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes, and suppositories. The form depends on the intended mode of administration and therapeutic application. In some embodiments, the disclosed ADCs can be incorporated into a pharmaceutical composition suitable for parenteral administration. The injectable solution may be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule, or pre-filled syringe, or other known delivery or storage device. In some embodiments, one or more of the ADCs or pharmaceutical compositions is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject.

[386] Typically, a therapeutically effective amount or efficacious amount of a disclosed composition, e.g., a disclosed ADC, is employed in the pharmaceutical compositions of the present disclosure. The composition, e.g., one comprising an ADC, may be formulated into a pharmaceutically acceptable dosage form by conventional methods known in the art. Dosages and administration protocols for the treatment of cancers using the foregoing methods will vary with the method and the target cancer, and will generally depend on a number of other factors appreciated in the art.

[387] Dosage regimens for compositions disclosed herein, e.g., those comprising ADCs alone or in combination with at least one additional inactive and/or active therapeutic agent, may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus of one or both agents may be administered at one time, several divided doses may be administered over a predetermined period of time, or the dose of one or both agents may be proportionally increased or decreased as indicated by the exigencies of the therapeutic situation. In some embodiments, treatment involves single bolus or repeated administration of the ADC preparation via an acceptable route of administration. In some embodiments, the ADC is administered to the patient daily, weekly, monthly, or any time period in between. For any particular subject, specific dosage regimens may be adjusted over time according to the individual's need, and the professional judgment of the treating clinician. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit

contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[388] Dosage values for compositions comprising an ADC and/or any additional therapeutic agent(s), may be selected based on the unique characteristics of the active compound(s), and the particular therapeutic effect to be achieved. A physician or veterinarian can start doses of the ADC employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, effective doses of the compositions of the present disclosure, for the treatment of a cancer may vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. The selected dosage level may also depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt, or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors. Treatment dosages may be titrated to optimize safety and efficacy.

[389] Toxicity and therapeutic efficacy of compounds provided herein can be determined by standard pharmaceutical procedures in cell culture or in animal models. For example, LD50, ED50, EC50, and IC50 may be determined, and the dose ratio between toxic and therapeutic effects (LD50/ED50) may be calculated as the therapeutic index. The data obtained from *in vitro* and *in vivo* assays can be used in estimating or formulating a range of dosage for use in humans. For example, the compositions and methods disclosed herein may initially be evaluated in xenogeneic cancer models (e.g., an NCI-H929 multiple myeloma mouse model).

[390] In some embodiments, an ADC or composition comprising an ADC is administered on a single occasion. In other embodiments, an ADC or composition comprising an ADC is administered on multiple occasions. Intervals between single dosages can be, e.g., daily, weekly, monthly, or yearly. Intervals can also be irregular, based on measuring blood levels of the administered agent (e.g., the ADC) in the patient in order to maintain a relatively consistent plasma concentration of the agent. The dosage and frequency of administration of an ADC or composition comprising an ADC may also vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage may be administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a

relatively higher dosage at relatively shorter intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of one or more symptoms of disease. Thereafter, the patient may be administered a lower, e.g., prophylactic regime.

[391] The above therapeutic approaches can be combined with any one of a wide variety of additional surgical, chemotherapy, or radiation therapy regimens. In some embodiments, the ADCs or compositions disclosed herein are co-formulated and/or co-administered with one or more additional therapeutic agents, e.g., one or more chemotherapeutic agents, one or more standard-of-care agents for the particular condition being treated.

[392] Kits for use in the therapeutic and/or diagnostic applications described herein are also provided. Such kits may comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method disclosed herein. A label may be present on or with the container(s) to indicate that an ADC or composition within the kit is used for a specific therapy or non-therapeutic application, such as a prognostic, prophylactic, diagnostic, or laboratory application. A label may also indicate directions for either *in vivo* or *in vitro* use, such as those described herein. Directions and or other information may also be included on an insert(s) or label(s), which is included with or on the kit. The label may be on or associated with the container. A label may be on a container when letters, numbers, or other characters forming the label are molded or etched into the container itself. A label may be associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. The label may indicate that an ADC or composition within the kit is used for diagnosing or treating a condition, such as a cancer a described herein.

[393] In some embodiments, a kit comprises an ADC or composition comprising an ADC. In some embodiments, the kit further comprises one or more additional components, including but not limited to: instructions for use; other reagents, e.g., a therapeutic agent (e.g., a standard-of-care agent); devices, containers, or other materials for preparing the ADC for administration; pharmaceutically acceptable carriers; and devices, containers, or other materials for administering the ADC to a subject. Instructions for use can include guidance for therapeutic applications including suggested dosages and/or modes of administration, e.g., in a patient having or suspected of having a cancer. In some embodiments, the kit comprises an ADC and instructions for use of the ADC in treating, preventing, and/or diagnosing a cancer.

[394] It is known that elevated Bcl-xL expression correlates with resistance to radiation therapy and chemotherapy. Antibody-drug conjugates (ADCs) that may not be sufficiently effective as monotherapy to treat cancer can be administered in combination with other

therapeutic agents (including non-targeted and targeted therapeutic agents) or radiation therapy (including radioligand therapy) to provide therapeutic benefit. Without wishing to be bound by theory, it is believed that the ADCs described herein sensitize tumor cells to the treatment with other therapeutic agents (including standard of care chemotherapeutic agents to which the tumor cells may have developed resistance) and/or radiation therapy. In some embodiments, antibody drug conjugates described herein, are administered to a subject having cancer in an amount effective to sensitize the tumor cells. As used herein, the term "sensitize" means that the treatment with ADC increases the potency or efficacy of the treatment with other therapeutic agents and/or radiation therapy against tumor cells.

COMBINATION THERAPIES

[395] In some embodiments, the present disclosure provides methods of treatment wherein the antibody-drug conjugates disclosed herein are administered in combination with one or more (*e.g.*, 1 or 2) additional therapeutic agents. Exemplary combination partners are disclosed herein.

[396] In certain embodiments, a combination described herein comprises a PD-1 inhibitor. In some embodiments, the PD-1 inhibitor is chosen from PDR001 (Novartis), Nivolumab (Bristol-Myers Squibb), Pembrolizumab (Merck & Co), Pidilizumab (CureTech), MEDI0680 (Medimmune), REGN2810 (Regeneron), TSR-042 (Tesaro), PF-06801591 (Pfizer), BGB-A317 (Beigene), BGB-108 (Beigene), INCSHR1210 (Incyte), or AMP-224 (Amplimmune). In some embodiments, the PD-1 inhibitor is PDR001. PDR001 is also known as Spartalizumab.

[397] In certain embodiments, a combination described herein comprises a LAG-3 inhibitor. In some embodiments, the LAG-3 inhibitor is chosen from LAG525 (Novartis), BMS-986016 (Bristol-Myers Squibb), or TSR-033 (Tesaro).

[398] In certain embodiments, a combination described herein comprises a TIM-3 inhibitor. In some embodiments, the TIM-3 inhibitor is MBG453 (Novartis), TSR-022 (Tesaro), LY-3321367 (Eli Lily), Sym23 (Symphogen), BGB-A425 (Beigene), INCAGN-2390 (Agenus), BMS-986258 (BMS), RO-7121661 (Roche), or LY-3415244 (Eli Lilly).

[399] In certain embodiments, a combination descdribed herein comprises a PDL1 inhibitor. In one embodiment, the PDL1 inhibitor is chosen from FAZ053 (Novartis), atezolizumab (Genentech), durvalumab (Astra Zeneca), or avelumab (Pfizer).

[400] In certain embodiments, a combination described herein comprises a GITR agonist. In some embodiments, the GITR agonist is chosen from GWN323 (NVS), BMS-986156, MK-4166 or MK-1248 (Merck), TRX518 (Leap Therapeutics), INCAGN1876 (Incyte/Agenus), AMG 228 (Amgen) or INBRX-110 (Inhibrx).

[401] In some embodiments, a combination described herein comprises an IAP inhibitor. In some embodiments, the IAP inhibitor comprises LCL161 or a compound disclosed in International Application Publication No. WO 2008/016893.

[402] In an embodiment, the combination comprises an mTOR inhibitor, *e.g.*, RAD001 (also known as everolimus).

[403] In an embodiment, the combination comprises a HDAC inhibitor, *e.g.*, LBH589. LBH589 is also known as panobinostat.

[404] In an embodiment, the combination comprises an IL-17 inhibitor, *e.g.*, CJM112.

[405] In certain embodiments, a combination described herein comprises an estrogen receptor (ER) antagonist. In some embodiments, the estrogen receptor antagonist is used in combination with a PD-1 inhibitor, a CDK4/6 inhibitor, or both. In some embodiments, the combination is used to treat an ER positive (ER+) cancer or a breast cancer (*e.g.*, an ER+ breast cancer).

[406] In some embodiments, the estrogen receptor antagonist is a selective estrogen receptor degrader (SERD). SERDs are estrogen receptor antagonists which bind to the receptor and result in *e.g.*, degradation or down-regulation of the receptor (Boer K. *et al.*, (2017) *Therapeutic Advances in Medical Oncology* 9(7): 465-479). ER is a hormone-activated transcription factor important for *e.g.*, the growth, development and physiology of the human reproductive system. ER is activated by, *e.g.*, the hormone estrogen (17beta estradiol). ER expression and signaling is implicated in cancers (*e.g.*, breast cancer), *e.g.*, ER positive (ER+) breast cancer. In some embodiments, the SERD is chosen from LSZ102, fulvestrant, brilanestrant, or elacestrant.

[407] In some embodiments, the SERD comprises a compound disclosed in International Application Publication No. WO 2014/130310, which is hereby incorporated by reference in its entirety.

[408] In some embodiments, the SERD comprises LSZ102. LSZ102 has the chemical name: (E)-3-(4-((2-(2-(1,1-difluoroethyl)-4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenyl)acrylic acid. In some embodiments, the SERD comprises fulvestrant (CAS Registry Number: 129453-61-8), or a compound disclosed in International Application Publication No. WO 2001/051056, which is hereby incorporated by reference in its entirety. In some embodiments, the SERD comprises elacestrant (CAS Registry Number: 722533-56-4), or a compound disclosed in U.S. Patent No. 7,612,114, which is incorporated by reference in its entirety. Elacestrant is also known as RAD1901, ER-306323 or (6R)-6-{2-[Ethyl({4-[2-(ethylamino)ethyl]phenyl}methyl)amino]-4-methoxyphenyl}-5,6,7,8-tetrahydronaphthalen-2-ol. Elacestrant is an orally bioavailable, non-steroidal combined selective estrogens receptor modulator (SERM) and a SERD. Elacestrant is also disclosed,

e.g., in Garner F *et al.*, (2015) *Anticancer Drugs* 26(9):948-56. In some embodiments, the SERD is brilanestrant (CAS Registry Number: 1365888-06-7), or a compound disclosed in International Application Publication No. WO 2015/136017, which is incorporated by reference in its entirety.

[409] In some embodiments, the SERD is chosen from RU 58668, GW7604, AZD9496, bazedoxifene, pipendoxifene, arzoxifene, OP-1074, or acolbifene, *e.g.*, as disclosed in McDonell *et al.* (2015) *Journal of Medicinal Chemistry* 58(12) 4883-4887.

[410] Other exemplary estrogen receptor antagonists are disclosed, *e.g.*, in WO 2011/156518, WO 2011/159769, WO 2012/037410, WO 2012/037411, and US 2012/0071535, all of which are hereby incorporated by reference in their entirety

[411] In certain embodiments, a combination described herein comprises an inhibitor of Cyclin-Dependent Kinases 4 or 6 (CDK4/6). In some embodiments, the CDK4/6 inhibitor is used in combination with a PD-1 inhibitor, an estrogen receptor (ER) antagonist, or both. In some embodiments, the combination is used to treat an ER positive (ER+) cancer or a breast cancer (*e.g.*, an ER+ breast cancer). In some embodiments, the CDK4/6 inhibitor is chosen from ribociclib, abemaciclib (Eli Lilly), or palbociclib.

[412] In some embodiments, the CDK4/6 inhibitor comprises ribociclib (CAS Registry Number: 1211441-98-3), or a compound disclosed in U.S. Patent Nos. 8,415,355 and 8,685,980, which are incorporated by reference in their entirety.

[413] In some embodiments, the CDK4/6 inhibitor comprises a compound disclosed in International Application Publication No. WO 2010/020675 and U.S. Patent Nos. 8,415,355 and 8,685,980, which are incorporated by reference in their entirety.

[414] In some embodiments, the CDK4/6 inhibitor comprises ribociclib (CAS Registry Number: 1211441-98-3). Ribociclib is also known as LEE011, KISQALI®, or 7-cyclopentyl-N,N-dimethyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide.

[415] In some embodiments, the CDK4/6 inhibitor comprises abemaciclib (CAS Registry Number: 1231929-97-7). Abemaciclib is also known as LY835219 or N-[5-[(4-Ethyl-1-piperazinyl)methyl]-2-pyridinyl]-5-fluoro-4-[4-fluoro-2-methyl-1-(1-methylethyl)-1H-benzimidazol-6-yl]-2-pyrimidinamine. Abemaciclib is a CDK inhibitor selective for CDK4 and CDK6 and is disclosed, *e.g.*, in Torres-Guzman R *et al.* (2017) *Oncotarget* 10.18632/oncotarget.17778.

[416] In some embodiments, the CDK4/6 inhibitor comprises palbociclib (CAS Registry Number: 571190-30-2). Palbociclib is also known as PD-0332991, IBRANCE® or 6-Acetyl-8-cyclopentyl-5-methyl-2-{[5-(1-piperazinyl)-2-pyridinyl]amino}pyrido[2,3-d]pyrimidin-7(8H)-one. Palbociclib inhibits CDK4 with an IC50 of 11nM, and inhibits CDK6

with an IC50 of 16nM, and is disclosed, *e.g.*, in Finn *et al.* (2009) *Breast Cancer Research* 11(5):R77.

[417] In certain embodiments, a combination described herein comprises an inhibitor of chemokine (C-X-C motif) receptor 2 (CXCR2). In some embodiments, the CXCR2 inhibitor is chosen from 6-chloro-3-((3,4-dioxo-2-(pentan-3-ylamino)cyclobut-1-en-1-yl)amino)-2-hydroxy-N-methoxy-N-methylbenzenesulfonamide, danirixin, reparixin, or navarixin.

[418] In some embodiments, the CSF-1/1R binding agent is chosen from an inhibitor of macrophage colony-stimulating factor (M-CSF), *e.g.*, a monoclonal antibody or Fab to M-CSF (*e.g.*, MCS110), a CSF-1R tyrosine kinase inhibitor (*e.g.*, 4-((2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-yl)oxy)-N-methylpicolinamide or BLZ945), a receptor tyrosine kinase inhibitor (RTK) (*e.g.*, pexidartinib), or an antibody targeting CSF-1R (*e.g.*, emactuzumab or FPA008). In some embodiments, the CSF-1/1R inhibitor is BLZ945. In some embodiments, the CSF-1/1R binding agent is MCS110. In other embodiments, the CSF-1/1R binding agent is pexidartinib.

[419] In certain embodiments, a combination described herein comprises a c-MET inhibitor. c-MET, a receptor tyrosine kinase overexpressed or mutated in many tumor cell types, plays key roles in tumor cell proliferation, survival, invasion, metastasis, and tumor angiogenesis. Inhibition of c-MET may induce cell death in tumor cells overexpressing c-MET protein or expressing constitutively activated c-MET protein. In some embodiments, the c-MET inhibitor is chosen from capmatinib (INC280), JNJ-3887605, AMG 337, LY2801653, MSC2156119J, crizotinib, tivantinib, or golvatinib.

[420] In certain embodiments, a combination described herein comprises a transforming growth factor beta (also known as TGF-β TGFβ, TGFb, or TGF-beta, used interchangeably herein) inhibitor. In some embodiments, the TGF-β inhibitor is chosen from fresolimumab or XOMA 089.

[421] In certain embodiments, a combination described herein comprises an adenosine A2a receptor (A2aR) antagonist (*e.g.,* an inhibitor of A2aR pathway, *e.g.,* an adenosine inhibitor, *e.g.,* an inhibitor of A2aR or CD-73). In some embodiments, the A2aR antagonist is used in combination with a PD-1 inhibitor, and one or more (*e.g.*, two, three, four, five, or all) of a CXCR2 inhibitor, a CSF-1/1R binding agent, LAG-3 inhibitor, a GITR agonist, a c-MET inhibitor, or an IDO inhibitor. In some embodiments, the combination is used to treat a pancreatic cancer, a colorectal cancer, a gastric cancer, or a melanoma (*e.g.*, a refractory melanoma). In some embodiments, the A2aR antagonist is chosen from PBF509 (NIR178) (Palobiofarma/Novartis), CPI444/V81444 (Corvus/Genentech), AZD4635/HTL-1071 (AstraZeneca/Heptares), Vipadenant (Redox/Juno), GBV-2034 (Globavir), AB928 (Arcus Biosciences), Theophylline, Istradefylline (Kyowa Hakko Kogyo),

Tozadenant/SYN-115 (Acorda), KW-6356 (Kyowa Hakko Kogyo), ST-4206 (Leadiant Biosciences), or Preladenant/SCH 420814 (Merck/Schering). Without wishing to be bound by theory, it is believed that in some embodiments, inhibition of A2aR leads to upregulation of IL-1b.

[422] In certain embodiments, a combination described herein comprises an inhibitor of indoleamine 2,3-dioxygenase (IDO) and/or tryptophan 2,3-dioxygenase (TDO). In some embodiments, the IDO inhibitor is used in combination with a PD-1 inhibitor, and one or more (*e.g.*, two, three, four, or all) of a TGF-β inhibitor, an A2aR antagonist, a CSF-1/1R binding agent, a c-MET inhibitor, or a GITR agonist. In some embodiments, the combination is used to treat a pancreatic cancer, a colorectal cancer, a gastric cancer, or a melanoma (*e.g.*, a refractory melanoma). In some embodiments, the IDO inhibitor is chosen from (4E)-4-[(3-chloro-4-fluoroanilino)-nitrosomethylidene]-1,2,5-oxadiazol-3-amine (also known as epacadostat or INCB24360), indoximod (NLG8189), (1-methyl-D-tryptophan), α-cyclohexyl-5H-Imidazo[5,1-a]isoindole-5-ethanol (also known as NLG919), indoximod, BMS-986205 (formerly F001287).

[423] In certain embodiments, a combination described herein comprises a Galectin, *e.g.*, Galectin-1 or Galectin-3, inhibitor. In some embodiments, the combination comprises a Galectin-1 inhibitor and a Galectin-3 inhibitor. In some embodiments, the combination comprises a bispecific inhibitor (*e.g.*, a bispecific antibody molecule) targeting both Galectin-1 and Galectin-3. In some embodiments, the Galectin inhibitor is used in combination with one or more therapeutic agents described herein. In some embodiments, the Galectin inhibitor is chosen from an anti-Galectin antibody molecule, GR-MD-02 (Galectin Therapeutics), Galectin-3C (Mandal Med), Anginex, or OTX-008 (OncoEthix, Merck).

[424] In some embodiments, a combination described herein comprises an inhibitor of the MAP kinase pathway including ERK inhibitors, MEK inhibitors and RAF inhibitors.

[425] In some embodiments, a combination described herein comprises a MEK inhibitor. In some embodiments, the MEK inhibitor is chosen from Trametinib, selumetinib, AS703026, BIX 02189, BIX 02188, CI-1040, PD0325901, PD98059, U0126, XL-518, G-38963, or G02443714.

[426] In some embodiments, the MEK inhibitor is trametinib. Trametinib is also known as JTP-74057, TMT212, N-(3-{3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl}phenyl)acetamide, or Mekinist (CAS Number 871700-17-3).

[427] In some embodiments, the MEK inhibitor comprises selumetinib which has the chemical name: (5-[(4-bromo-2-chlorophenyl)amino]-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide. Selumetinib is also known as AZD6244 or ARRY 142886, e.g., as described in PCT Publication No. WO2003077914.

[428] In some embodiments, the MEK inhibitor comprises AS703026, BIX 02189 or BIX 02188.

[429] In some embodiments, the MEK inhibitor comprises 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352), e.g., as described in PCT Publication No. WO2000035436).

[430] In some embodiments, the MEK inhibitor comprises N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]- benzamide (also known as PD0325901), e.g., as described in PCT Publication No. WO2002006213).

[431] In some embodiments, the MEK inhibitor comprises 2'-amino-3'-methoxyflavone (also known as PD98059) which is available from Biaffin GmbH & Co., KG, Germany.

[432] In some embodiments, the MEK inhibitor comprises 2,3-bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126), e.g., as described in US Patent No. 2,779,780).

[433] In some embodiments, the MEK inhibitor comprises XL-518 (also known as GDC-0973) which has a CAS No. 1029872-29-4 and is available from ACC Corp.

[434] In some embodiments, the MEK inhibitor comprises G-38963.

[435] In some embodiments, the MEK inhibitor comprises G02443714 (also known as AS703206)

[436] Additional examples of MEK inhibitors are disclosed in WO 2013/019906, WO 03/077914, WO 2005/121142, WO 2007/04415, WO 2008/024725 and WO 2009/085983, the contents of which are incorporated herein by reference. Further examples of MEK inhibitors include, but are not limited to, 2,3-Bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126 and described in US Patent No. 2,779,780); (3S,4R,5Z,8S,9S,11E)-14-(Ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9, 19-tetrahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione] (also known as E6201, described in PCT Publication No. WO2003076424); vemurafenib (PLX-4032, CAS 918504-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione (TAK-733, CAS 1035555-63-5); pimasertib (AS-703026, CAS 1204531-26-9); 2-(2-Fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide (AZD 8330); and 3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-N-(2-hydroxyethoxy)-5-[(3-oxo-[1,2]oxazinan-2-yl)methyl]benzamide (CH 4987655 or Ro 4987655).

[437] In some embodiments, a combination described herein comprises a RAF inhibitor.

[438] RAF inhibitors include, but are not limited to, Vemurafenib (or Zelboraf®, PLX-4032, CAS 918504-65-1), GDC-0879, PLX-4720 (available from Symansis), Dabrafenib (or

GSK2118436), LGX 818, CEP-32496, UI-152, RAF 265, Regorafenib (BAY 73-4506), CCT239065, or Sorafenib (or Sorafenib Tosylate, or Nexavar®).

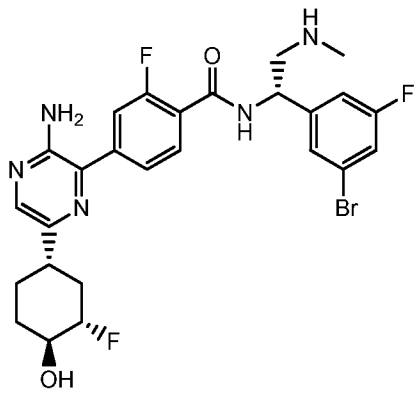
[439] In some embodiments, the RAF inhibitor is Dabrafenib.

[440] In some embodiments, the RAF inhibitor is LXH254.

[441] In some embodiments, a combination described herein comprises an ERK inhibitor.

[442] ERK inhibitors include, but are not limited to, LTT462, ulixertinib (BVD-523), LY3214996, GDC-0994, KO-947 and MK-8353.

[443] In some embodiments, the ERK inhibitor is LTT462. LTT462 is 4-(3-amino-6-((1S,3S,4S)-3-fluoro-4-hydroxy¬cyclohexyl)pyrazin-2-yl)-N-((S)-1-(3-bromo-5-fluorophenyl)-2-(methylamino)¬ethyl)-2-fluorobenzamide and is the compound of the following structure:



[444] The preparation of LTT462 is described in PCT patent application publication WO2015/066188. LTT462 is an inhibitor of extracellular signal-regulated kinases 1 and 2 (ERK 1/2).

[445] In some embodiments, a combination described herein comprises a taxane, a *vinca* alkaloid, a MEK inhibitor, an ERK inhibitor, or a RAF inhibitor.

[446] In some embodiments, a combination described herein comprises at least two inhibitors selected, independently, from a MEK inhibitor, an ERK inhibitor, and a RAF inhibitor.

[447] In some embodiments, a combination described herein comprises an anti-mitotic drug.

[448] In some embodiments, a combination described herein comprises a taxane.

[449] Taxanes include, but are not limited to, docetaxel, paclitaxel, or cabazitaxel. In some embodiments, the taxane is docetaxel.

[450] In some embodiments, a combination described herein comprises a *vinca* alkaloid.

[451] Vinca alkaloids include, but are not limited to, vincristine, vinblastine, and leurosine.

[452] In some embodiments, a combination described herein comprises a topoisomerase inhibitor.

[453] Topoisomerase inhibitors include, but are not limited to, topotecan, irinotecan, camptothecin, diflomotecan, lamellarin D, ellipticines, etoposide (VP-16), teniposide, doxorubicin, daunorubicin, mitoxantrone, amsacrine, aurintricarboxylic acid, and HU-331.

[454] In one embodiment, a combination described herein includes an interleukin-1 beta (IL-1β) inhibitor. In some embodiments, the IL-1β inhibitor is chosen from canakinumab, gevokizumab, Anakinra, or Rilonacept.

[455] In certain embodiments, a combination described herein comprises an IL-15/IL-15Ra complex. In some embodiments, the IL-15/IL-15Ra complex is chosen from NIZ985 (Novartis), ATL-803 (Altor) or CYP0150 (Cytune).

[456] In certain embodiments, a combination described herein comprises a mouse double minute 2 homolog (MDM2) inhibitor. The human homolog of MDM2 is also known as HDM2. In some embodiments, an MDM2 inhibitor described herein is also known as a HDM2 inhibitor. In some embodiments, the MDM2 inhibitor is chosen from HDM201 or CGM097.

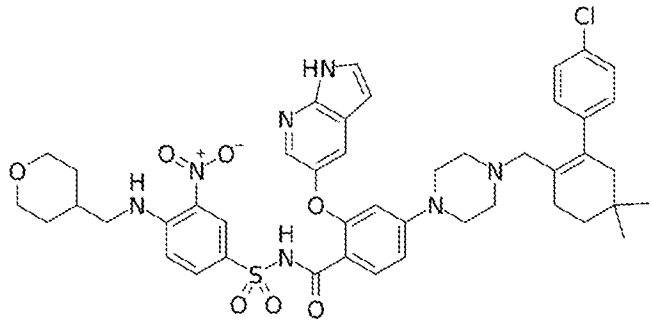
[457] In an embodiment the MDM2 inhibitor comprises (S)-1-(4-chlorophenyl)-7-isopropoxy-6-methoxy-2-(4-(methyl(((1r,4S)-4-(4-methyl-3-oxopiperazin-1-yl)cyclohexyl)methyl)amino)phenyl)-1,2-dihydroisoquinolin-3(4H)-one (also known as CGM097) or a compound disclosed in PCT Publication No. WO 2011/076786 to treat a disorder, *e.g.*, a disorder described herein). In one embodiment, a therapeutic agent disclosed herein is used in combination with CGM097.

[458] In some embodiments, a combination described herein comprises a hypomethylating agent (HMA). In some embodiments, the HMA is chosen from decitabine or azacitidine.

[459] In some embodiments, a combination described herein comprises a glucocorticoid. In some embodiments, the glucocorticoid is dexamethasone.

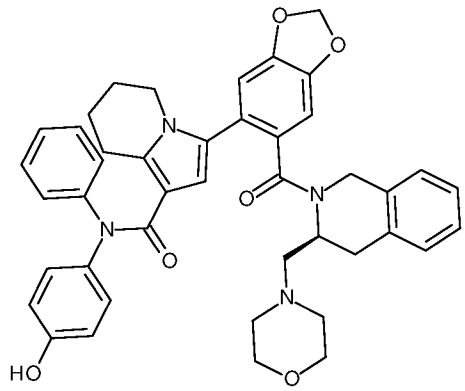
[460] In some embodiments, a combination described herein comprises asparaginase.

[461] In certain embodiments, a combination described herein comprises an inhibitor acting on any pro-survival proteins of the Bcl2 family. In certain embodiments, a combination described herein comprises a Bcl-2 inhibitor. In some embodiments, the Bcl-2 inhibitor is venetoclax (also known as ABT-199):



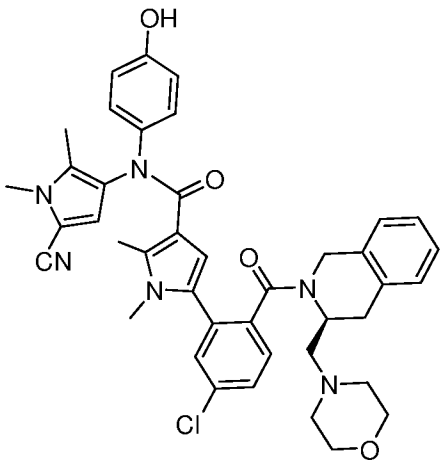
(venetoclax).

In one embodiment, the Bcl-2 inhibitor is selected from the compounds described in WO 2013/110890 and WO 2015/011400. In some embodiments, the Bcl-2 inhibitor comprises navitoclax (ABT-263), ABT-737, BP1002, SPC2996, APG-1252, obatoclax mesylate (GX15-070MS), PNT2258, Zn-d5, BGB-11417, or oblimersen (G3139). In some embodiments, the Bcl-2 inhibitor is N-(4-hydroxyphenyl)-3-[6-[(3S)-3-(morpholinomethyl)-3,4-dihydro-1H-isoquinoline-2-carbonyl]-1,3-benzodioxol-5-yl]-N-phenyl-5,6,7,8-tetrahydroindolizine-1-carboxamide, compound A1:



(compound A1).

In some embodiments, the Bcl-2 inhibitor is (S)-5-(5-chloro-2-(3-(morpholinomethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)phenyl)-N-(5-cyano-1,2-dimethyl-1H-pyrrol-3-yl)-N-(4-hydroxyphenyl)-1,2-dimethyl-1H-pyrrole-3-carboxamide), compound A2:



(compound A2).

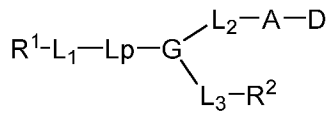
[462] In one embodiment, the antibody-drug conjugates or combinations disclosed herein are suitable for the treatment of cancer *in vivo*. For example, the combination can be used to inhibit the growth of cancerous tumors. The combination can also be used in combination with one or more of: a standard of care treatment (*e.g.*, for cancers or infectious disorders), a vaccine (*e.g.*, a therapeutic cancer vaccine), a cell therapy, a hormone therapy (e.g., with anti-estrogens or anti-androgens), a radiation therapy, surgery, or any other therapeutic agent or modality, to treat a disorder herein. For example, to achieve antigen-specific enhancement of immunity, the combination can be administered together with an antigen of interest. A combination disclosed herein can be administered in either order or simultaneously.

ADDITIONAL EMBODIMENTS

[463] The disclosure provides the following additional embodiments for linker-drug groups, antibody-drug conjugates, linker groups, and methods of conjugation.

Linker-Drug Group

In some embodiments, the Linker-Drug group of the invention may be a compound having the structure of Formula (A'), or a pharmaceutically acceptable salt thereof:



Formula (A')

wherein:

R^1 is a reactive group;

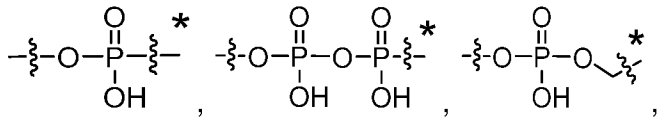
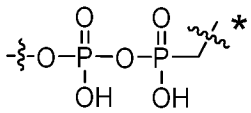
L_1 is a bridging spacer;

Lp is a bivalent peptide spacer;

G-L_2-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety; and

D is a Drug moiety that is capable of inhibiting the activity of the Bcl-xL protein when, e.g., released from the Antibody Drug Conjugates or immunoconjugates disclosed herein.

Certain aspects and examples of the Linker-Drug group of the invention are provided in the following listing of enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

Embodiment 1. The compound of Formula (A'), or pharmaceutically acceptable salt thereof, wherein:

R^1 is a reactive group;

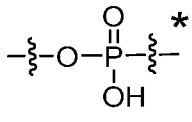
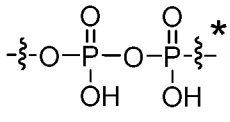
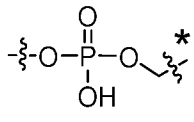
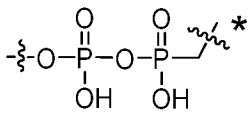
L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

G-L_2-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety; and

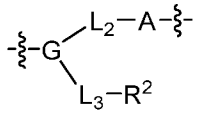
D is a Drug moiety as defined herein, e.g., a Bcl-xL inhibitor.

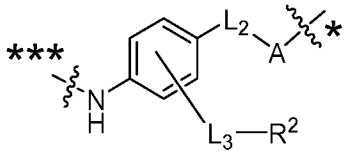
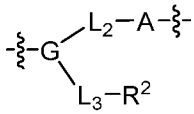
Embodiment 2. The compound of Formula (A'), or pharmaceutically acceptable salt thereof, wherein:

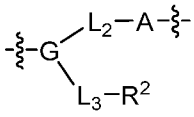
R^1 is a reactive group;

L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

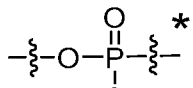
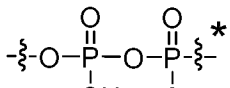
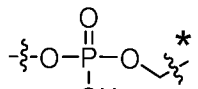
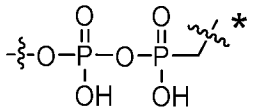
the  group is selected from:

, wherein the * of indicates the point of attachment to D (e.g., to an N or a O of the Drug moiety), the *** of  indicates the point of attachment to Lp;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

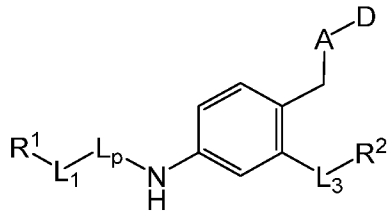
A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety; and

D is a Drug moiety as defined herein, e.g., a Bcl-xL inhibitor.

Embodiment 3. The compound of Formula (A'), or pharmaceutically acceptable salt thereof, having the structure of Formula (B'):



Formula (B')

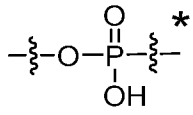
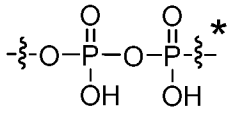
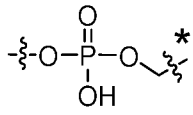
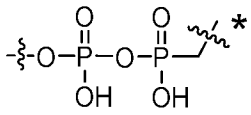
wherein:

R^1 is a reactive group;

L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

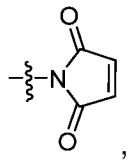
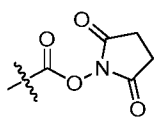
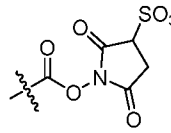
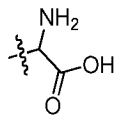
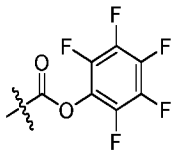
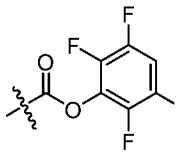
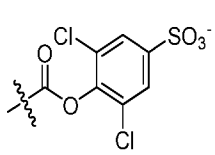
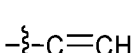
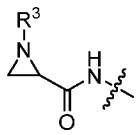
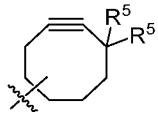
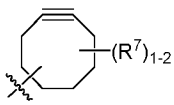
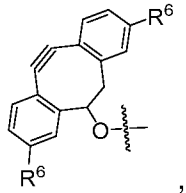
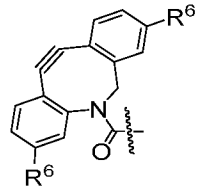
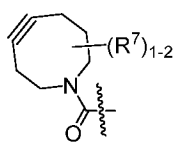
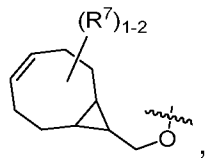
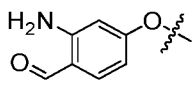
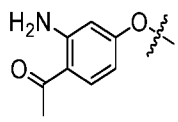
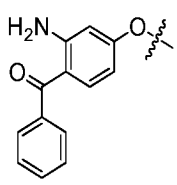
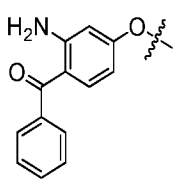
R^2 is a hydrophilic moiety;

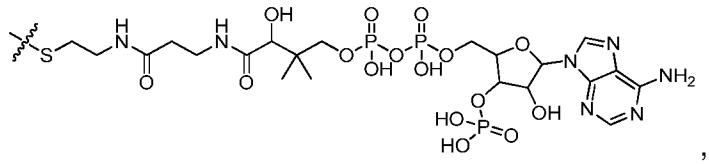
A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety; and

D is a Drug moiety as defined herein and comprising an N, wherein D is connected to A via a direct bond from A to the N of the Drug moiety.

Embodiment 4. The compound of Formula (A') or of any one of Embodiments 1 to 3, or pharmaceutically acceptable salt thereof, wherein:

R^1 is  , -ONH$_2$, -NH$_2$,  ,  ,  ,  ,  ,  , -N$_3$,  , -SH, -SR^3, -SSR^4, -S(=O)$_2$(CH=CH$_2$), -(CH$_2$)$_2$S(=O)$_2$(CH=CH$_2$), -NHS(=O)$_2$(CH=CH$_2$), -NHC(=O)CH$_2$Br, -NHC(=O)CH$_2$I,  , -C(O)NHNH$_2$,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,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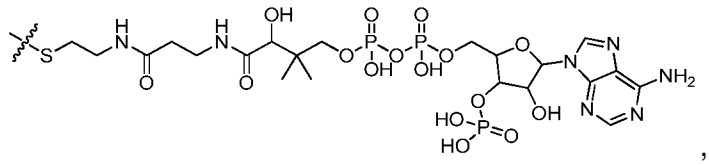
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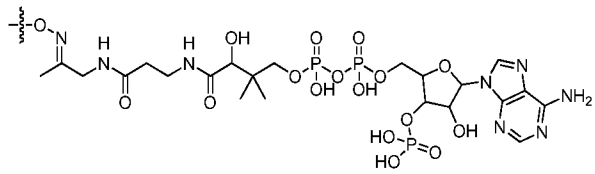
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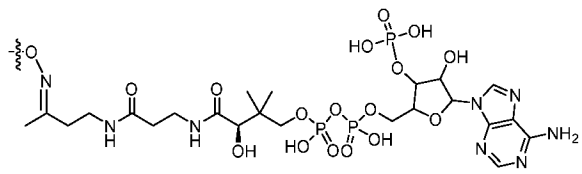
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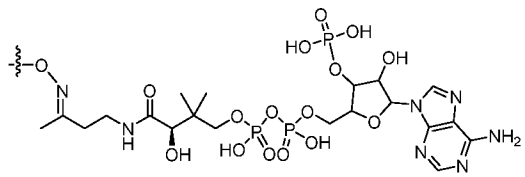
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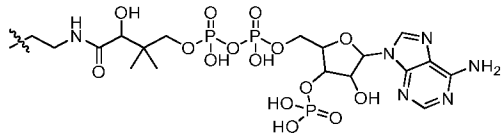
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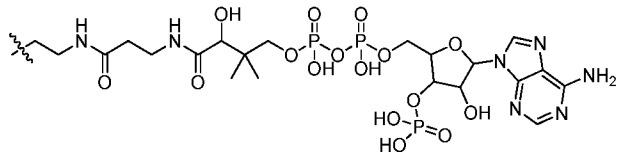
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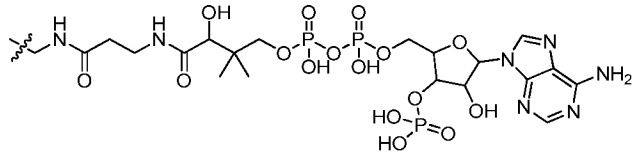

;

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**;

*-C(=O)$(CH_2)_m$-**;

*-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-**;

*-C(=O)O$(CH_2)_m$SSC$(R^3)_2$$(CH_2)_m$C(=O)N$R^3$$(CH_2)_mNR^3$C(=O)$(CH_2)_m$-**;

*-C(=O)O$(CH_2)_m$C(=O)NH$(CH_2)_m$-**;

*-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**;

*-C(=O)$(CH_2)_m$$X_1$$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$$X_1$$(CH_2)_n$-**;

*-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$-**;

*-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**;

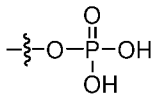
*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$C(=O)NH$(CH_2)_m$-**;

*-C(=O)$(CH_2)_m$C(R^3)$_2$-**; or

*-C(=O)$(CH_2)_m$C(=O)NH$(CH_2)_m$-**,

where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

each R^3 is independently selected from H and C_1-C_6alkyl;

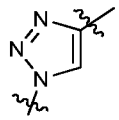
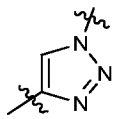
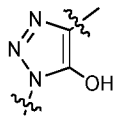
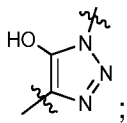
R^4 is 2-pyridyl or 4-pyridyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, -NH_2, -OCH_3, -OCH_2CH_3, -$N(CH_3)_2$, -CN, -NO_2 and –OH;

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with

–C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH;

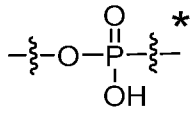
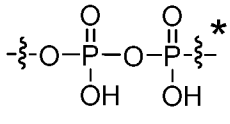
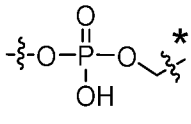
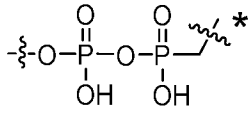
X_1 is  ,  ,  or  ;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

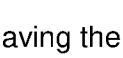
each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer comprising an amino acid residue selected from glycine, valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

$-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety having the structure  ,

where

W is $-CH_2O-**$, $-CH_2N(R^b)C(=O)O-**$, $-NHC(=O)C(R^b)_2NHC(=O)O-**$, $-NHC(=O)C(R^b)_2NH-**$, $-NHC(=O)C(R^b)_2NHC(=O)-**$, $-CH_2N(X-R^2)C(=O)O-**$, $-C(=O)N(X-R^2)-**$, $-CH_2N(X-R^2)C(=O)-**$, $-C(=O)NR^b-**$, $-C(=O)NH-**$, $-CH_2NR^bC(=O)-**$, $-CH_2NR^bC(=O)NH-**$, $-CH_2NR^bC(=O)NR^b-**$, $-NHC(=O)-**$, $-NHC(=O)O-**$, $-NHC(=O)NH-**$, $-OC(=O)NH-**$, $-S(O)_2NH-**$, $-NHS(O)_2-**$, $-C(=O)-$, $-C(=O)O-**$ or $-NH-$, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

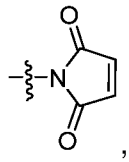
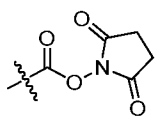
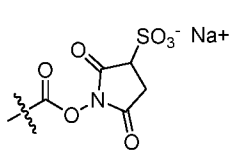
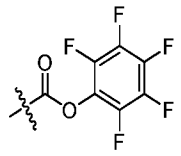
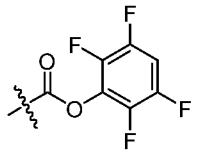
X is a bond, triazolyl or $***-CH_2$-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2; and

the * of L_3 indicates the point of attachment to R^2;

and

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety.

Embodiment 5. The compound of Formula (A') or of any one of Embodiments 1 to 4, or pharmaceutically acceptable salt thereof, wherein:

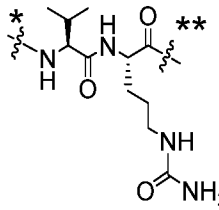
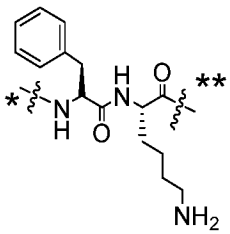
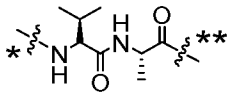
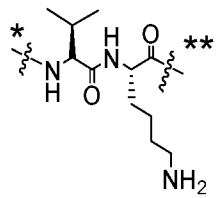
R^1 is  , $-ONH_2$,  ,  ,  or  ;

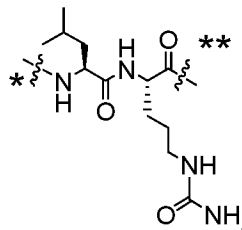
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1;

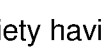
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit),  (PheLys),  (ValAla), 

 (ValLys) and (LeuCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the –NH- group of G;

L_3 is a spacer moiety having the structure  ,

where

W is $-CH_2O$-**, $-CH_2N(R^b)C(=O)O$-**, -NHC(=O)CH_2NHC(=O)O-**, -NHC(=O)CH_2NH-**, -NHC(=O)CH_2NHC(=O)-**, $-CH_2N(X-R^2)C(=O)O$-**, $-C(=O)N(X-R^2)$-**, $-CH_2N(X-R^2)C(=O)$-**,

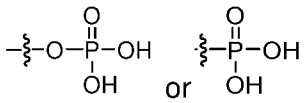
-C(=O)NR^b-**, -C(=O)NH-**, -CH$_2$NR^{b}C(=O)-**, -CH$_2$NR^{b}C(=O)NH-**, -CH$_2$NR^{b}C(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C$_1$-C$_6$alkyl or C$_3$-C$_8$cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

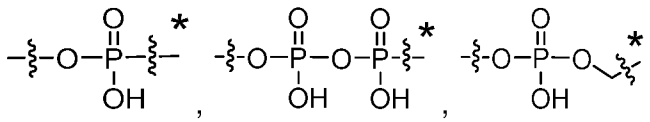
the * of L$_3$ indicates the point of attachment to R^2;

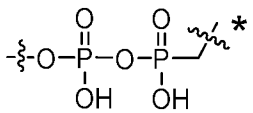
R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide, C$_2$-C$_6$alkyl substituted with 1 to 3



;

A is a bond, -OC(=O)-*,



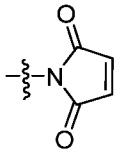


,

-OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety.

Embodiment 6. The compound of Formula (A') or of any one of Embodiments 1 to 5, or pharmaceutically acceptable salt thereof, wherein:

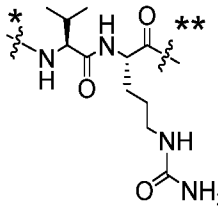
R^1 is  ;

L$_1$ is *-C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-**; *-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-**; *-C(=O)(CH$_2$)$_m$-**; or *-C(=O)NH((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-, where the * of L$_1$ indicates the point of attachment to Lp, and the ** of L$_1$ indicates the point of attachment to R^1;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the –NH- group of G;

L_3 is a spacer moiety having the structure  ,

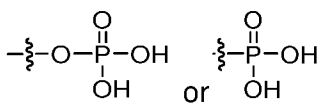
where

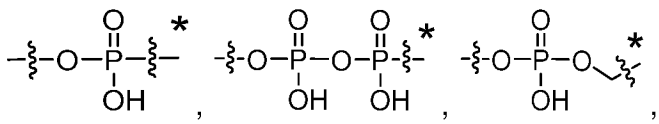
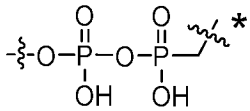
W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -$OC(=O)NH$-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide, C_2-C_6alkyl substituted with 1 to 3  groups;

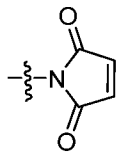
A is a bond, -OC(=O)-*,  ,  , -$OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or

-OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

and

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety.

Embodiment 7. The compound of Formula (A') or of any one of Embodiments 1 to 6, or pharmaceutically acceptable salt thereof, wherein:

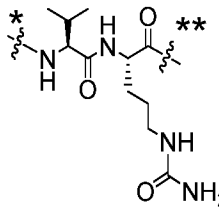
R^1 is  ;

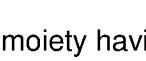
L$_1$ is *-C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-**; *-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-**; *-C(=O)(CH$_2$)$_m$-**; or *-C(=O)NH((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-, where the * of L$_1$ indicates the point of attachment to Lp and the ** of L$_1$ indicates the point of attachment to R^1;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L$_1$ and the ** of Lp indicates the attachment point to the -NH- group of G;

L$_3$ is a spacer moiety having the structure  ,

where

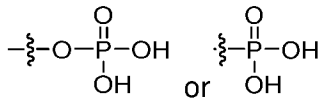
W is -CH$_2$O-**, -CH$_2$N(R^b)C(=O)O-**, -NHC(=O)CH$_2$NHC(=O)O-**, -CH$_2$N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH$_2$NR^{b}C(=O)-**, -CH$_2$NR^{b}C(=O)NH-**, -CH$_2$NR^{b}C(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, or -NHC(=O)NH-**, wherein each R^b is independently selected from H, C$_1$-C$_6$alkyl or C$_3$-C$_8$cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide, C_2-C_6alkyl substituted with 1 to 3



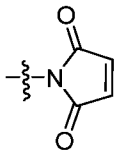
;

A is a bond or -OC(=O)*, in which * indicates the attachment point to D;

and

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety.

Embodiment 8. The compound of Formula (A') or of any one of Embodiments 1 to 7, or pharmaceutically acceptable salt thereof, wherein:

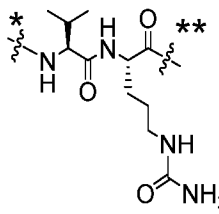
R^1 is  ;

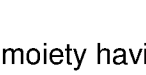
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp and the ** of L_1 indicates the point of attachment to R^1;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the –NH- group of G;

L_3 is a spacer moiety having the structure  ,

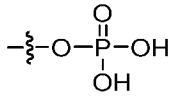
where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, or -$C(=O)N(X\text{-}R^2)$-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

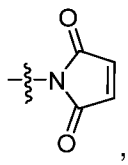
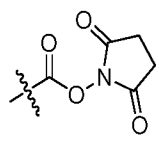
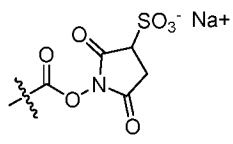
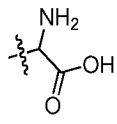
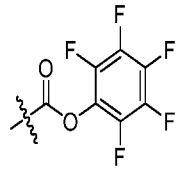
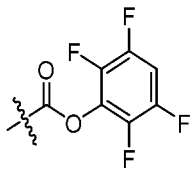
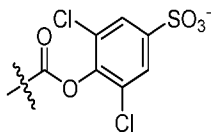
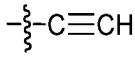
A is a bond or -OC(=O)* in which * indicates the attachment point to D;

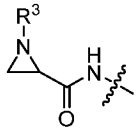
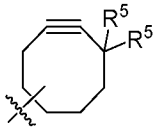
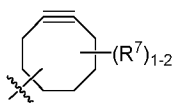
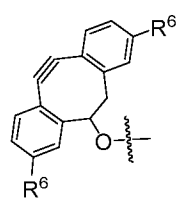
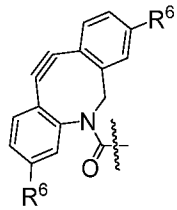
and

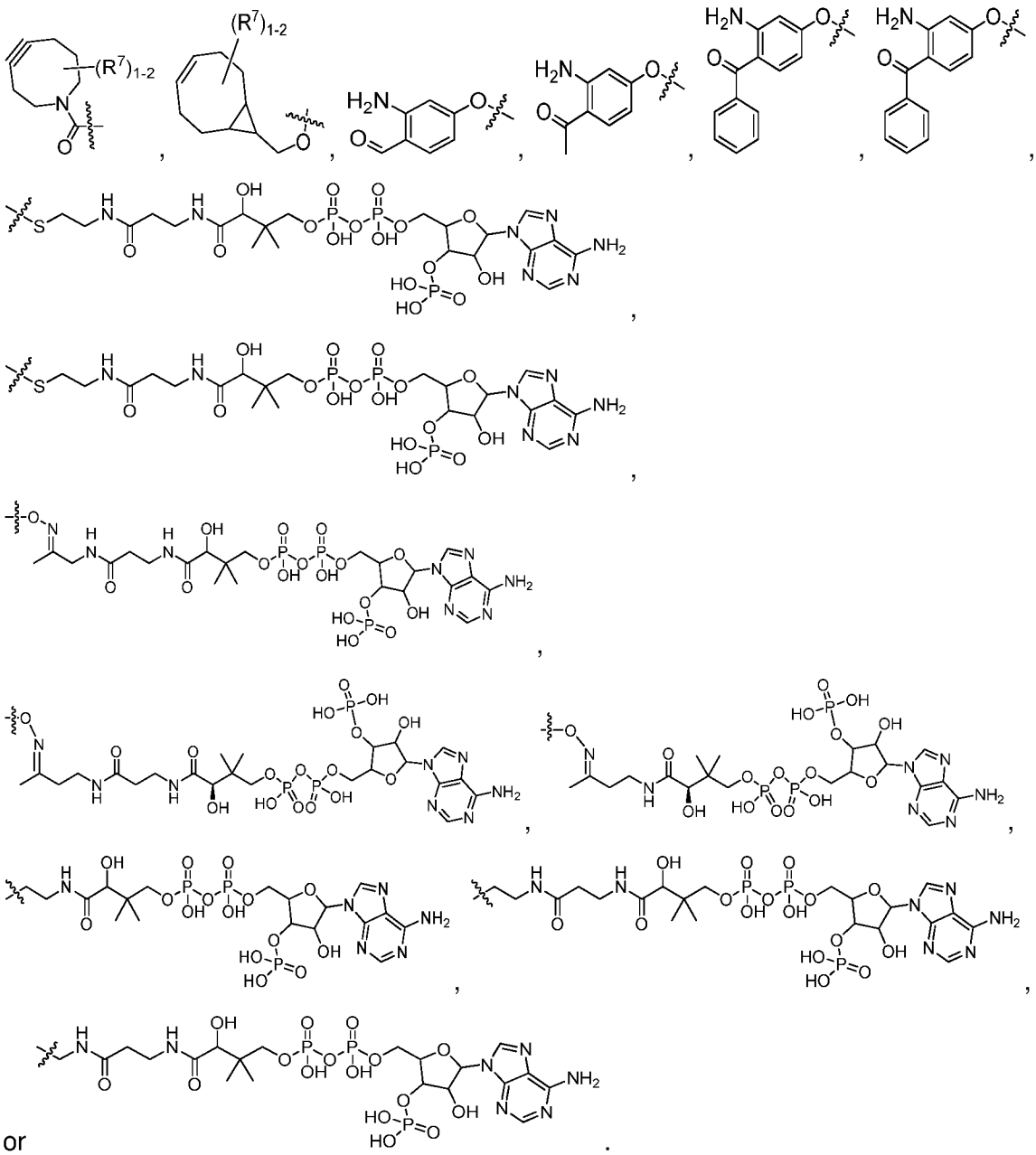
D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety.

Embodiment 9. The compound of Formula (A') or of any one of Embodiments 1 to 8, or pharmaceutically acceptable salt thereof, wherein R^1 is a reactive group selected from Table 8.

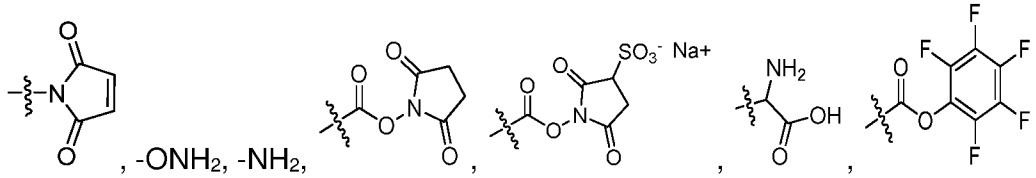
Embodiment 10. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein:

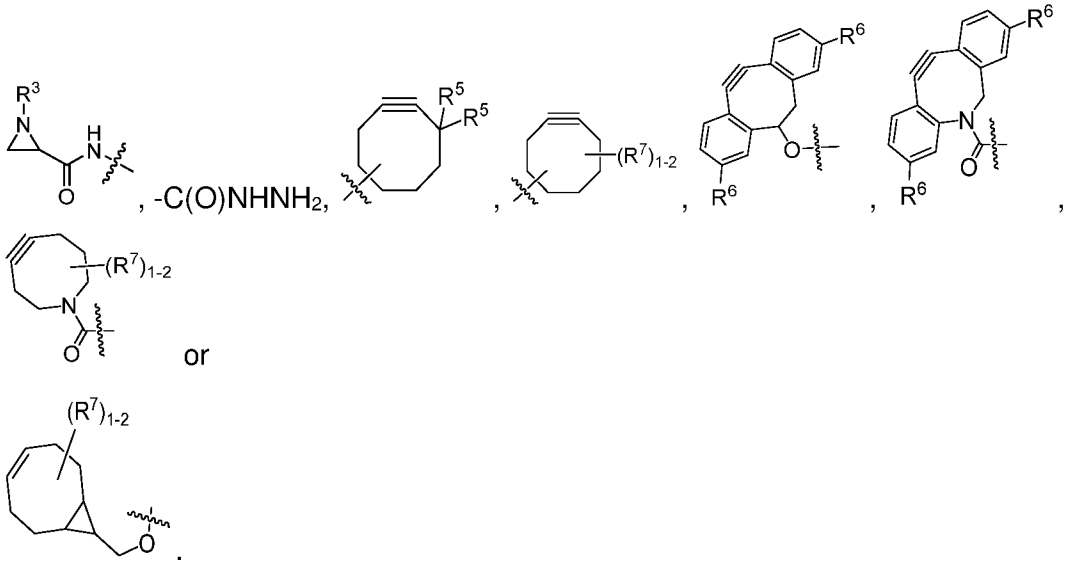
R^1 is  , -ONH_2, -NH_2,  ,  ,  ,  ,  ,  , -N_3,  , -SH, -SR^3, -SSR^4, -$S(=O)_2(CH=CH_2)$, -$(CH_2)_2S(=O)_2(CH=CH_2)$, -$NHS(=O)_2(CH=CH_2)$, -$NHC(=O)CH_2Br$, -$NHC(=O)CH_2I$,

 , -$C(O)NHNH_2$,  ,  ,  ,  ,

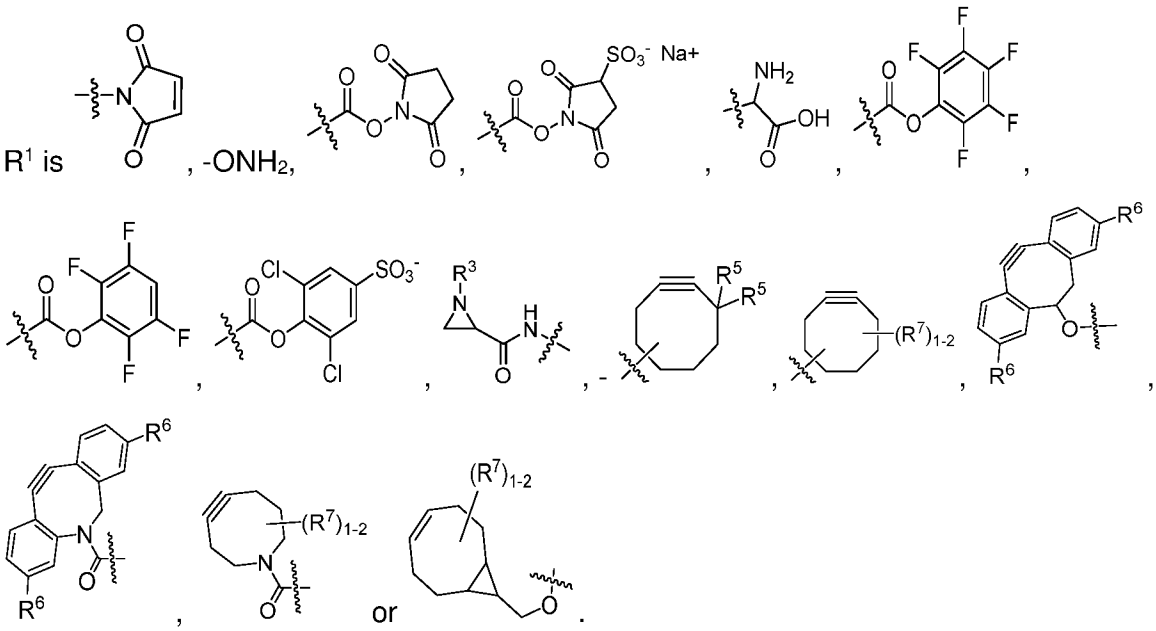


Embodiment 11. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein:

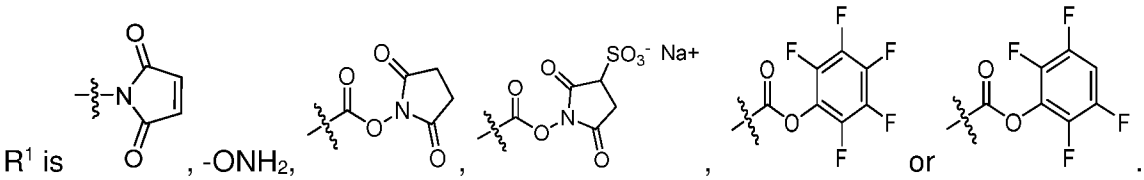
R^1 is  , $-ONH_2$, $-NH_2$, , $-N_3$,  , -SH, $-SR^3$, $-SSR^4$, $-S(=O)_2(CH=CH_2)$, $-(CH_2)_2S(=O)_2(CH=CH_2)$, $-NHS(=O)_2(CH=CH_2)$, $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$,



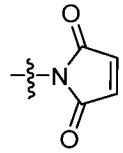
Embodiment 12. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein:



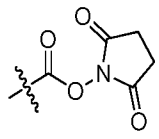
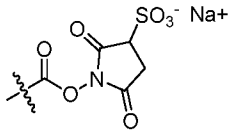
Embodiment 13. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein:



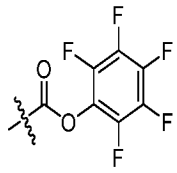
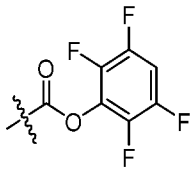
Embodiment 14. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein R^1 is



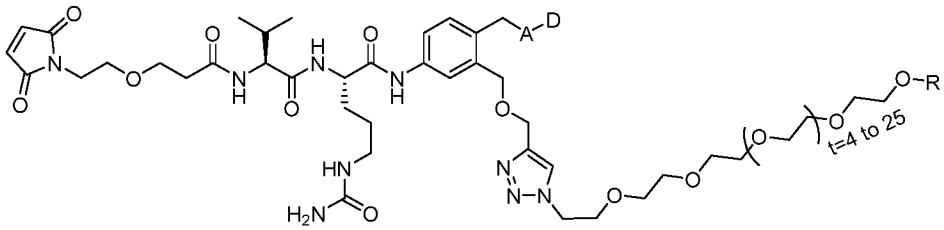
Embodiment 15. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein R^1 is $-ONH_2$.

Embodiment 16. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein: R^1 is  or  .

Embodiment 17. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, wherein:

R^1 is  or  .

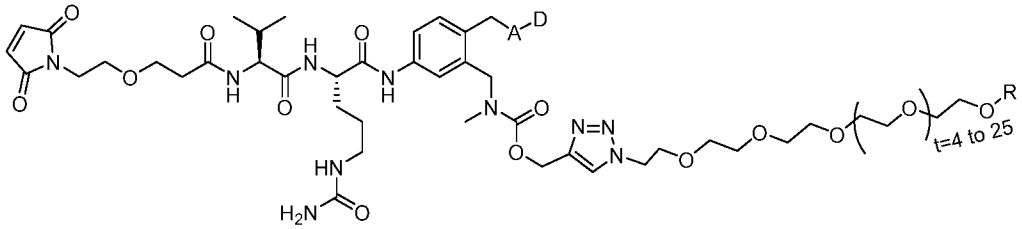
Embodiment 18. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

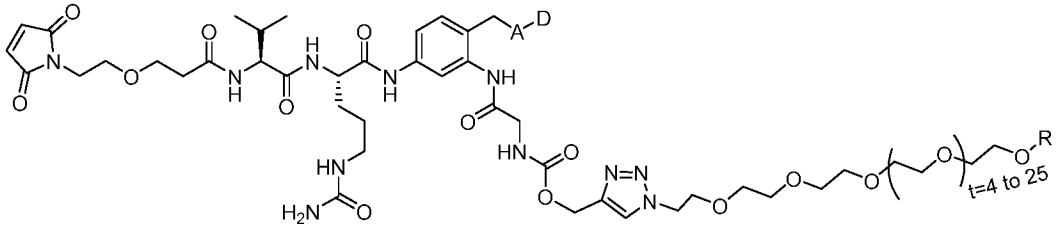
Embodiment 19. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

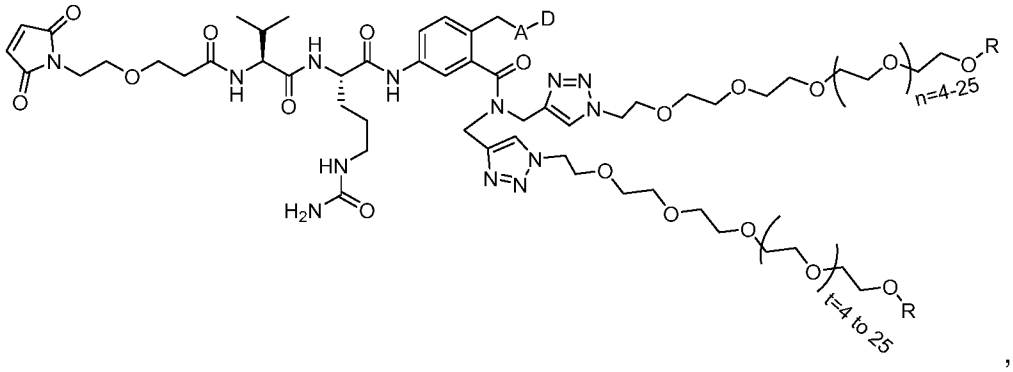
Embodiment 20. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

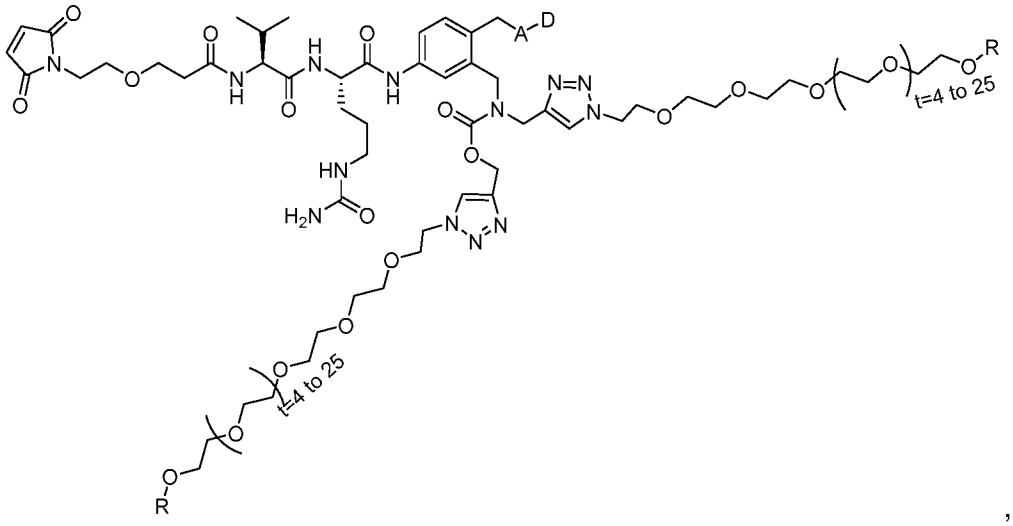
Embodiment 21. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$.

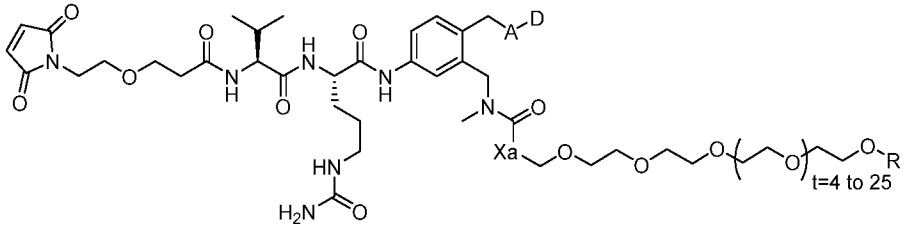
Embodiment 22. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$.

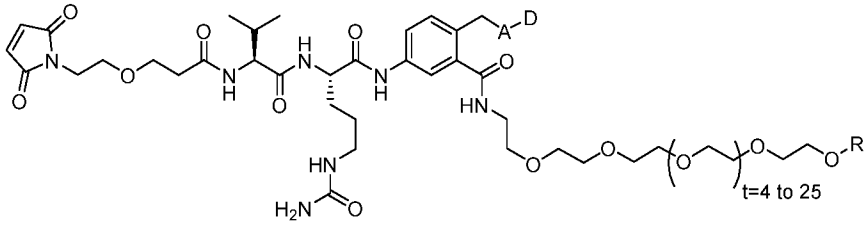
Embodiment 23. The compound of Formula (A') or of any one of Embodiments 1 to 9 or pharmaceutically acceptable salt thereof, having the structure:



, where

Xa is $–CH_2-$, $-OCH_2-$, $-NHCH_2-$ or $–NRCH_2-$ and each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

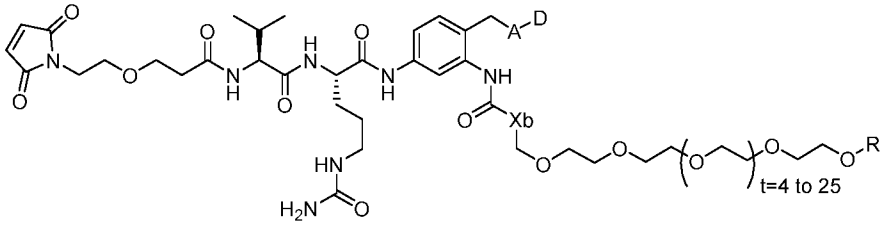
Embodiment 24. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

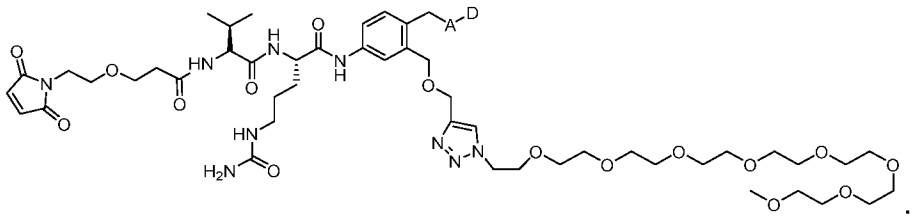
Embodiment 25. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where

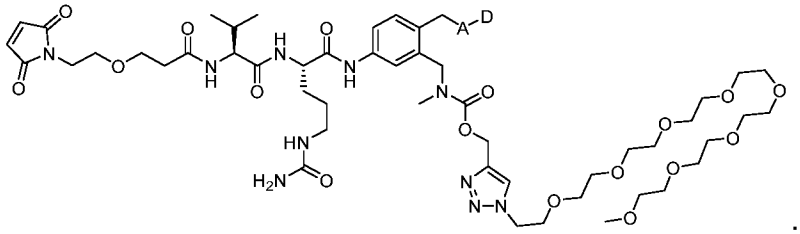
Xb is $-CH_2-$, $-OCH_2-$, $-NHCH_2-$ or $–NRCH_2-$ and each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

Embodiment 26. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:

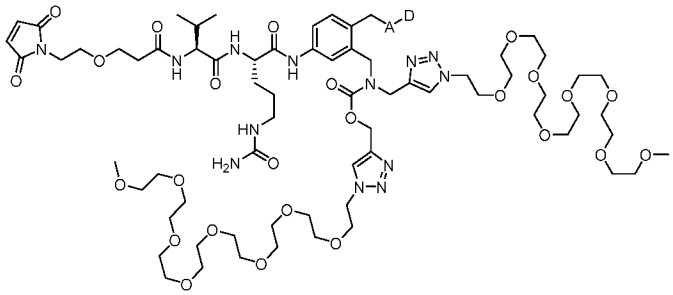


.

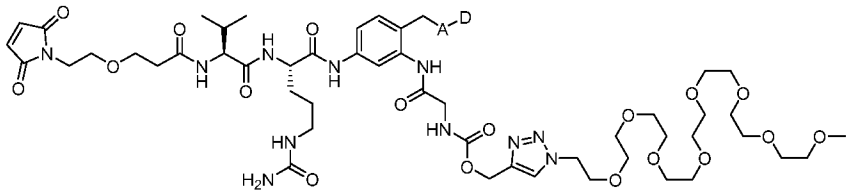
Embodiment 27. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



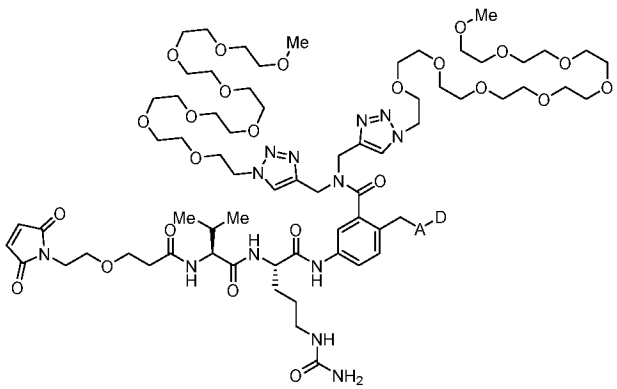
Embodiment 28. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



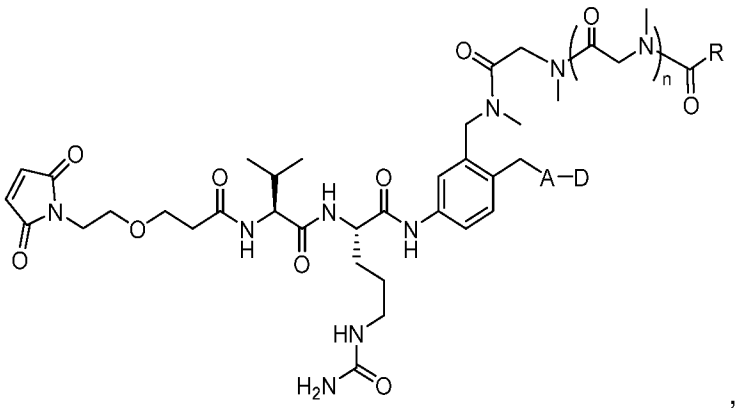
Embodiment 29. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



Embodiment 30. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



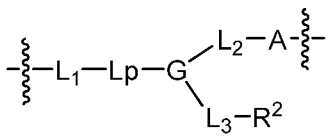
Embodiment 31. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure:



, where n is an integer between 2 and 24.

Embodiment 32. The compound of Formula (A') or of any one of Embodiments 1 to 9, or pharmaceutically acceptable salt thereof, having the structure of a compound in Table B.

Embodiment 33. A linker of the Linker-Drug group of Formula (A') having the structure of Formula (C'),



Formula (C')

wherein

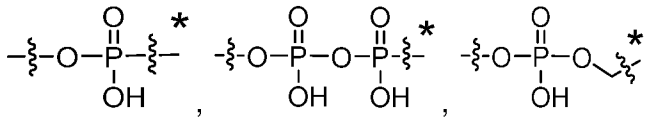
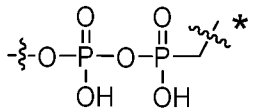
L_1 is a bridging spacer;

Lp is a bivalent peptide spacer;

G-L_2-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D,

and

L_3 is a spacer moiety.

Embodiment 34. The linker of Embodiment 33, wherein:

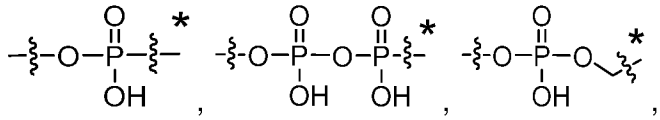
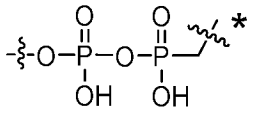
L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

G-L_2-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D,

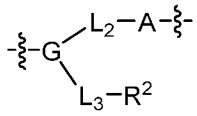
and

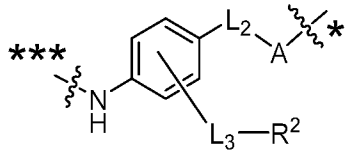
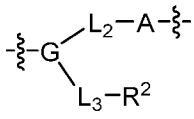
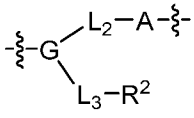
L_3 is a spacer moiety.

Embodiment 35. The linker of Embodiment 33 or 34, wherein:

L_1 is a bridging spacer;

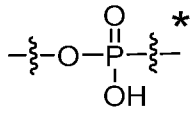
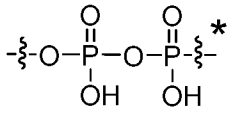
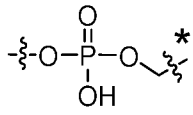
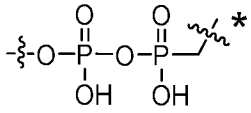
Lp is a bivalent peptide spacer comprising two to four amino acid residues;

the  group is selected from:

 , wherein the * of  indicates the point of attachment to D (e.g., to an N or a O of the Drug moiety), the *** of  indicates the point of attachment to Lp;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

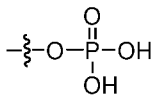
-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D,

and

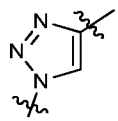
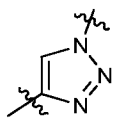
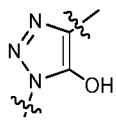
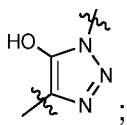
L_3 is a spacer moiety.

Embodiment 36. The linker of any one of Embodiments 33 to 35, wherein:

L_1 is *-C(=O)(CH_2)$_m$O(CH_2)$_m$-**; *-C(=O)((CH_2)$_m$O)$_t$(CH_2)$_n$-**; *-C(=O)(CH_2)$_m$-**; *-C(=O)NH((CH_2)$_m$O)$_t$(CH_2)$_n$-**; *-C(=O)O(CH_2)$_m$SSC(R^3)$_2$(CH_2)$_m$C(=O)NR^3(CH_2)$_m$NR^3C(=O)(CH_2)$_m$-**; *-C(=O)O(CH_2)$_m$C(=O)NH(CH_2)$_m$-**; *-C(=O)(CH_2)$_m$NH(CH_2)$_m$-**; *-C(=O)(CH_2)$_m$NH(CH_2)$_n$C(=O)-**; *-C(=O)(CH_2)$_m$$X_1$($CH_2$)$_m$-**; *-C(=O)(($CH_2$)$_m$O)$_t$($CH_2$)$_n$$X_1$($CH_2$)$_n$-**; *-C(=O)($CH_2$)$_m$NHC(=O)($CH_2$)$_n$-**; *-C(=O)(($CH_2$)$_m$O)$_t$($CH_2$)$_n$NHC(=O)($CH_2$)$_n$-**; *-C(=O)($CH_2$)$_m$NHC(=O)($CH_2$)$_n$$X_1$($CH_2$)$_n$-**; *-C(=O)(($CH_2$)$_m$O)$_t$($CH_2$)$_n$NHC(=O)($CH_2$)$_n$$X_1$($CH_2$)$_n$-**; *-C(=O)(($CH_2$)$_m$O)$_t$($CH_2$)$_n$C(=O)NH($CH_2$)$_m$-**; *-C(=O)($CH_2$)$_m$C($R^3$)$_2$-** or *-C(=O)($CH_2$)$_m$C(=O)NH($CH_2$)$_m$-**, where the * of L_1 indicates the point of attachment to Lp;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

each R^3 is independently selected from H and C_1-C_6alkyl;

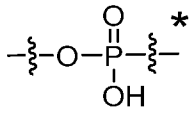
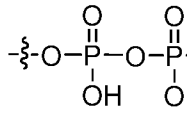
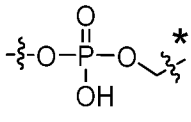
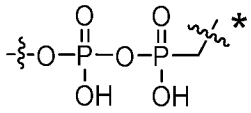
X_1 is  ,  ,  or  ;

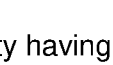
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer comprising an amino acid residue selected from glycine, valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine;

A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety having the structure  ,

where

W is -CH$_2$O-**, -CH$_2$N(R^b)C(=O)O-**, -NHC(=O)C(R^b)$_2$NHC(=O)O-**, -NHC(=O)C(R^b)$_2$NH-**, -NHC(=O)C(R^b)$_2$NHC(=O)-**, -CH$_2$N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH$_2$N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH$_2$NR^{b}C(=O)-**, -CH$_2$NR^{b}C(=O)NH-**, -CH$_2$NR^{b}C(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C$_1$-C$_6$alkyl or C$_3$-C$_8$cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH$_2$-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L$_3$ indicates the point of attachment to R^2.

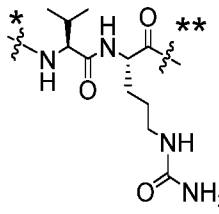
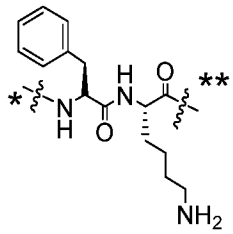
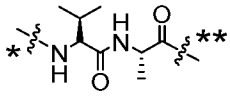
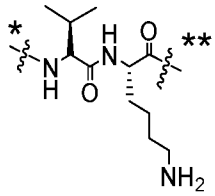
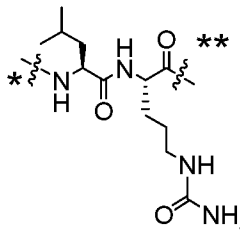
Embodiment 37. The linker of any one of Embodiments 33 to 36, wherein:

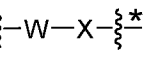
L$_1$ is *-C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-**; *-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-**; *-C(=O)(CH$_2$)$_m$-**; or *-C(=O)NH((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$-, where the * of L$_1$ indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit),  (PheLys),  (ValAla),  (ValLys) and  (LeuCit), where the * of Lp indicates the attachment point to L_1;

L_3 is a spacer moiety having the structure  ,

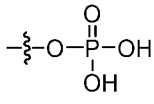
where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$NHC(=O)CH_2NH$-**, -$NHC(=O)CH_2NHC(=O)$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -$OC(=O)NH$-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

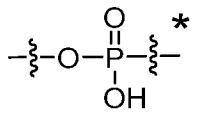
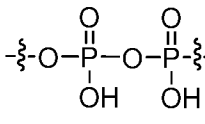
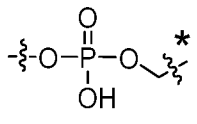
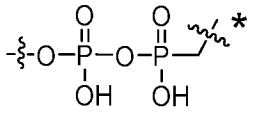
X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D.

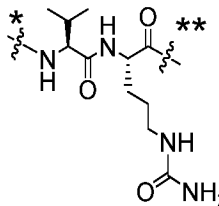
Embodiment 38. The linker of any one of Embodiments 33 to 37, wherein:

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

where

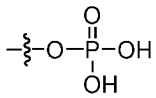
W is -CH_2O-**, -CH2N(Rb)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -

$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

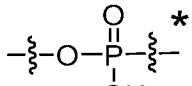
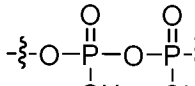
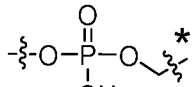
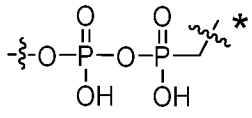
X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D.

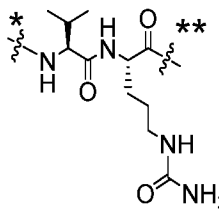
Embodiment 39. The linker of any one of Embodiments 33 to 38, wherein:

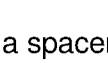
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($CH_2)_m$O)$_t$($CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH(($CH_2)_m$O)$_t$($CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1;

L_3 is a spacer moiety having the structure  ,

where

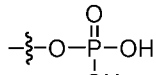
W is -CH_2O-**, -CH2N(Rb)C(=O)O-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$C(=O)NR^b$-**, -$C(=O)NH$-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -$NHC(=O)$-**, -$NHC(=O)O$-**, or -$NHC(=O)NH$-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to

3  groups;

and

A is a bond or -$OC(=O)$* in which * indicates the attachment point to D.

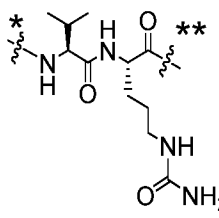
Embodiment 40. The linker of any one of Embodiments 33 to 39, wherein:

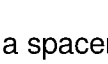
L_1 is *-$C(=O)(CH_2)_mO(CH_2)_m$-**; *-$C(=O)((CH_2)_mO)_t(CH_2)_n$-**; *-$C(=O)(CH_2)_m$-**; or *-$C(=O)NH((CH_2)_mO)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1;

L_3 is a spacer moiety having the structure  ,

where

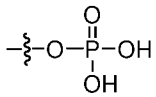
W is -CH_2O-**, -CH2N(Rb)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, or -C(=O)N(X-R^2)-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to

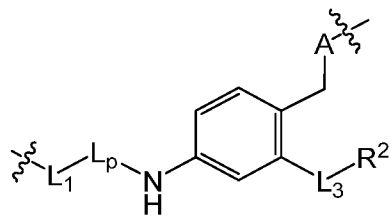


3 groups;

and

A is a bond or -OC(=O)* in which * indicates the attachment point to D.

Embodiment 41. The linker of Formula (C') having the structure having the structure of Formula (D'),



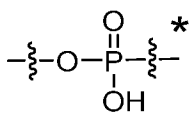
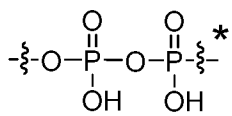
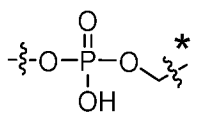
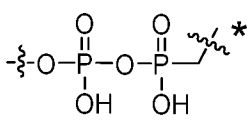
Formula (D')

wherein

L_1 is a bridging spacer;

Lp is a bivalent peptide spacer;

R^2 is a hydrophilic moiety;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is

independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D,

and

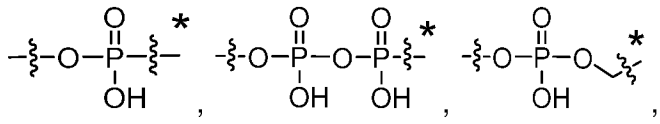
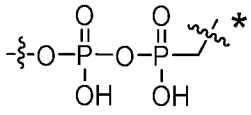
L_3 is a spacer moiety.

Embodiment 42. The linker of Embodiments 41, wherein:

L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

R^2 is a hydrophilic moiety;

A is a bond, -OC(=O)-*,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D,

and

L_3 is a spacer moiety.

Embodiment 43. The linker of Embodiment 41 or 42, wherein:

L_1 is *-C(=O)(CH_2)$_m$O(CH_2)$_m$-**; *-C(=O)(((CH_2)$_m$O)$_t$(CH_2)$_n$-**; *-C(=O)(CH_2)$_m$-**;

*-C(=O)NH(((CH_2)$_m$O)$_t$(CH_2)$_n$-**;

*-C(=O)O(CH_2)$_m$SSC(R^3)$_2$(CH_2)$_m$C(=O)NR^3(CH_2)$_m$NR^3C(=O)(CH_2)$_m$-**;

*-C(=O)O(CH_2)$_m$C(=O)NH(CH_2)$_m$-**; *-C(=O)(CH_2)$_m$NH(CH_2)$_m$-**;

*-C(=O)(CH_2)$_m$NH(CH_2)$_n$C(=O)-**; *-C(=O)(CH_2)$_m$$X_1$($CH_2$)$_m$-**;

*-C(=O)(((CH_2)$_m$O)$_t$(CH_2)$_n$$X_1$($CH_2$)$_n$-**; *-C(=O)($CH_2$)$_m$NHC(=O)($CH_2$)$_n$-**;

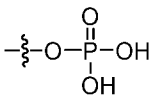
*-C(=O)(((CH_2)$_m$O)$_t$(CH_2)$_n$NHC(=O)(CH_2)$_n$-**;

*-C(=O)(CH_2)$_m$NHC(=O)(CH_2)$_n$$X_1$($CH_2$)$_n$-**;

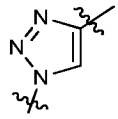
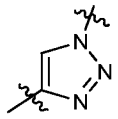
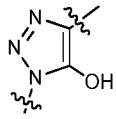
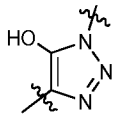
*-C(=O)(((CH_2)$_m$O)$_t$(CH_2)$_n$NHC(=O)(CH_2)$_n$$X_1$($CH_2$)$_n$-**;

*-C(=O)(((CH_2)$_m$O)$_t$(CH_2)$_n$C(=O)NH(CH_2)$_m$-**; *-C(=O)(CH_2)$_m$C(R^3)$_2$-** or

*-C(=O)(CH_2)$_m$C(=O)NH(CH_2)$_m$-**, where the * of L_1 indicates the point of attachment to Lp;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

each R^3 is independently selected from H and C_1-C_6alkyl;

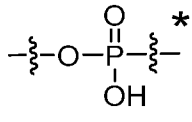
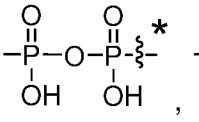
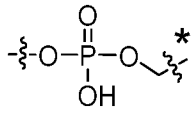
X_1 is  ,  ,  or  ;

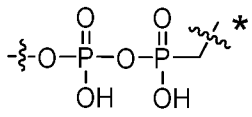
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

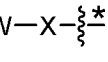
Lp is a bivalent peptide spacer comprising an amino acid residue selected from glycine, valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine;

A is a bond, -OC(=O)-*,  ,  ,  ,

 ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety having the structure  ,

where

W is -CH_2O-**, -CH2N(Rb)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

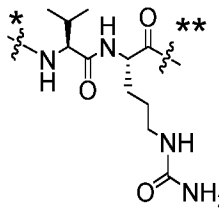
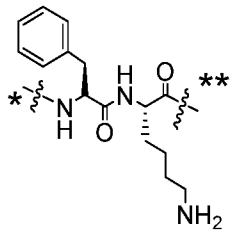
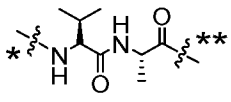
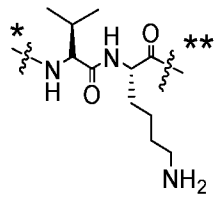
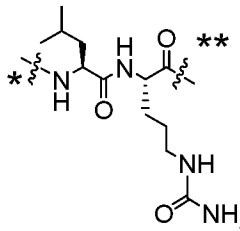
Embodiment 44. The linker of any one of Embodiments 41 to 43, wherein:

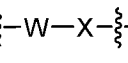
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH($(CH_2)_m$O)$_t$$(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit),  (PheLys),  (ValAla),  (ValLys) and  (LeuCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

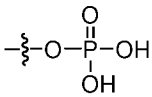
where

W is -CH_2O-**, -CH2N(Rb)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

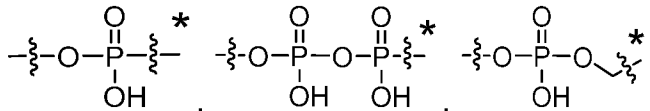
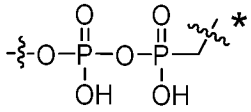
X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond, -OC(=O)-*,  ,  , -OC(=O)$N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -OC(=O)$N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D.

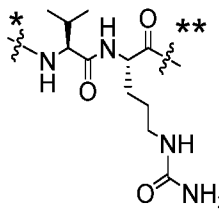
Embodiment 45. The linker of any one of Embodiments 41 to 44, wherein:

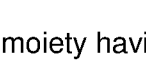
L_1 is *-$C(=O)(CH_2)_mO(CH_2)_m$-**; *-$C(=O)((CH_2)_mO)_t(CH_2)_n$-**; *-$C(=O)(CH_2)_m$-**; or *-$C(=O)NH((CH_2)_mO)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

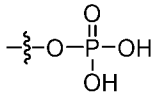
where

W is -CH_2O-**, -$CH_2N(Rb)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -$OC(=O)NH$-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

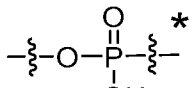
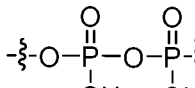
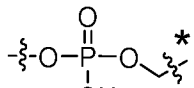
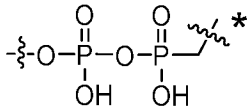
X is a bond, triazolyl or ***-CH2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-$OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -$OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D.

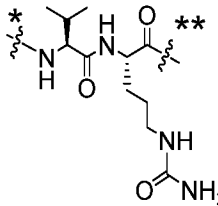
Embodiment 46. The linker of any one of Embodiments 41 to 45, wherein:

L_1 is *-$C(=O)(CH_2)_mO(CH_2)_m$-**; *-$C(=O)((CH_2)_mO)_t(CH_2)_n$-**; *-$C(=O)(CH_2)_m$-**; or *-$C(=O)NH((CH_2)_mO)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp;

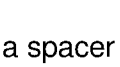
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;



Lp is a bivalent peptide spacer selected from (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

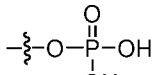
where

W is $-CH_2O-$**, $-CH_2N(Rb)C(=O)O-$**, $-NHC(=O)CH_2NHC(=O)O-$**, $-CH_2N(X-R^2)C(=O)O-$**, $-C(=O)N(X-R^2)-$**, $-C(=O)NR^b-$**, $-C(=O)NH-$**, $-CH_2NR^bC(=O)-$**, $-CH_2NR^bC(=O)NH-$**, $-CH_2NR^bC(=O)NR^b-$**, $-NHC(=O)-$**, $-NHC(=O)O-$**, or $-NHC(=O)NH-$**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond or -OC(=O)* in which * indicates the attachment point to D.

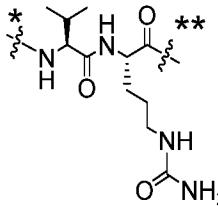
Embodiment 47. The linker of any one of Embodiments 41 to 46, wherein:

L_1 is *-$C(=O)(CH_2)_mO(CH_2)_m-$**; *-$C(=O)((CH_2)_mO)_t(CH_2)_n-$**; *-$C(=O)(CH_2)_m-$**; or *-$C(=O)NH((CH_2)_mO)_t(CH_2)_n-$, where the * of L_1 indicates the point of attachment to Lp;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;



Lp is a bivalent peptide spacer selected from (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure ,

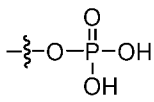
where

W is $-CH_2O-$**, $-CH_2N(Rb)C(=O)O-$**, $-NHC(=O)CH_2NHC(=O)O-$**, $-CH_2N(X-R^2)C(=O)O-$**, or $-C(=O)N(X-R^2)-$**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

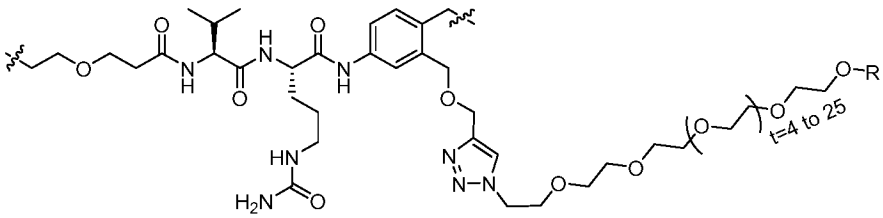
the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

and

A is a bond or -OC(=O)* in which * indicates the attachment point to D.

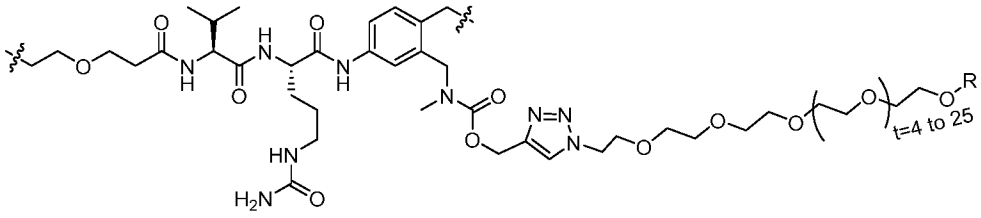
Embodiment 48. The linker of any one of Embodiments 33 to 47, having the structure:



, where

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$.

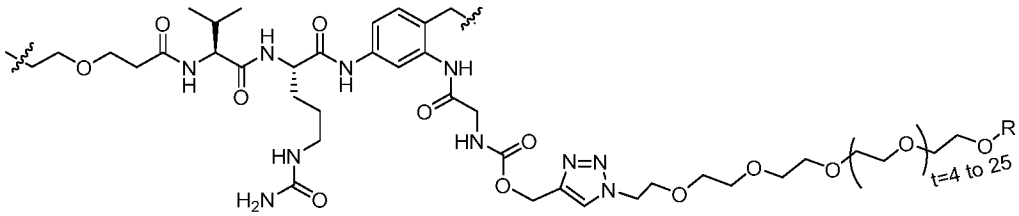
Embodiment 49. The linker of any one of Embodiments 33 to 47, having the structure:



, where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

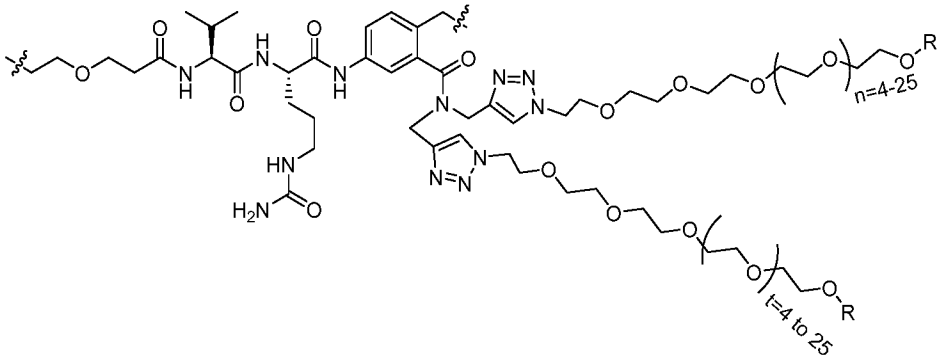
Embodiment 50. The linker of any one of Embodiments 33 to 47, having the structure:



, where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

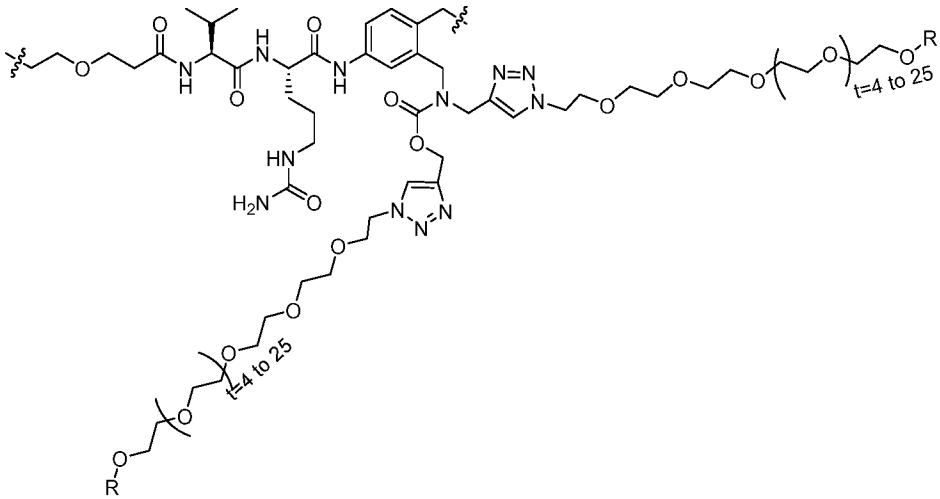
Embodiment 51. The linker of any one of Embodiments 33 to 47, having the structure:



, where

each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$.

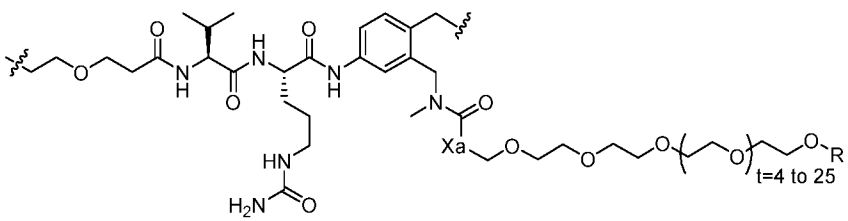
Embodiment 52. The linker of any one of Embodiments 37 to 47, having the structure:



, where

each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$.

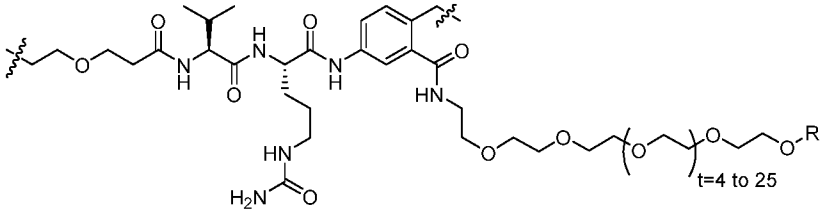
Embodiment 53. The linker of any one of Embodiments 33 to 47, having the structure:



, where

Xa is –CH_2-, -OCH_2-, -$NHCH_2$- or –$NRCH_2$- and each R independently is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

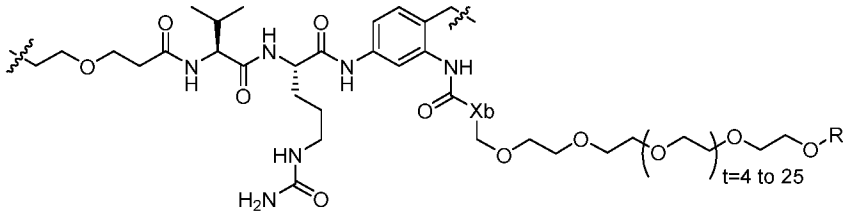
Embodiment 54. The linker of any one of Embodiments 33 to 47, having the structure:



, where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

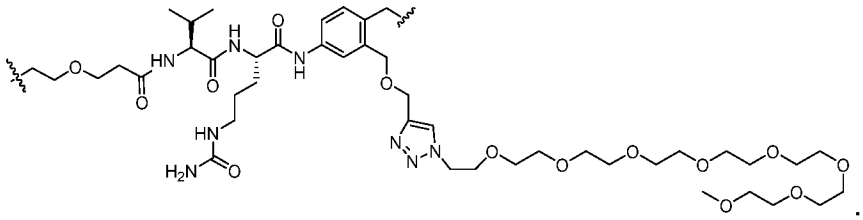
Embodiment 55. The linker of any one of Embodiments 33 to 47, having the structure:



, where

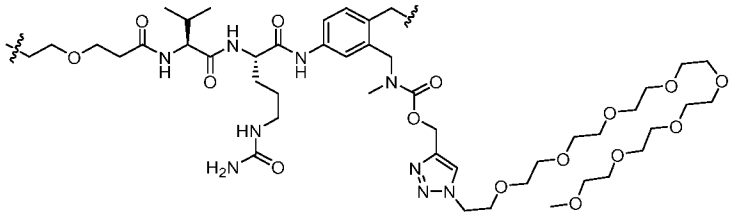
Xb is -CH_2-, -OCH_2-, -$NHCH_2$- or –$NRCH_2$- and each R independently is H, -CH_3 or -$CH_2CH_2C(=O)OH$.

Embodiment 56. The linker of any one of Embodiments 33 to 47, having the structure:



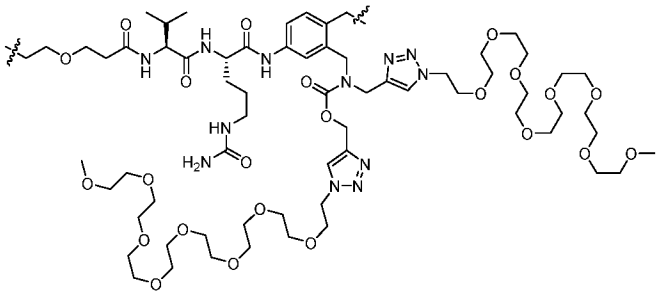
.

Embodiment 57. The linker of any one of Embodiments 33 to 47, having the structure:



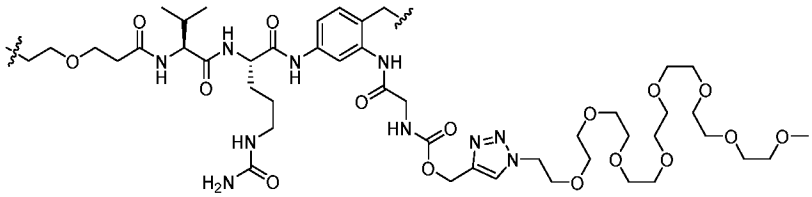
.

Embodiment 58. The linker of any one of Embodiments 37 to 47, having the structure:

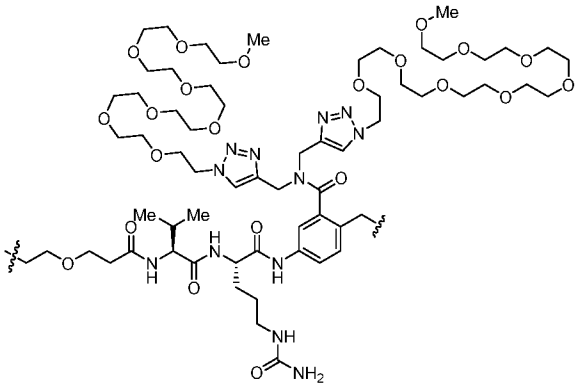


.

Embodiment 59. The linker of any one of Embodiments 33 to 47, having the structure:

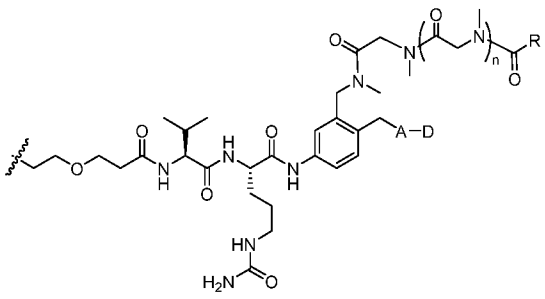


Embodiment 60. The linker of any one of Embodiments 33 to 47, having the structure:



.

Embodiment 61. The linker of any one of Embodiments 33 to 47, having the structure:

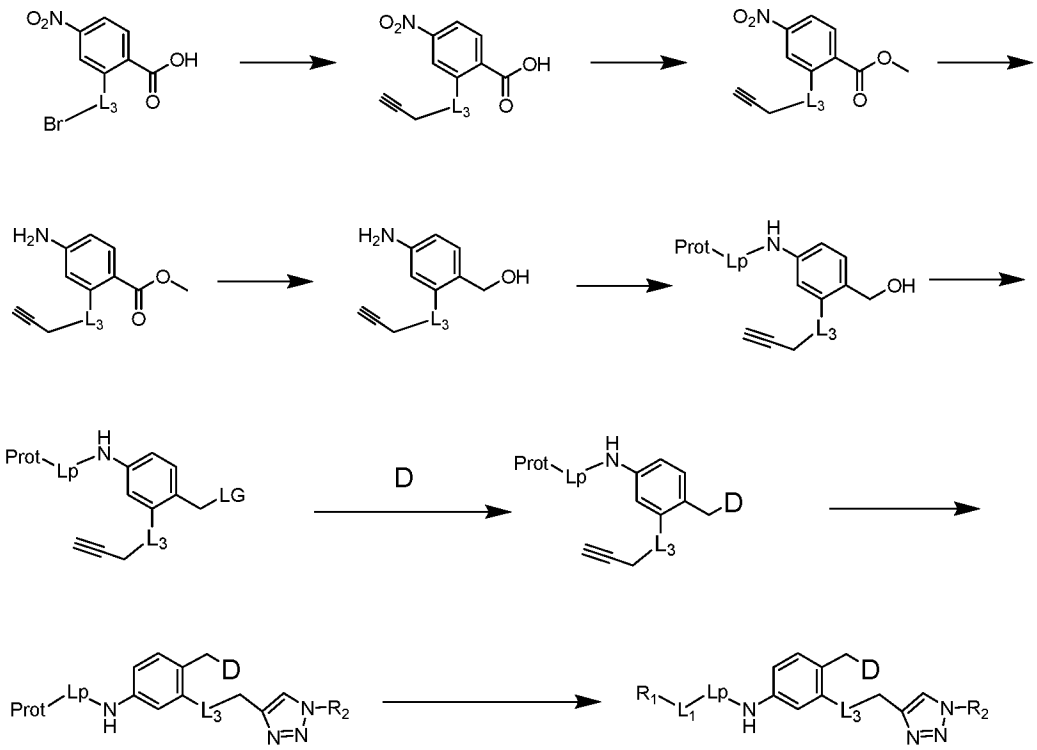


, where n is an integer between 2 and 24

For illustrative purposes, the general reaction schemes depicted herein provide potential routes for synthesizing the compounds of the present invention as well as key intermediates. For a more detailed description of the individual reaction steps, see the Examples section below. Although specific starting materials and reagents are depicted in the schemes and discussed below, other starting materials and reagents can be easily substituted to provide a variety of derivatives and/or reaction conditions. In addition, many of the compounds prepared by the methods described below can be further modified in light of this disclosure using conventional chemistry well known to those skilled in the art.

By way of example, a general synthesis for compounds of Formula (B') is shown below in Scheme 1.

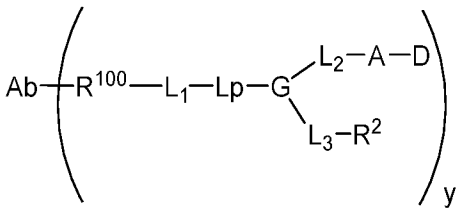
Scheme 1



Antibody Drug Conjugates of the Invention

The present invention provides Antibody Drug Conjugates, also referred to herein as immunoconjugates, which comprise linkers which comprise one or more hydrophilic moieties.

The Antibody Drug Conjugates of the invention have the structure of Formula (E'):



Formula (E')

wherein:

Ab is an antibody or fragment thereof;

R^{100} is a coupling group;

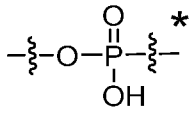
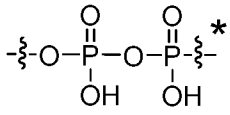
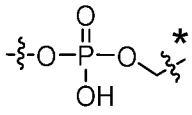
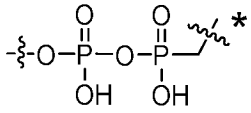
L_1 is a bridging spacer;

Lp is a bivalent peptide spacer;

G-L_2-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or

-OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety;

D is a Drug moiety as defined herein, e.g., a Bcl-xL inhibitor, and may comprise an N, wherein D can be connected to A via a direct bond from A to the N of the Drug moiety,

and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Certain aspects and examples of the Antibody Drug Conjugates of the invention are provided in the following listing of enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

Embodiment 62. The immunoconjugate of Formula (E') wherein:

Ab is an antibody or fragment thereof;

R^{100} is a coupling group;

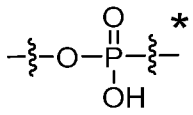
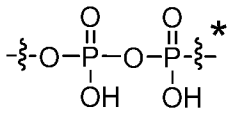
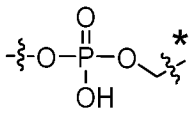
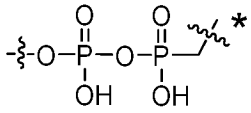
L$_1$ is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

G-L$_2$-A is a self-immolative spacer;

R^2 is a hydrophilic moiety;

L$_2$ is a bond, a methylene, a neopentylene or a C$_2$-C$_3$alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or

-OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*, wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety;

D is a Drug moiety as defined herein wherein D is connected to A via a direct bond from A to D (e.g., an N of the Drug moiety),

and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

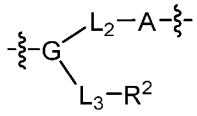
Embodiment 63. The immunoconjugate of Formula (E') or Embodiment 62, wherein:

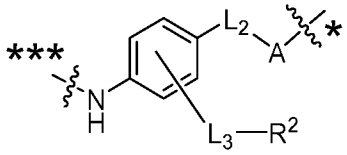
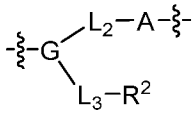
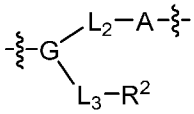
Ab is an antibody or fragment thereof;

R^{100} is a coupling group;

L_1 is a bridging spacer;

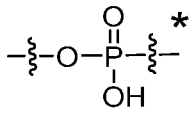
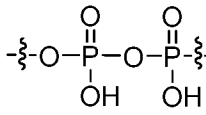
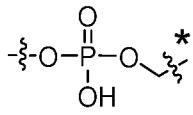
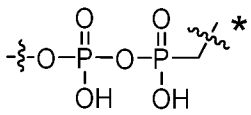
Lp is a bivalent peptide spacer comprising two to four amino acid residues;

the  group is selected from:

 , wherein the * of  indicates the point of attachment to D (e.g., to an N or a O of the Drug moiety), the *** of  indicates the point of attachment to Lp;

R^2 is a hydrophilic moiety;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

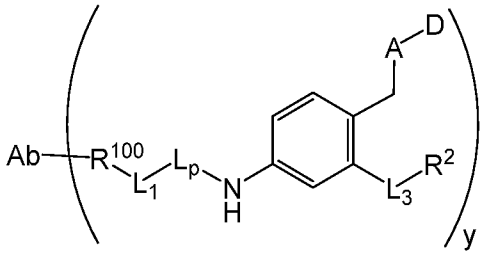
L_3 is a spacer moiety;

D is a Drug moiety as defined herein and comprising an N, wherein D is connected to A via a direct bond from A to the N of the Drug moiety,

and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 64. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 63 having the structure of Formula (F'),



Formula (F')

wherein:

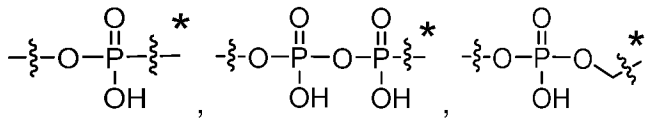
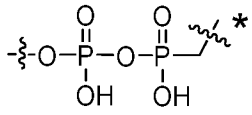
Ab is an antibody or fragment thereof;

R^{100} is a coupling group;

L_1 is a bridging spacer;

Lp is a bivalent peptide spacer comprising two to four amino acid residues;

R^2 is a hydrophilic moiety;

A is a bond, -OC(=O)-*,  ,  , -OC(=O)$N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or -OC(=O)$N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety;

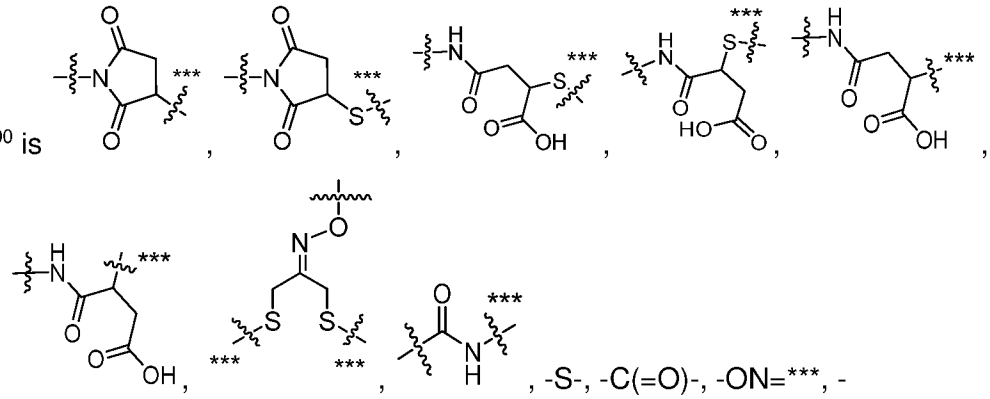
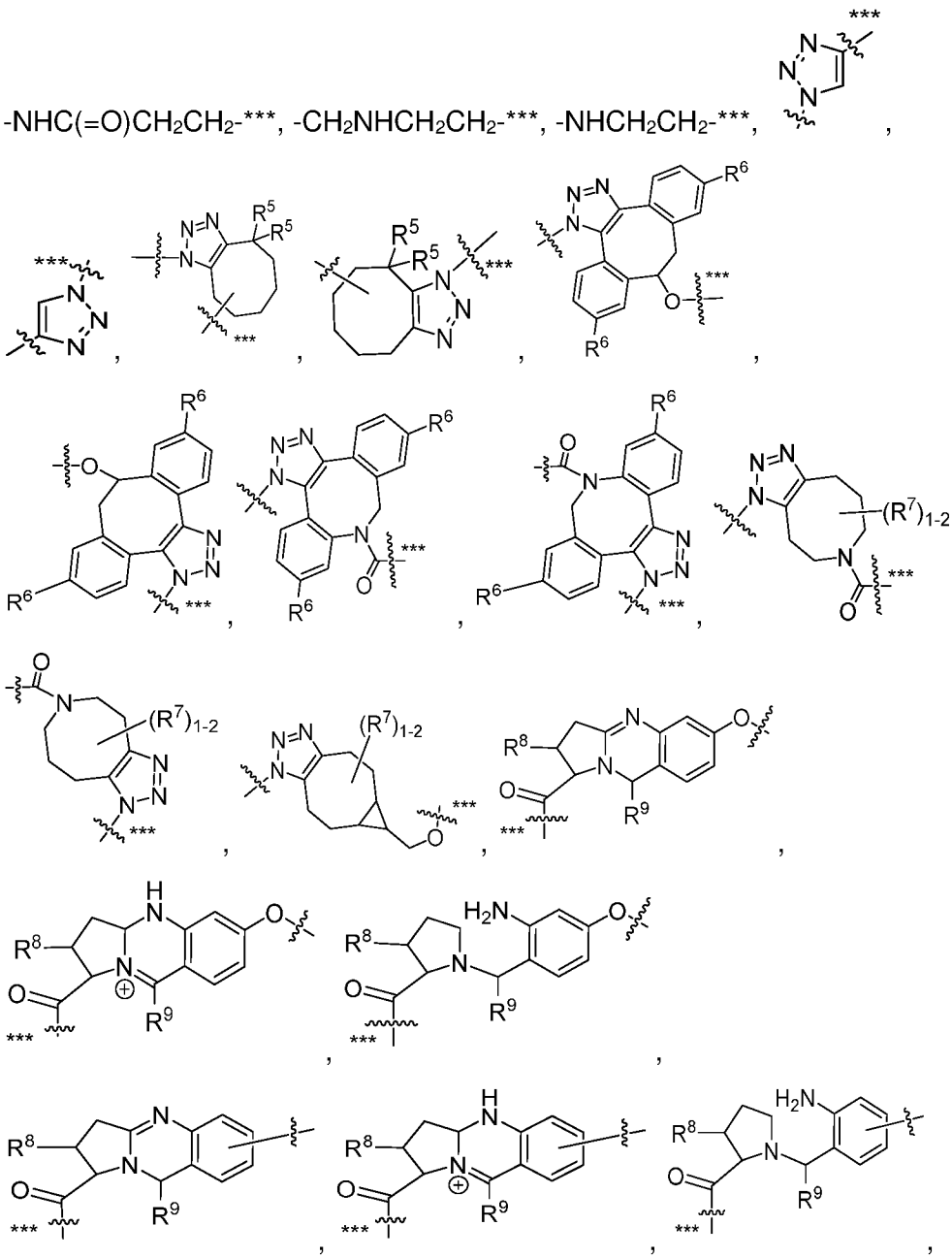
D is a Drug moiety as defined herein and comprising an N, wherein D is connected to A via a direct bond from A to the N of the Drug moiety,

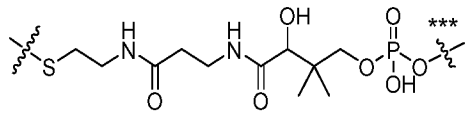
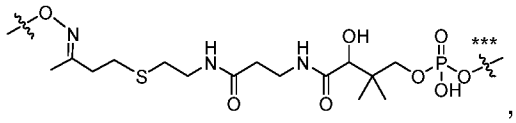
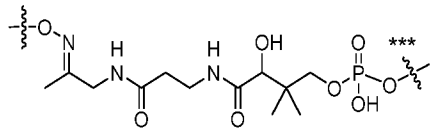
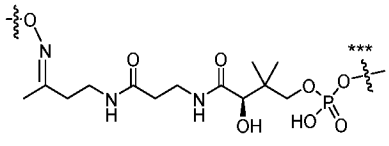
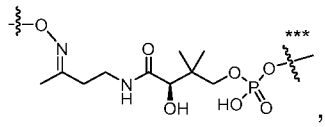
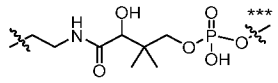
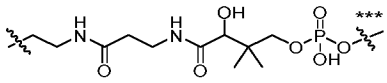
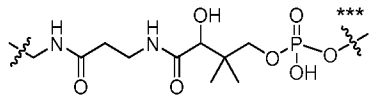
and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 65. The immunoconjugate of Formula (D') or any one of Embodiments 62 to 64, wherein:

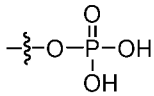
Ab is an antibody or fragment thereof;

R^{100} is  , -S-, -C(=O)-, -ON=***, -NHC(=O)CH_2-***, -S(=O)$_2CH_2CH_2$-***, -$(CH_2)_2$S(=O)$_2CH_2CH_2$-***, -NHS(=O)$_2CH_2CH_2$-***, -NHC(=O)CH_2CH_2-***, -$CH_2NHCH_2CH_2$-***, -$NHCH_2CH_2$-***, 

, or , where the *** of R^{100} indicates the point of attachment to Ab;

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; *-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)O$(CH_2)_mSSC(R^3)_2$$(CH_2)_m$C(=O)N$R^3$$(CH_2)_mNR^3$C(=O)$(CH_2)_m$-**; *-C(=O)O$(CH_2)_m$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_mNH(CH_2)_m$-**; *-C(=O)$(CH_2)_mNH(CH_2)_n$C(=O)-**; *-C(=O)$(CH_2)_m$$X_1$$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_mC(R^3)_2$-** or *-C(=O)$(CH_2)_m$C(=O)NH$(CH_2)_m$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^{100};

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

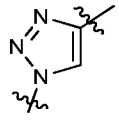
each R^3 is independently selected from H and C_1-C_6alkyl;

R^4 is 2-pyridyl or 4-pyridyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and -OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and -OH;

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with -C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH;

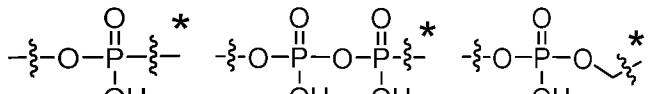
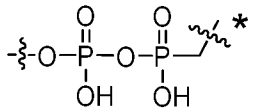
X_1 is  ;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer comprising an amino acid residue selected from valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine;

A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

L_3 is a spacer moiety having the structure ,

where

W is -CH_2O-**, -CH_2N(Rb)C(=O)O-**, -NHC(=O)C(R^b)$_2$NHC(=O)O-**, -NHC(=O)C(R^b)$_2$NH-**, -NHC(=O)C(R^b)$_2$NHC(=O)-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

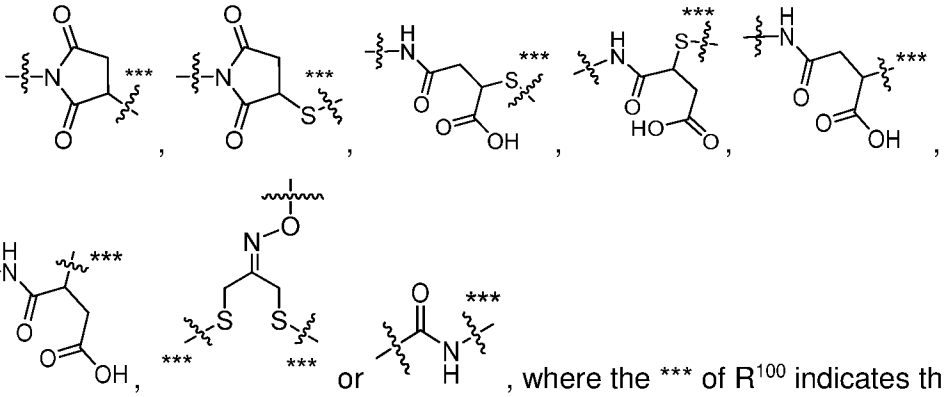
D is a Drug moiety as defined herein and comprising an N, wherein D is connected to A via a direct bond from A to the N of the Drug moiety,

and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 66. The immunoconjugate of Formula (D') or any one of Embodiments 62 to 65, wherein:

Ab is an antibody or fragment thereof;

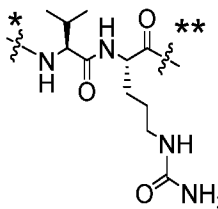
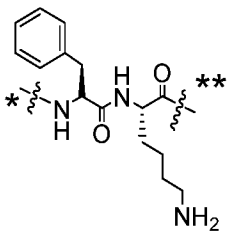
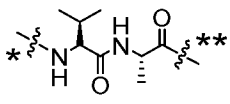
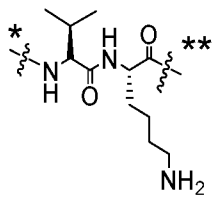
R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab;

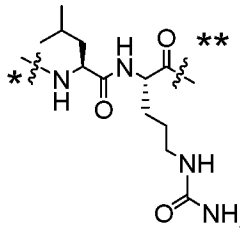
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^{100};

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit),  (PheLys),  (ValAla), 

 (ValLys) and (LeuCit), where the * of Lp indicates the

attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

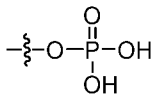
where

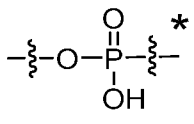
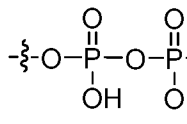
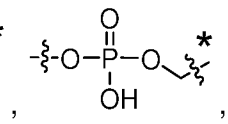
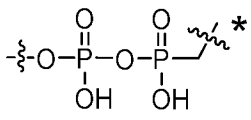
W is $-CH_2O-$**, $-CH_2N(Rb)C(=O)O-$**, $-NHC(=O)CH_2NHC(=O)O-$**, $-NHC(=O)CH_2NH-$**, $-NHC(=O)CH_2NHC(=O)-$**, $-CH_2N(X-R^2)C(=O)O-$**, $-C(=O)N(X-R^2)-$**, $-CH_2N(X-R^2)C(=O)-$**, $-C(=O)NR^b-$**, $-C(=O)NH-$**, $-CH_2NR^bC(=O)-$**, $-CH_2NR^bC(=O)NH-$**, $-CH_2NR^bC(=O)NR^b-$**, $-NHC(=O)-$**, $-NHC(=O)O-$**, $-NHC(=O)NH-$**, $-OC(=O)NH-$**, $-S(O)_2NH-$**, $-NHS(O)_2-$**, $-C(=O)-$, $-C(=O)O-$** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

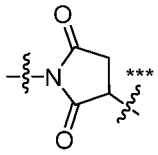
D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety,

and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 67. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 66, wherein:

Ab is an antibody or fragment thereof;

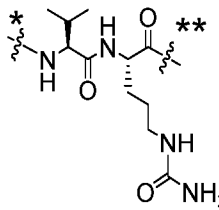
R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab;

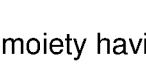
L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^{100};

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

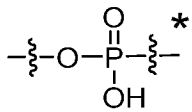
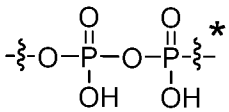
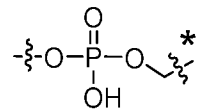
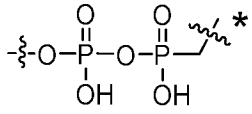
where

W is -CH_2O-**, -CH_2N(Rb)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3 A is a bond, -OC(=O)-*,  ,  ,  ,  ,

-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

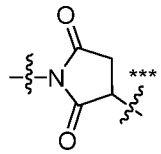
-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*, wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D;

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety, and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 68. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 67, wherein:

Ab is an antibody or fragment thereof;

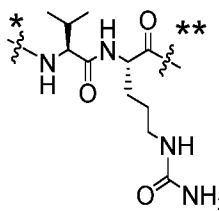
R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab;

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp and the ** of L_1 indicates the point of attachment to R^{100};

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the –NH- group of G;

L_3 is a spacer moiety having the structure  ,

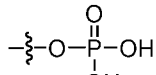
where

W is -CH_2O-**, -$CH_2N(Rb)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, or -NHC(=O)NH-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

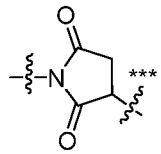
A is a bond or -OC(=O)* in which * indicates the attachment point to D;

D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety, and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 69. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 68, wherein:

Ab is an antibody or fragment thereof;

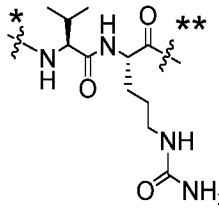
R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab;

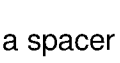
L_1 is *-C(=O)$(CH_2)_mO(CH_2)_m$-**; *-C(=O)$((CH_2)_mO)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; or *-C(=O)NH$((CH_2)_mO)_t(CH_2)_n$-, where the * of L_1 indicates the point of attachment to Lp and the ** of L_1 indicates the point of attachment to R^{100};

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

each t is independently selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

Lp is a bivalent peptide spacer selected from  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of G;

L_3 is a spacer moiety having the structure  ,

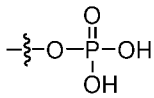
where

W is $-CH_2O-$**, $-CH_2N(Rb)C(=O)O-$**, $-NHC(=O)CH_2NHC(=O)O-$**, $-CH_2N(X-R^2)C(=O)O-$**, or $-C(=O)N(X-R^2)-$**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2;

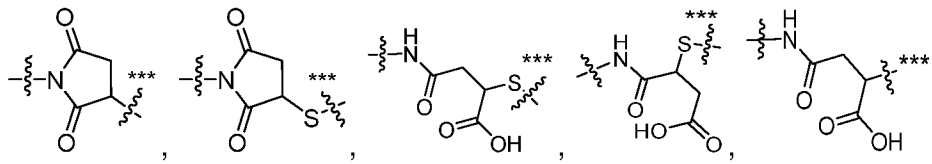
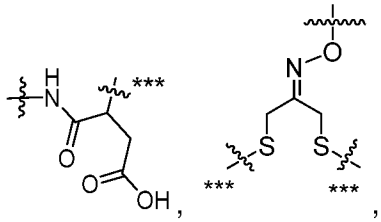
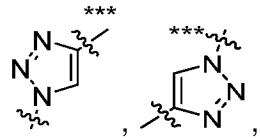
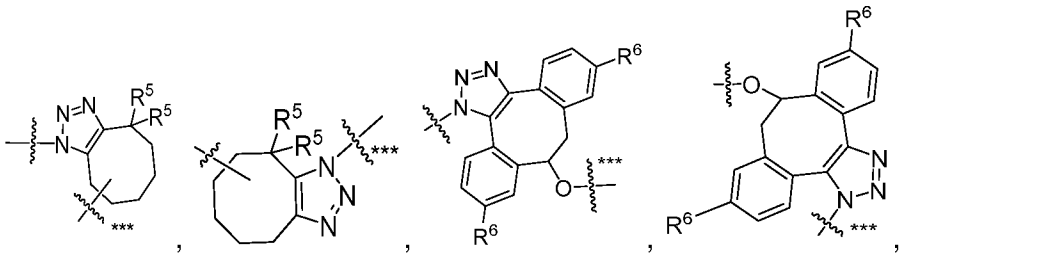
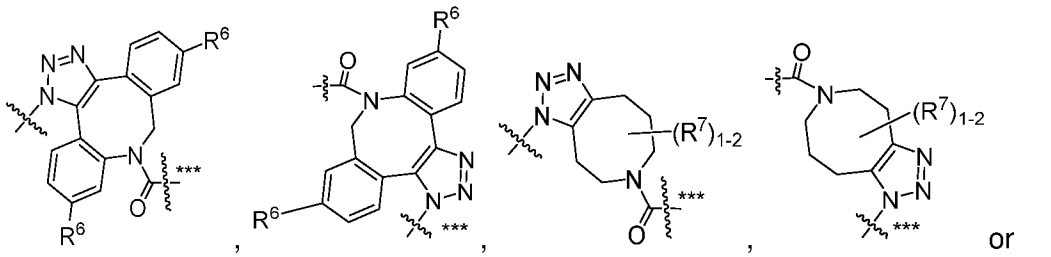
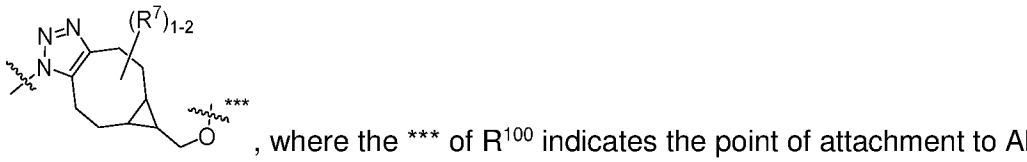
R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups;

A is a bond or -OC(=O)* in which * indicates the attachment point to D;

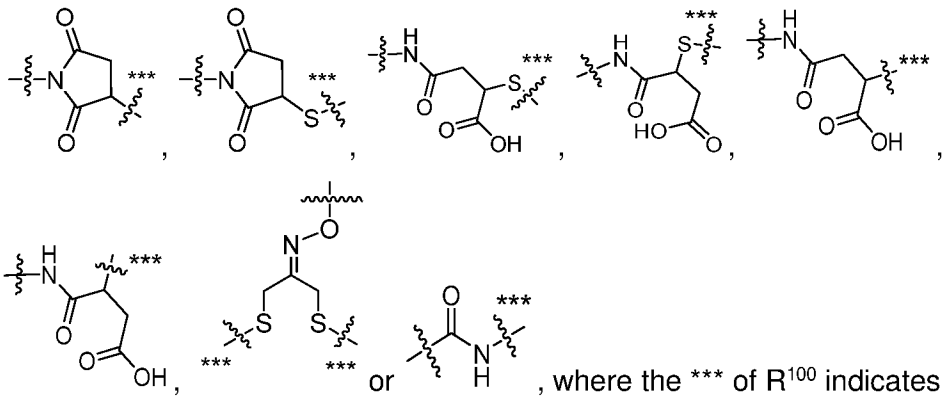
D is a Drug moiety as defined herein and comprising an N or an O, wherein D is connected to A via a direct bond from A to the N or the O of the Drug moiety, and

y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

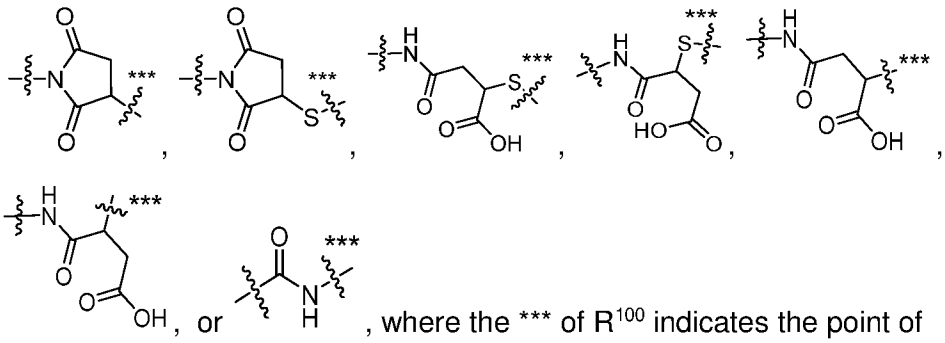
Embodiment 70. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 65, wherein

R^{100} is  ,  , -S-, -C(=O)-, -ON=***, -NHC(=O)CH_2-***, -S(=O)$_2CH_2CH_2$-***, -$(CH_2)_2$S(=O)$_2CH_2CH_2$-***, -NHS(=O)$_2CH_2CH_2$-***, -NHC(=O)CH_2CH_2-***, -$CH_2NHCH_2CH_2$-***, -$NHCH_2CH_2$-***,  ,  ,  ,  , or , where the *** of R^{100} indicates the point of attachment to Ab.

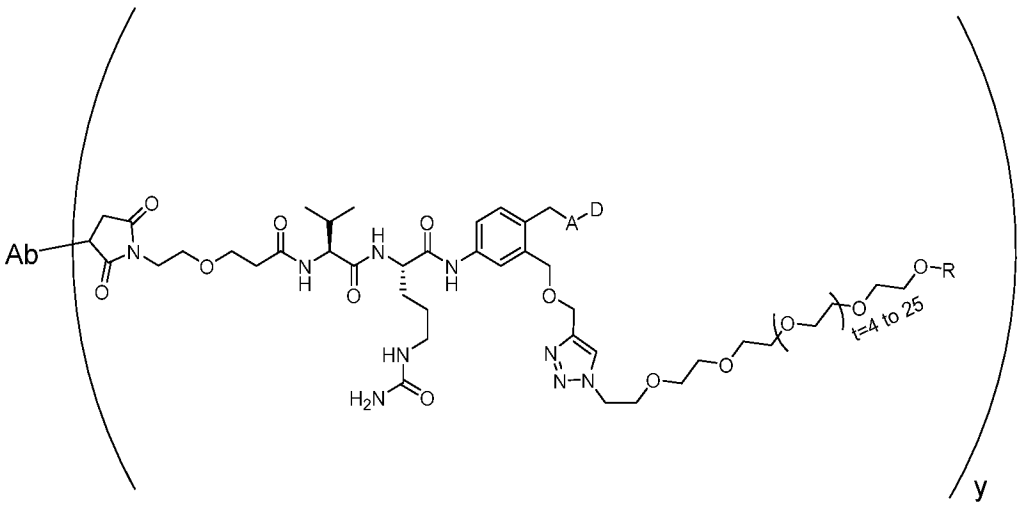
Embodiment 71. The immunoconjugate of Formula (E') or any one of Embodiments 60 to 63, wherein

R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab.

Embodiment 72. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 65, wherein

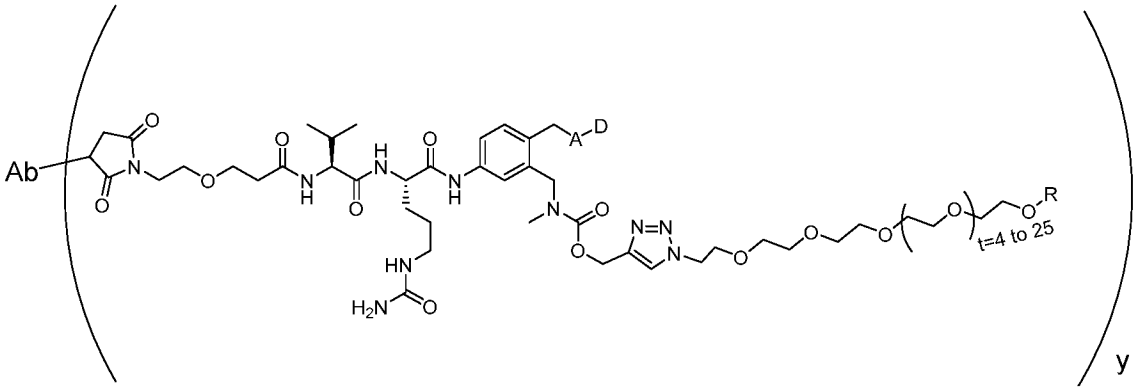
R^{100} is  , where the *** of R^{100} indicates the point of attachment to Ab.

Embodiment 73. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

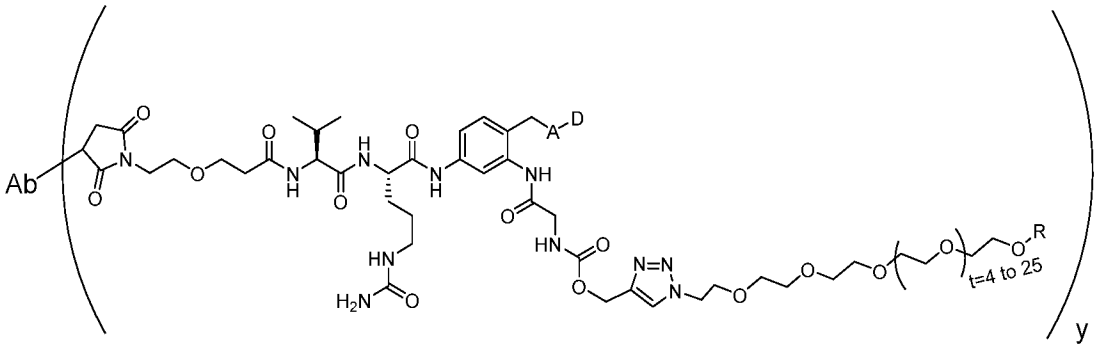
Embodiment 74. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

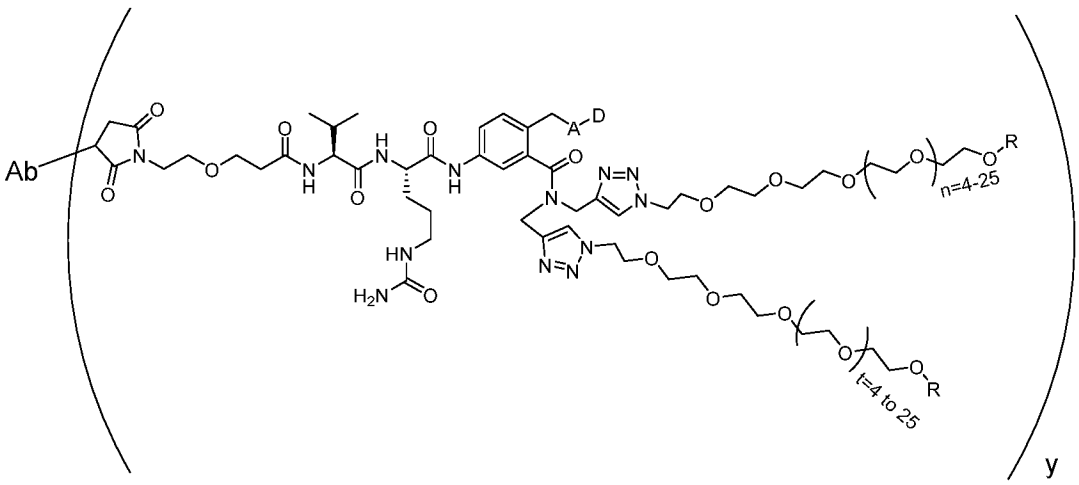
Embodiment 75. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where

R is H, -CH_3 or -$CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

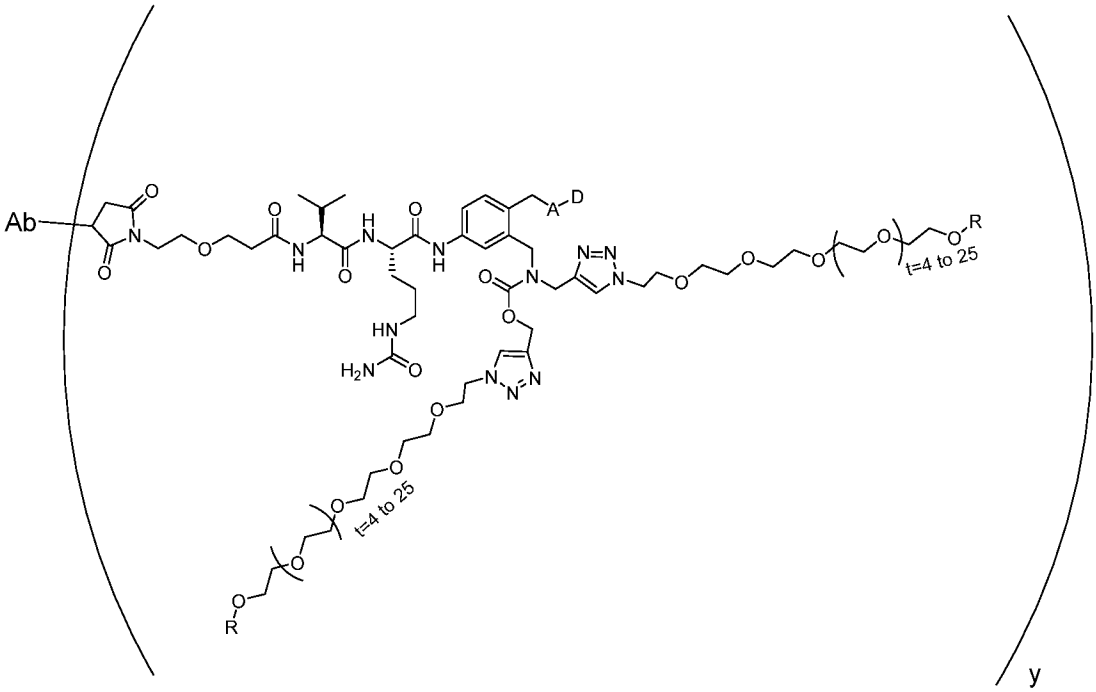
Embodiment 76. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where

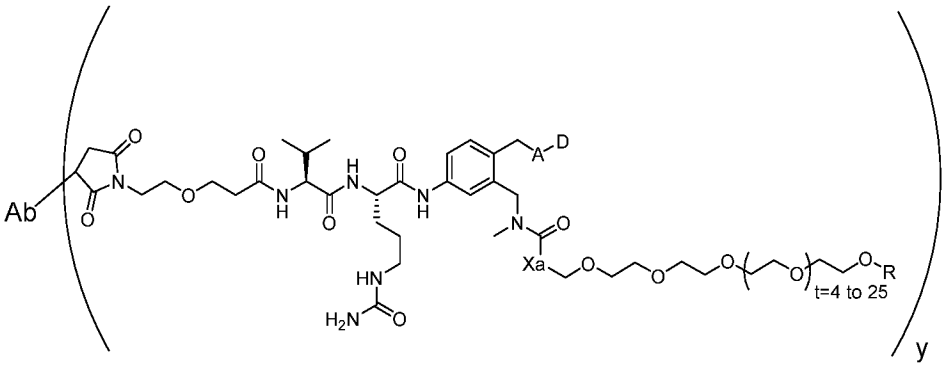
each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 77. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



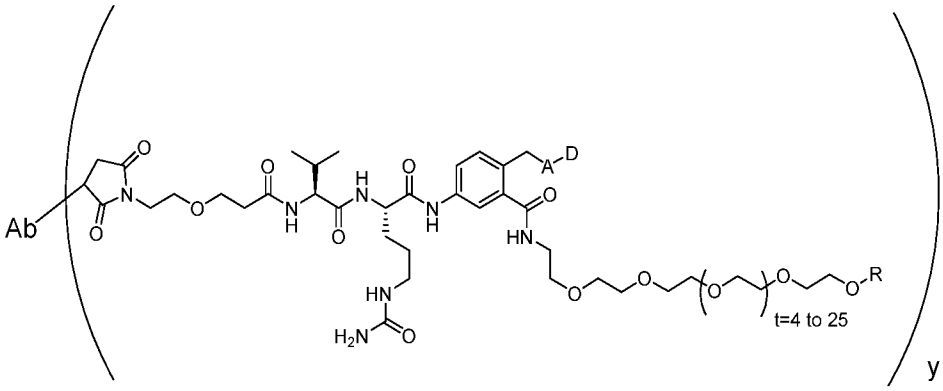
, where each R is independently selected from H, -CH_3 or -$CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 78. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where Xa is –CH_2-, -OCH_2-, -$NHCH_2$- or –$NRCH_2$- and each R is independently H, -CH_3 or -$CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

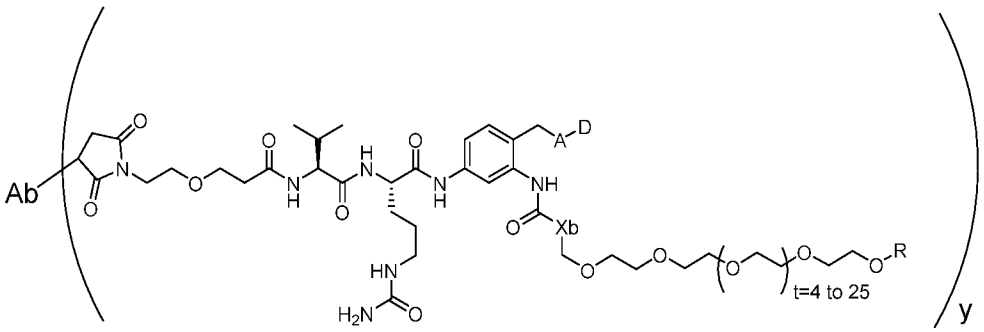
Embodiment 79. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

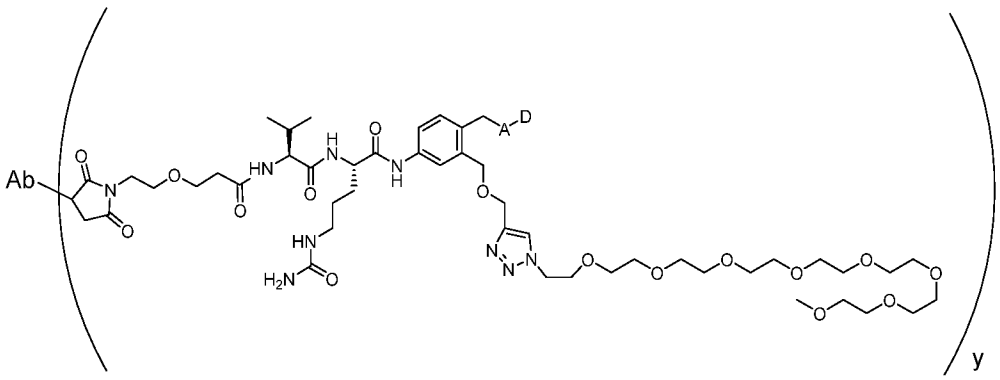
Embodiment 80. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where

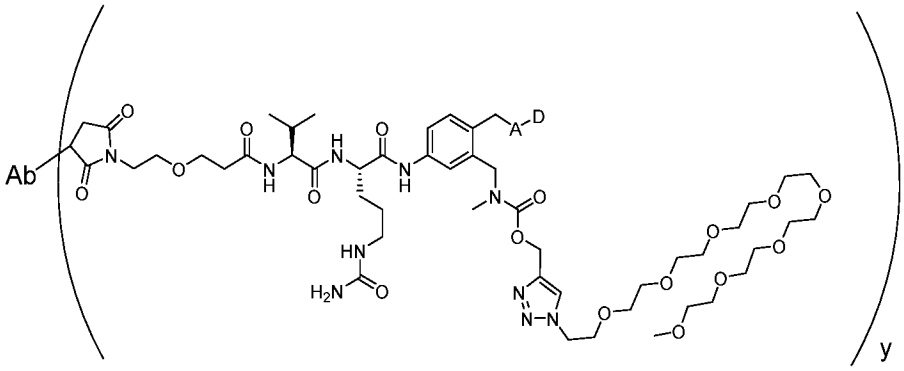
Xb is $-CH_2-$, $-OCH_2-$, $-NHCH_2-$ or $-NRCH_2-$ and each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$ and y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 81. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



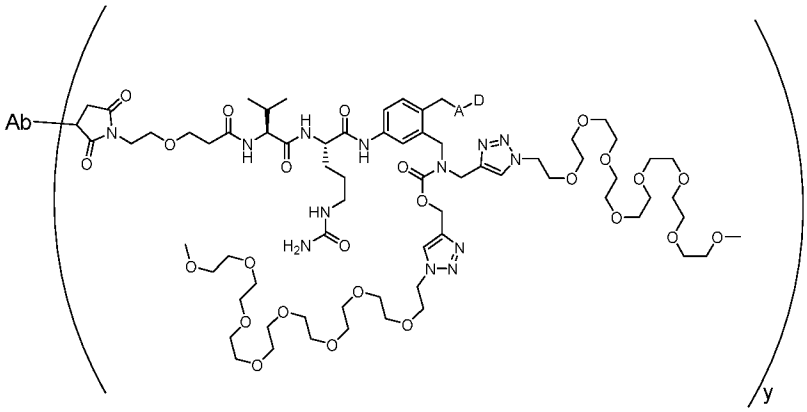
, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 82. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



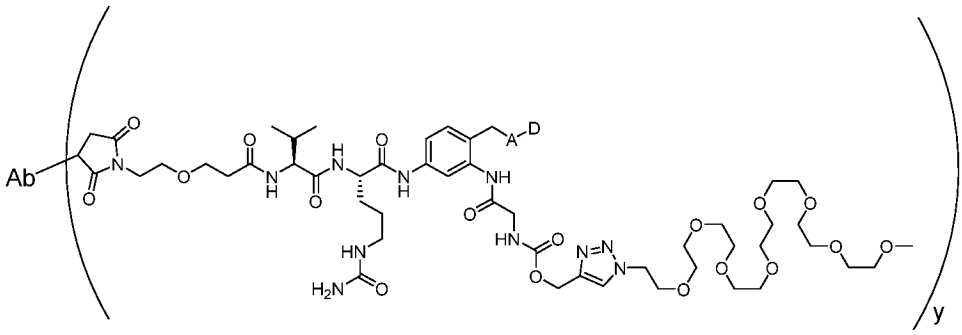
, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 83. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



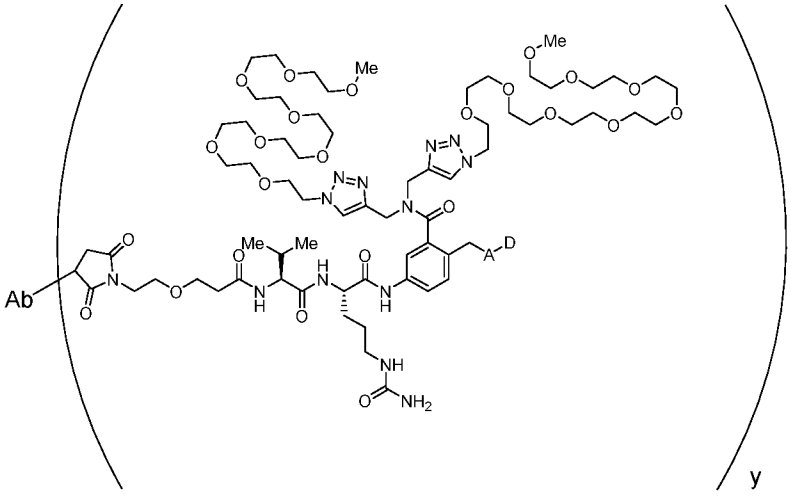
, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 84. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



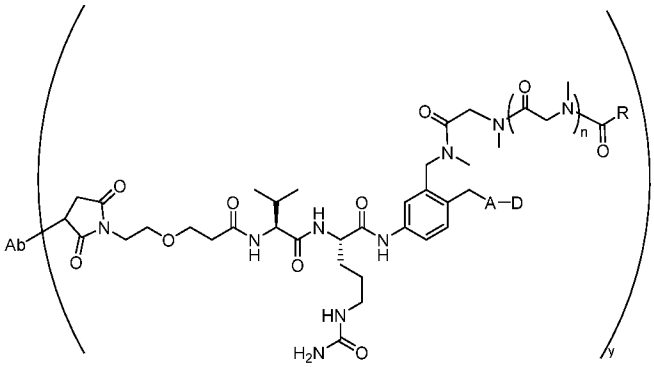
, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Embodiment 85. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16

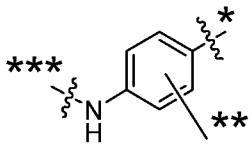
Embodiment 86. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72 having the structure:



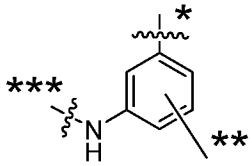
, where y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

Certain aspects and examples of the Linker-Drug groups, the Linkers and the Antibody Drug Conjugates of the invention are provided in the following listing of additional enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

Embodiment 87. The compound of Formula (A') or any one of Embodiments 1 to 2, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 40, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 63, wherein:

G is  , where the * of G indicates the point of attachment to L_2, and the ** of G indicates the point of attachment to L_3 and the *** of G indicates the point of attachment to Lp.

Embodiment 88. The compound of Formula (A') or any one of Embodiments 1 to 2, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 40, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 63, wherein:

G is  , where the * of G indicates the point of attachment to L_2, and the ** of G indicates the point of attachment to L_3 and the *** of G indicates the point of attachment to Lp.

Embodiment 89. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein:

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**;

*-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-**;

*-C(=O)O$(CH_2)_m$SSC$(R^3)_2$$(CH_2)_m$C(=O)N$R^3$$(CH_2)_mNR^3$C(=O)$(CH_2)_m$-**;

*-C(=O)O$(CH_2)_m$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**;

*-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**; *-C(=O)$(CH_2)_m$$X_1$$(CH_2)_m$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$NHC(=O)$(CH_2)_n$$X_1$$(CH_2)_n$-**;

*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_mC(R^3)_2$-** or

*-C(=O)$(CH_2)_m$C(=O)NH$(CH_2)_m$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 90. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein:

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$NHC(=O)$(CH_2)_n$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$C$(R^3)_2$-** or *-C(=O)$(CH_2)_m$C(=O)NH$(CH_2)_m$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 91. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein:

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**; *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**; or *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 92. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein:

L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-** or *-C(=O)NH$((CH_2)_m$O$)_t(CH_2)_n$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 93. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein L_1 is *-C(=O)$(CH_2)_m$O$(CH_2)_m$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 94. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein L_1 is *-C(=O)$((CH_2)_m$O$)_t(CH_2)_n$-**, where the * of L_1

indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 95. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, wherein L_1 is *-C(=O)$(CH_2)_m$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 96. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 32 to 46, and the immunoconjugate of Formula (E') or any one of Embodiments 60 to 70, wherein L_1 is *-C(=O)NH$((CH_2)_mO)_t(CH_2)_n$-**, where the * of L_1 indicates the point of attachment to Lp, and the ** of L_1 indicates the point of attachment to R^1 if present or the ** of L_1 indicates the point of attachment to R^{100} if present.

Embodiment 97. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 84 to 93, wherein Lp is an enzymatically cleavable bivalent peptide spacer.

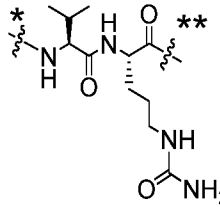
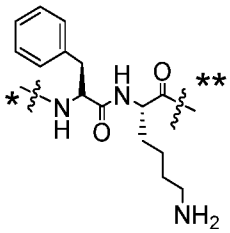
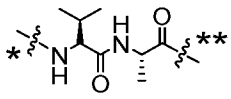
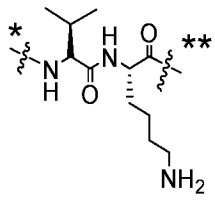
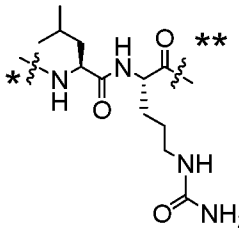
Embodiment 98. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 32 to 46, and the immunoconjugate of Formula (E') or any one of Embodiments 60 to 70, or any one of Embodiments 87 to 97, wherein Lp is a bivalent peptide spacer comprising an amino acid residue selected from glycine, valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine.

Embodiment 99. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 98, wherein Lp is a bivalent peptide spacer comprising two to four amino acid residues.

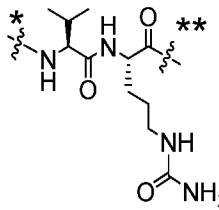
Embodiment 100. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 99, wherein Lp is a bivalent peptide spacer comprising two to four amino acid residues each independently selected

from glycine, valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine.

Embodiment 101. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 100, wherein:

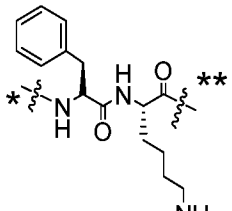
Lp is a bivalent peptide spacer selected from  (ValCit),  (PheLys),  (ValAla),  (ValLys) and  (LeuCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

Embodiment 102. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 101, wherein:

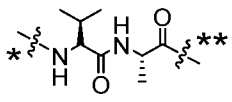
Lp is  (ValCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

Embodiment 103. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of

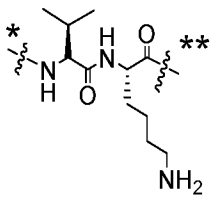
Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 101, wherein:

Lp is  (PheLys), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

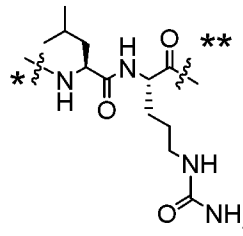
Embodiment 104. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 101, wherein:

Lp is  (ValAla), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

Embodiment 105. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 101, wherein:

Lp is  (ValLys), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to the -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

Embodiment 106. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 101, wherein:

Lp is  (LeuCit), where the * of Lp indicates the attachment point to L_1 and the ** of Lp indicates the attachment point to -NH- group of Formula (B') or the ** of Lp indicates the attachment point to the G of Formula (A').

Embodiment 107. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 106, wherein L_2 is a bond, a methylene, or a C_2-C_3alkenylene.

Embodiment 108. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 107, wherein L_2 is a bond or a methylene.

Embodiment 109. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 108, wherein L_2 is a bond.

Embodiment 110. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 108, wherein L_2 is a methylene.

Embodiment 111. The compound of Formula (A') or any one of Embodiments 1 to 30, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 85, or any one of Embodiments 87 to 110, wherein:

A is a bond, -OC(=O)-, -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)- or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-, wherein each R^a is independently selected from H, C_1-C_6alkyl or a C_3-C_8cycloalkyl.

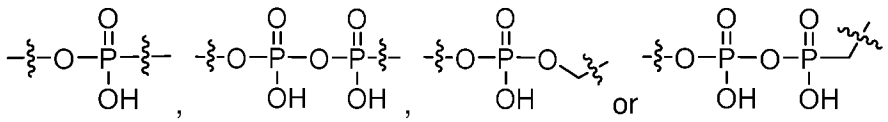
Embodiment 112. The compound of Formula (A') or any one of Embodiments 1 to 32, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 61, and the immunoconjugate of Formula (E') or any one of

Embodiments 62 to 86, or any one of Embodiments 87 to 111, wherein A is a bond or -OC(=O).

Embodiment 113. The compound of Formula (A') or any one of Embodiments 1 to 32, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 61, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 86, or any one of Embodiments 87 to 112, wherein A is a bond.

Embodiment 114. The compound of Formula (A') or any one of Embodiments 1 to 32, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 61, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 86, or any one of Embodiments 87 to 112, wherein A is -OC(=O).

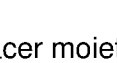
Embodiment 115. The compound of Formula (A') or any one of Embodiments 1 to 32, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 61, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 86, or any one of Embodiments 87 to 110, wherein:

A is  or .

Embodiment 116. The compound of Formula (A') or any one of Embodiments 1 to 32, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 61, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 85, or any one of Embodiments 86 to 110, wherein:

A is -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)- or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-, wherein each R^a is independently selected from H, C_1-C_6alkyl or a C_3-C_8cycloalkyl.

Embodiment 117. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 49, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 116, wherein:

L_3 is a spacer moiety having the structure  ,

where

W is -CH_2O-**, -CH_2N(R^b)C(=O)O-**, -NHC(=O)C(R^b)$_2$NHC(=O)O-**, -NHC(=O)C(R^b)$_2$NH-**, -NHC(=O)C(R^b)$_2$NHC(=O)-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, -CH_2N(X-R^2)C(=O)-**, -C(=O)NR^b-**, -C(=O)NH-**, -CH_2NR^bC(=O)-**, -CH_2NR^bC(=O)NH-**, -CH_2NR^bC(=O)NR^b-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -S(O)$_2$NH-**, -NHS(O)$_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from

H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 118. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 117, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -NHC(=O)CH_2NHC(=O)O-**, -NHC(=O)CH_2NH-**, -NHC(=O)CH_2NHC(=O)-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 119. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 118, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -NHC(=O)CH_2NHC(=O)O-**, -NHC(=O)CH_2NH-**, -NHC(=O)CH_2NHC(=O)-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -OC(=O)NH-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from

H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a triazolyl, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 120. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 118, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$NHC(=O)CH_2NH$-**, -$NHC(=O)CH_2NHC(=O)$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, -$CH_2N(X\text{-}R^2)C(=O)$-**, -$C(=O)NR^b$-**, -C(=O)NH-**, -$CH_2NR^bC(=O)$-**, -$CH_2NR^bC(=O)NH$-**, -$CH_2NR^bC(=O)NR^b$-**, -NHC(=O)-**, -NHC(=O)O-**, -NHC(=O)NH-**, -$OC(=O)NH$-**, -$S(O)_2NH$-**, -$NHS(O)_2$-**, -C(=O)-, -C(=O)O-** or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 121. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 118, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond, triazolyl or ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 122. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 118, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -CH_2N(R^b)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a bond ;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 123. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 83 to 118, wherein:

L_3 is a spacer moiety having the structure $-\S-W-X-\S^{*}$,

where

W is -CH_2O-**, -CH_2N(R^b)C(=O)O-**, -NHC(=O)CH_2NHC(=O)O-**, -CH_2N(X-R^2)C(=O)O-**, -C(=O)N(X-R^2)-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is a triazolyl, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 124. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 118, wherein:

L_3 is a spacer moiety having the structure  ,

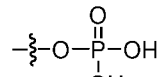
where

W is -CH_2O-**, -$CH_2N(R^b)C(=O)O$-**, -$NHC(=O)CH_2NHC(=O)O$-**, -$CH_2N(X\text{-}R^2)C(=O)O$-**, -$C(=O)N(X\text{-}R^2)$-**, wherein each R^b is independently selected from H, C_1-C_6alkyl or C_3-C_8cycloalkyl and wherein the ** of W indicates the point of attachment to X;

X is ***-CH_2-triazolyl-*, wherein the *** of X indicates the point of attachment to W and the * of X indicates the point of attachment to R^2;

and

the * of L_3 indicates the point of attachment to R^2.

Embodiment 125. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 86 to 124, wherein R^2 is a hydrophilic moiety selected from polyethylene glycol, polyalkylene glycol, a sugar, an oligosaccharide, a polypeptide or C_2-C_6alkyl substituted with 1 to 3  groups..

Embodiment 126. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a sugar.

Embodiment 127. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is an oligosaccharide.

Embodiment 128. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polypeptide.

Embodiment 129. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyalkylene glycol.

Embodiment 130. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyalkylene glycol having the structure -$(O(CH_2)_m)_tR'$, where R' is OH, OCH_3 or $OCH_2CH_2C(=O)OH$, m is 1-10 and t is 4-40.

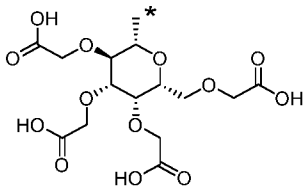
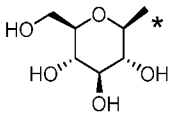
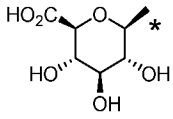
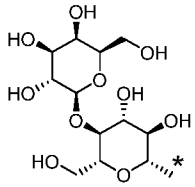
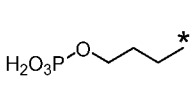
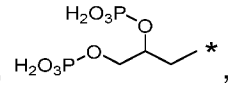
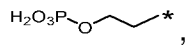
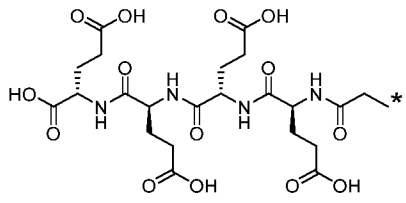
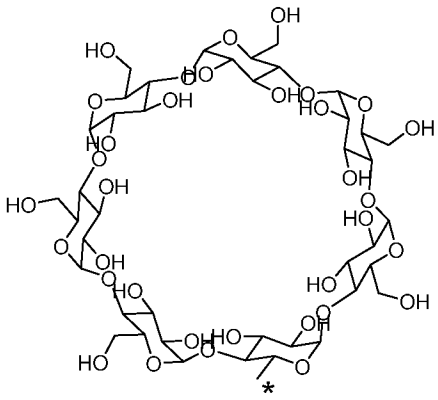
Embodiment 131. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyalkylene glycol having the structure -$((CH_2)_mO)_tR''$-, where R'' is H, CH_3 or $CH_2CH_2C(=O)OH$, m is 1-10 and t is 4-40.

Embodiment 132. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyethylene glycol.

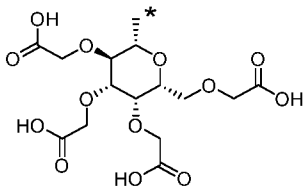
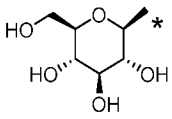
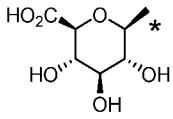
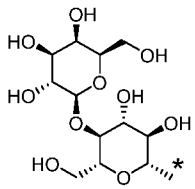
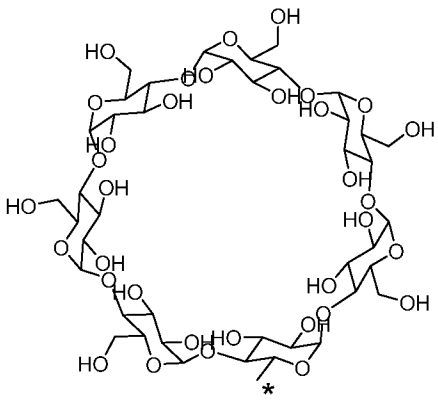
Embodiment 133. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyethylene glycol having the structure -$(OCH_2CH_2)_tR'$, where R' is OH, OCH_3 or $OCH_2CH_2C(=O)OH$ and t is 4-40,

Embodiment 134. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein R^2 is a polyethylene glycol having the structure -$(CH_2CH_2O)_tR''$-, where R'' is H, CH_3 or $CH_2CH_2C(=O)OH$ and t is 4-40.

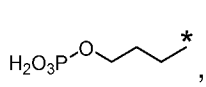
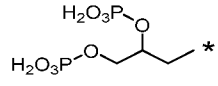
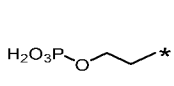
Embodiment 135. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein:

R^2 is  ,  ,  ,  ,  ,  ,  ,  , or  , where the * of R^2 indicates the point of attachment to X or L_3.

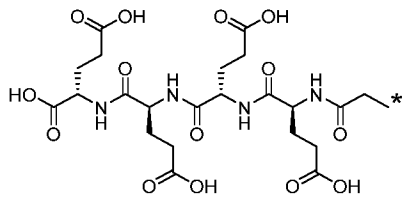
Embodiment 136. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein:

R^2 is  ,  ,  ,  or  , where the * of R^2 indicates the point of attachment to X or L_3.

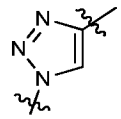
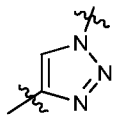
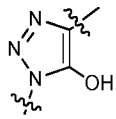
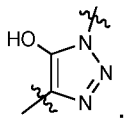
Embodiment 137. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein:

R^2 is  ,  or  , where the * of R^2 indicates the point of attachment to X or L_3.

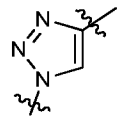
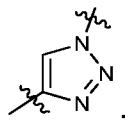
Embodiment 138. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 125, wherein:

R^2 is  , where the * of R^2 indicates the point of attachment to X or L_3.

Embodiment 139. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 138, wherein:

X_1 is  ,  ,  or  .

Embodiment 140. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 138, wherein:

X_1 is  or  .

Embodiment 141. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 140, wherein:

each m is independently selected from 1, 2, 3, 4, and 5.

Embodiment 142. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of

Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 140, wherein:

each m is independently selected from 1, 2 and 3.

Embodiment 143. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 142, wherein:

each n is independently selected from 1, 2, 3, 4 and 5.

Embodiment 144. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 142, wherein:

each n is independently selected from 1, 2 and 3.

Embodiment 145. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 144, wherein:

each t is independently selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30.

Embodiment 146. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 144, wherein:

each t is independently selected from 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25.

Embodiment 147. The compound of Formula (A') or any one of Embodiments 1 to 17, or pharmaceutically acceptable salt thereof, the linker of Formula (C') or any one of Embodiments 33 to 47, and the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 144, wherein:

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 148. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

Embodiment 149. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12.

Embodiment 150. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

Embodiment 151. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3, 4, 5, 6, 7 or 8.

Embodiment 152. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3, 4, 5 or 6.

Embodiment 153. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1, 2, 3 or 4.

Embodiment 154. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 1 or 2.

Embodiment 155. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 2.

Embodiment 156. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 4.

Embodiment 157. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 6.

Embodiment 158. The immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 147, wherein y is 8.

Embodiment 159. The compound of Formula (A') or any one of Embodiments 1 to 31, or pharmaceutically acceptable salt thereof, the immunoconjugate of Formula (E') or any one of Embodiments 62 to 72, or any one of Embodiments 87 to 158, wherein D is a Bcl-xL inhibitor when released from the immunoconjugates.

Other Linker Groups

Other examples of linker groups that are suitable for making ADCs or immunoconjugates of a Bcl-xL inhibitor disclosed herein includes those disclosed in international application publications such as WO2018200812, WO2017214456, WO2017214458, WO2017214462, WO2017214233, WO2017214282, WO2017214301, WO2017214322, WO2017214335, WO2017214339, WO2016094509, WO2016094517, and WO2016094505, the contents of each of which are incorporated by reference in their entireties.

For example, the immunoconjugates of Bcl-xL inhibitors disclosed herein can have a linker-payload ("-L-D") structure selected from:

$-\!\!\!\!\!\!\sim\!\!\!-(L_c)_x-C_E-D$, $-\!\!\!\!\!\!\sim\!\!\!-(L_c)_x-C_E-(L_c)_y-C_E-D$, and $-\!\!\!\!\!\!\sim\!\!\!-((L_c)_x-C_E)_p-(L_c)_y-C_E-D$,

wherein:

Lc is a linker component and each Lc is independently selected from a linker component as disclosed herein;

x is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20;

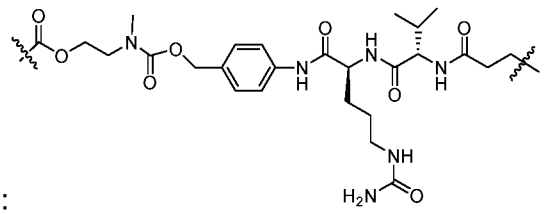
y is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20;

p is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

D is a Bcl-xL inhibitor disclosed herein;

and each cleavage element (C_E) is independently selected from a self-immolative spacer and a group that is susceptible to cleavage selected from acid-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, glycosidase induced cleavage, phosphodiesterase induced cleavage, phosphatase induced cleavage, protease induced cleavage, lipase induced cleavage or disulfide bond cleavage.

In some embodiments, L has a structure selected from the following, or L comprises a structural component selected from the following: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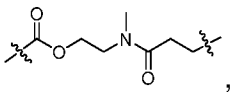
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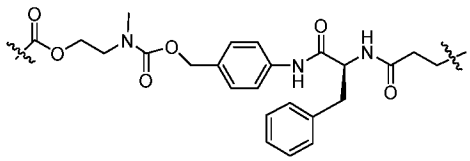
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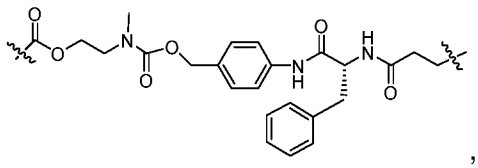
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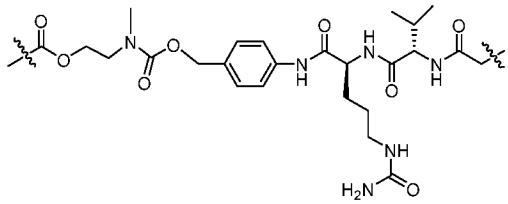
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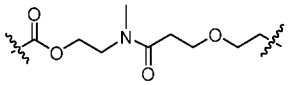
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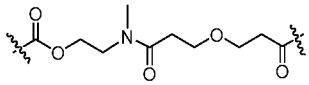
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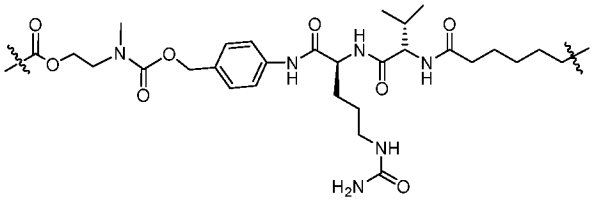
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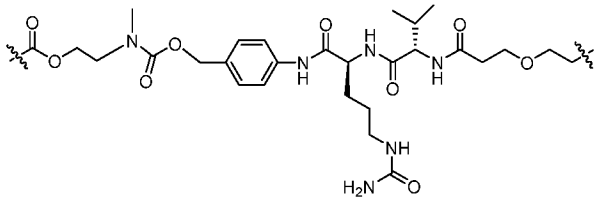
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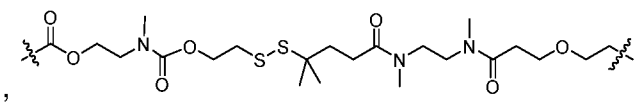
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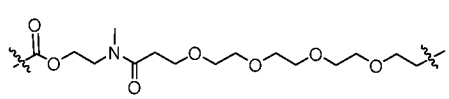
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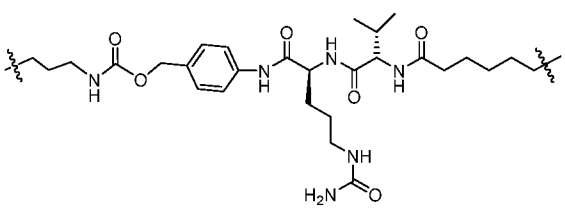
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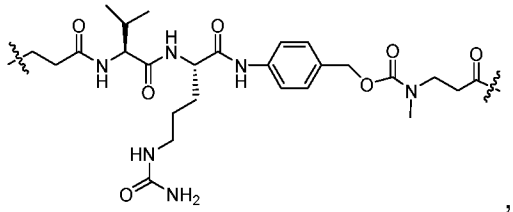
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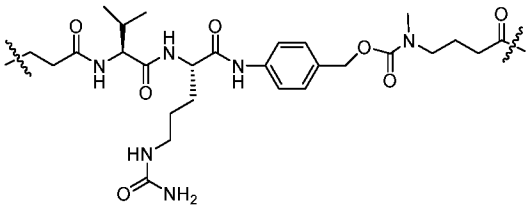
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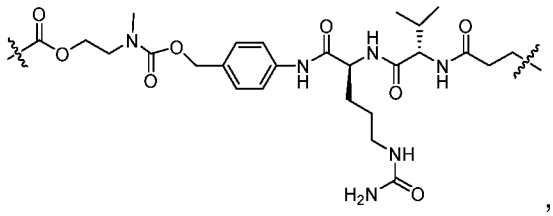
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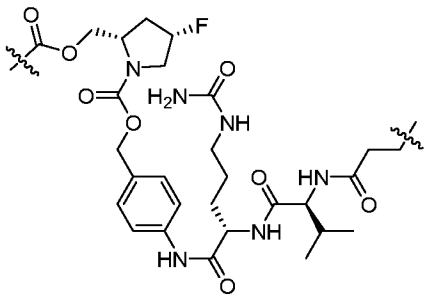
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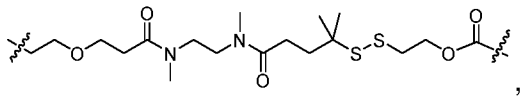
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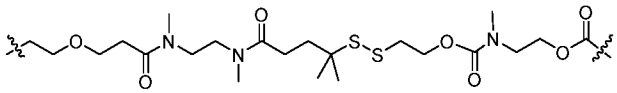
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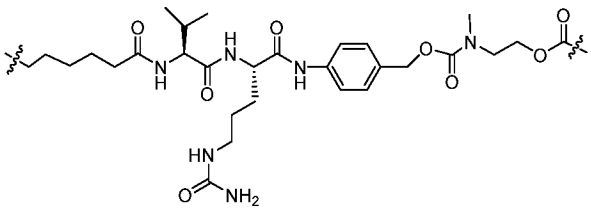
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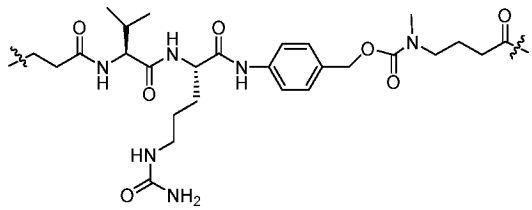
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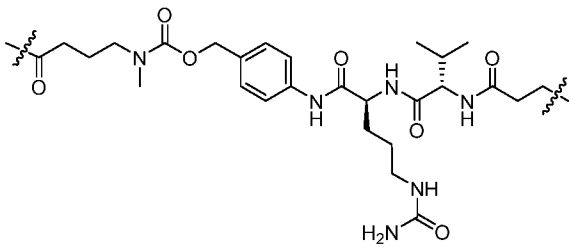
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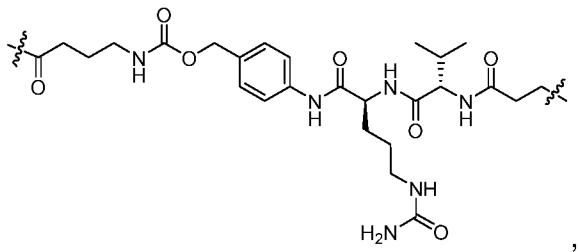
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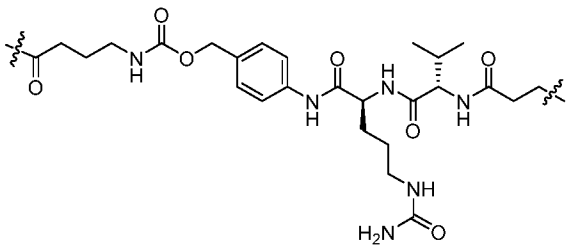
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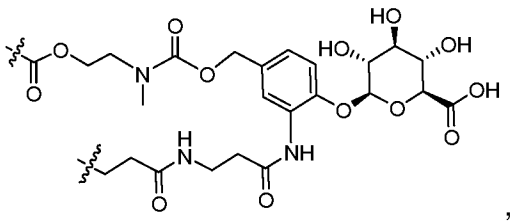
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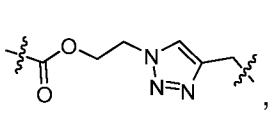
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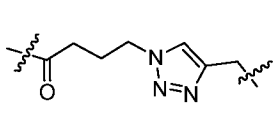
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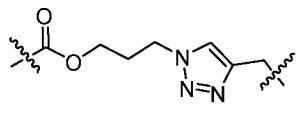
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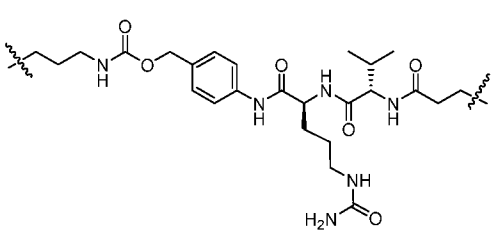
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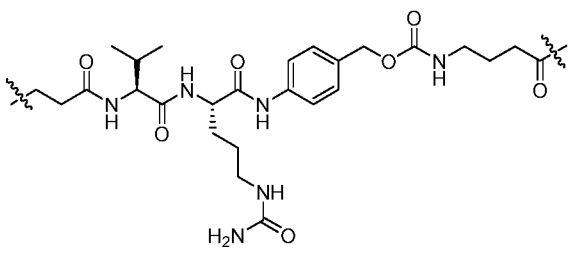
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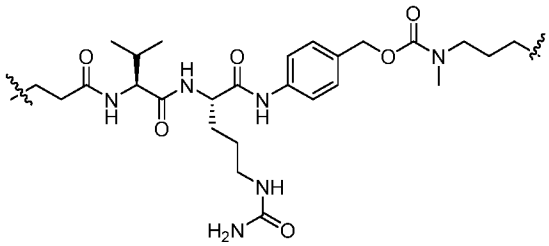
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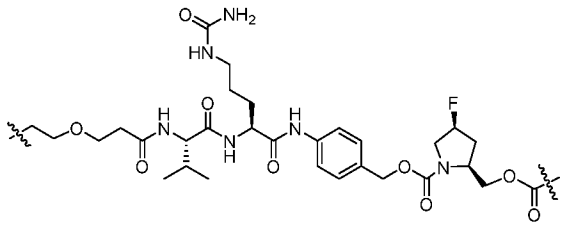
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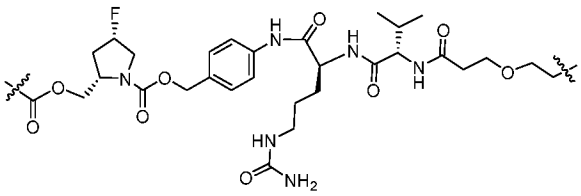
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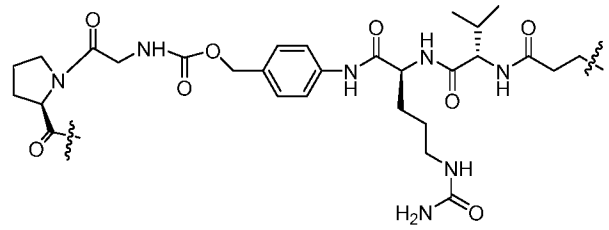
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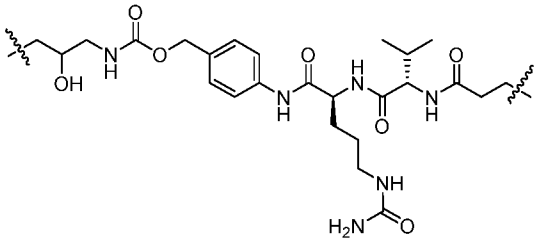
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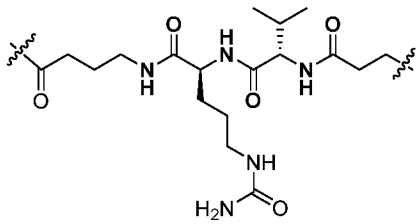
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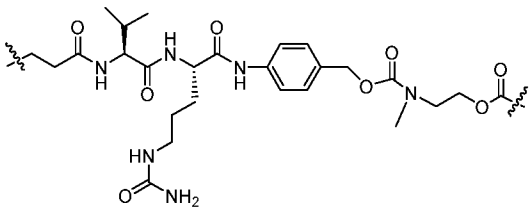
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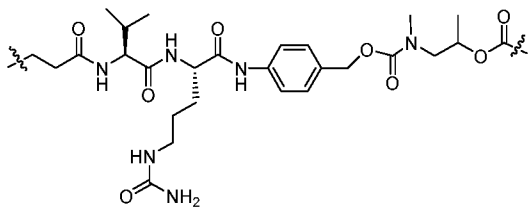
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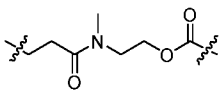
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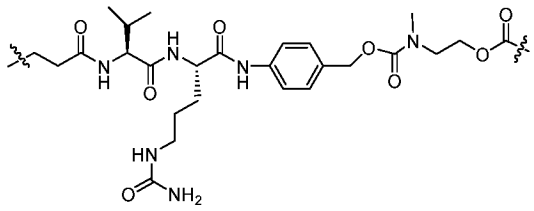
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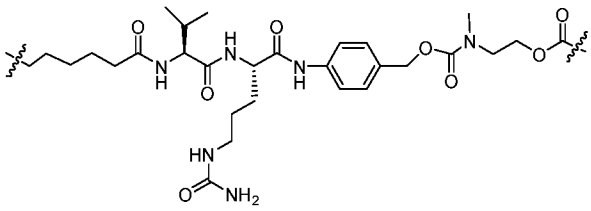
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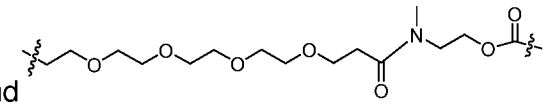
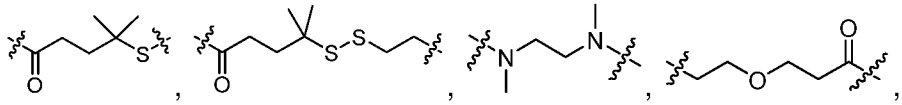

.

In some embodiments, Lc is a linker component and each Lc is independently selected from

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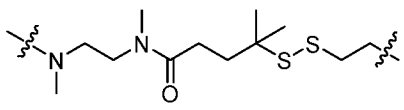
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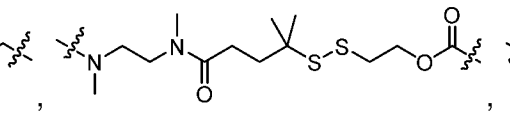
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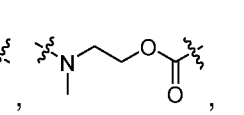
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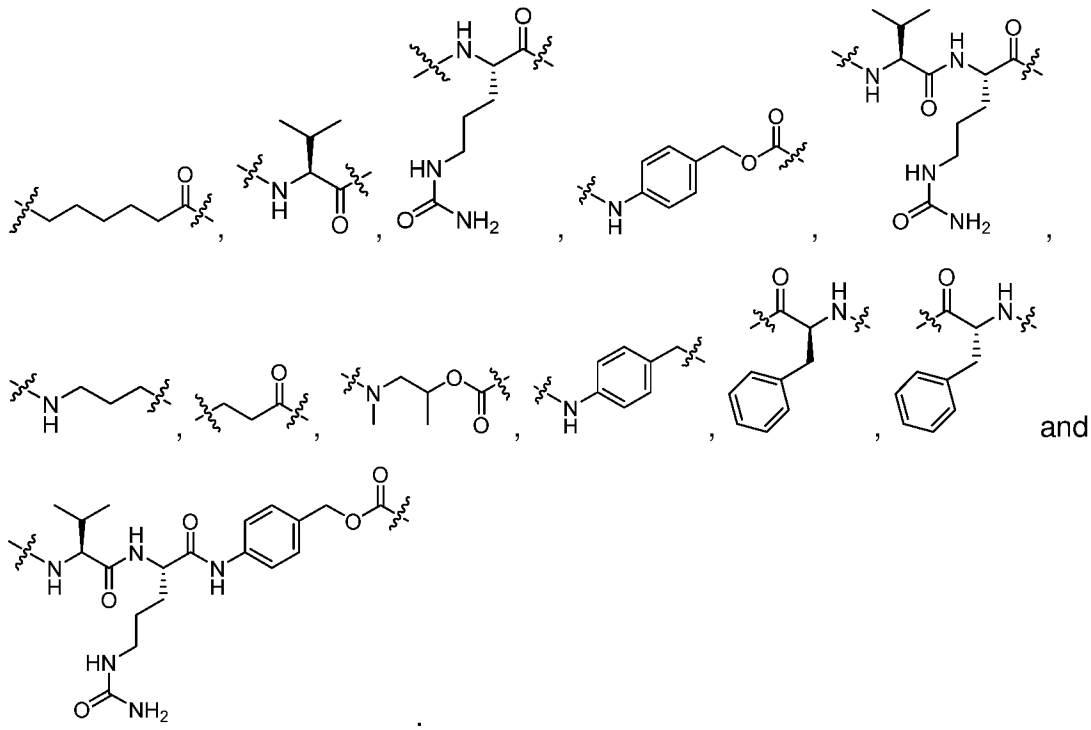


In some embodiments, the linker L comprises a linker component that is selected from:

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$-; -**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

-**C(=O)OC(R^{12})$_2$(CH$_2$)$_m$NR^{11}C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$C(=O)-;

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)X$_4$C(=O)NR^{11}(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$NR^{11}C(=O)X$_5$C(=O)(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$-;

-**C(=O)X$_4$C(=O)NR^{11}(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)(CH$_2$)$_m$NR^{11}C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

-**C(=O)(CH$_2$)$_m$NR^{11}C(=O)((CH$_2$)$_m$O)$_n$(CH$_2$)$_m$-

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)(CH$_2$)$_m$-; -**C(=O)O(CH$_2$)$_m$X$_6$C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$C(=O)-;

-**C(=O)O(CH$_2$)$_m$X$_6$C(=O)X$_4$C(=O)NR^{11}(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

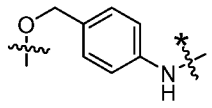
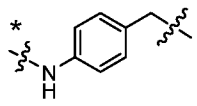
-**C(=O)X$_4$C(=O)X$_6$(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$O(CH$_2$)$_m$-;

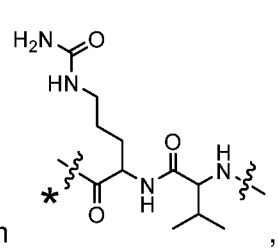
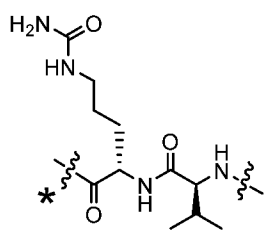
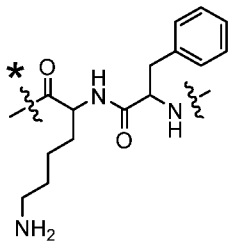
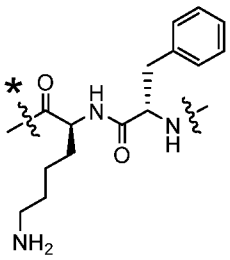
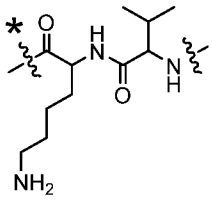
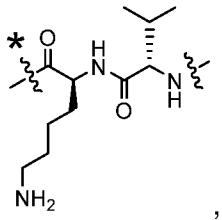
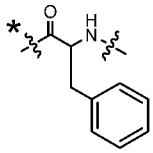
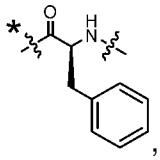
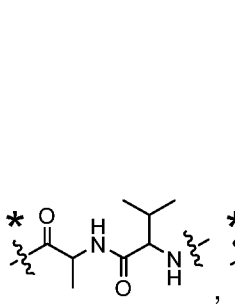
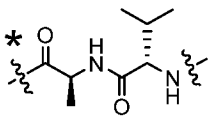
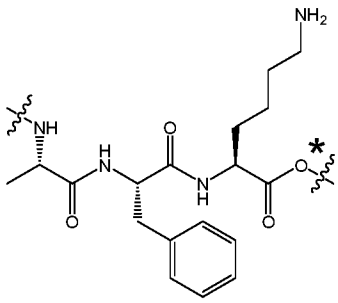
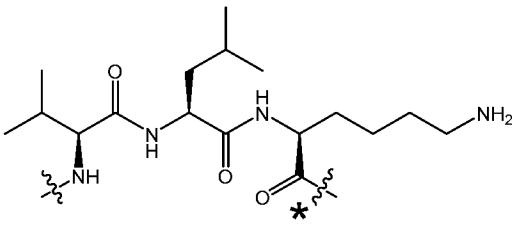
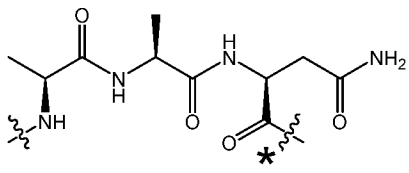
-**C(=O)(CH$_2$)$_m$X$_6$C(=O)X$_{1a}$X$_{2a}$C(=O)(CH$_2$)$_m$-;

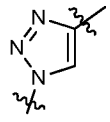
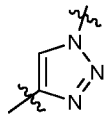
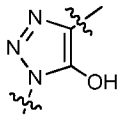
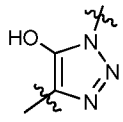
-**C(=O)O((CH$_2$)$_m$O)$_n$(CH$_2$)$_m$NR^{11}C(=O)X$_5$C(=O)(CH$_2$)$_m$-;

-**C(=O)O((CH$_2$)$_m$O)$_n$(CH$_2$)$_m$NR^{11}C(=O)X$_5$C(=O)(CH$_2$)$_m$NR^{11}C(=O)(CH$_2$)$_m$-;

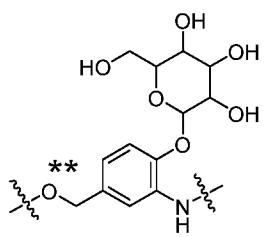
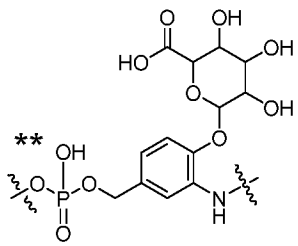
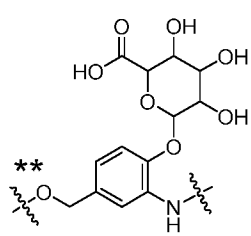
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)((CH2)mO)n(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)((CH2)mO)n(CH2)mNR11C(=O)(CH2)m-;
-
**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)((CH2)mO)n(CH2)mNR11C(=O)(CH2)mX3(CH2)
m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O))X5C(=O)((CH2)mO)n(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)(CH2)mNR11C(=O)((CH2)mO)n(CH2)m-;
-
**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)(CH2)mNR11C(=O)((CH2)mO)n(CH2)mX3(CH2)
m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5((CH2)mO)n(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5((CH2)mO)n(CH2)mNR11C(=O)(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5((CH2)mO)n(CH2)mNR11C(=O)(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5((CH2)mO)n(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5(CH2)mNR11((CH2)mO)n(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)(CH2)mNR11((CH2)mO)n(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5C(=O)((CH2)mO)n(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)X5(CH2)mX3(CH2)m-; -**C(=O)O(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)m-; -**C(=O)O(CH2)mNR11(CH2)m-;
-**C(=O)O(CH2)mNR11(CH2)mC(=O)X2aX1aC(=O)-;
-**C(=O)O(CH2)mX3(CH2)m-; -**C(=O)O((CH2)mO)n(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)(CH2)m-; -
**C(=O)O(CH2)mNR11C(=O(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mNR11C(=O)(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)nX3(CH2)m-; -**C(=O)O((CH2)mO)n(CH2)mX3(CH2)m-;
-**C(=O)O((CH2)mO)n(CH2)mC(=O)NR11(CH2)m-; -**C(=O)O(CH2)mC(R12)2-;
-**C(=O)OCH2)mC(R12)2SS(CH2)mNR11C(=O)(CH2)m-, and
-**C(=O)O(CH2)mC(=O)NR11(CH2)m-, where: ** indicates point of attachment to the drug moiety (D) and the other end can be connected to R^{100}, i.e., the coupling group as described herein;
wherein:

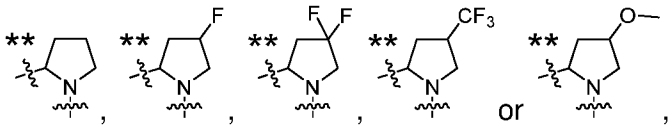
X_{1a} is  or  , where the * indicates the point of attachment to X_{2a};

X_{2a} is selected from  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and ; where the * indicates the point of attachment to X_{1a};

X_3 is  ,  ,  or  ;

X_4 is $-O(CH_2)_nSSC(R^{12})_2(CH_2)_n-$ or $-(CH_2)_nC(R^{12})_2SS(CH_2)_nO-$;

X_5 is  ,  or  , where the ** indicates orientation toward the Drug moiety;

X_6 is  , where the ** indicates orientation toward the Drug moiety;

each R^{11} is independently selected from H and C_1-C_6alkyl;

each R^{12} is independently selected from H and C_1-C_6alkyl;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

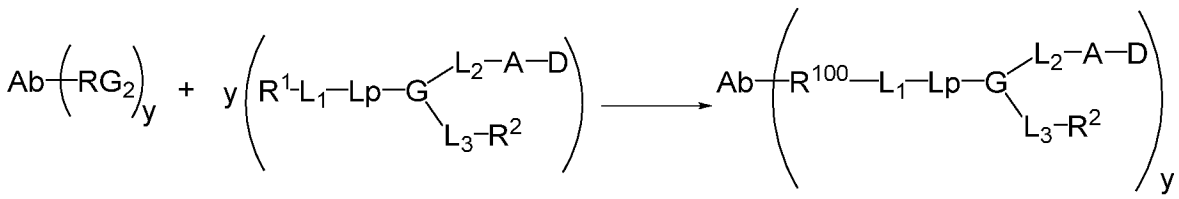
each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,17 and 18.

Methods of Conjugation

The present invention provides various methods of conjugating Linker-Drug groups of the invention to antibodies or antibody fragments to produce Antibody Drug Conjugates which comprise a linker having one or more hydrophilic moieties.

A general reaction scheme for the formation of Antibody Drug Conjugates of Formula (E') is shown in Scheme 2 below:

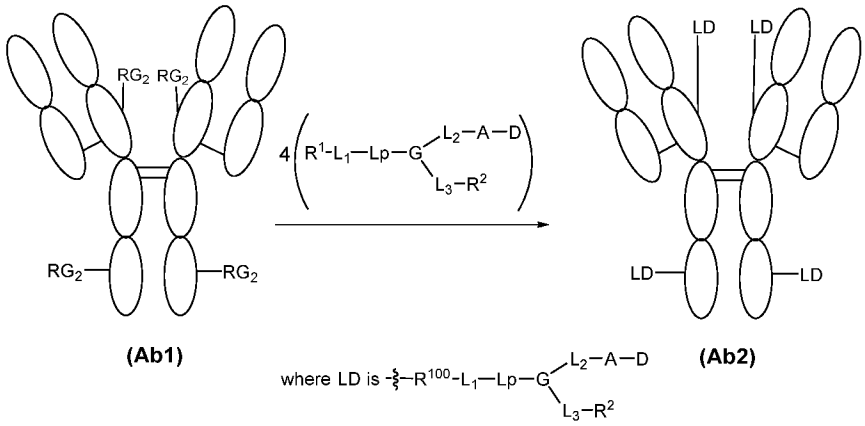
Scheme 2



where: RG_2 is a reactive group which reacts with a compatible R^1 group to form a corresponding R^{100} group (such groups are illustrated in Table 8 and Table 9). D, R^1, L_1, Lp, L_2, L_3, R^2, A, G, Ab, y and R^{100} are as defined herein.

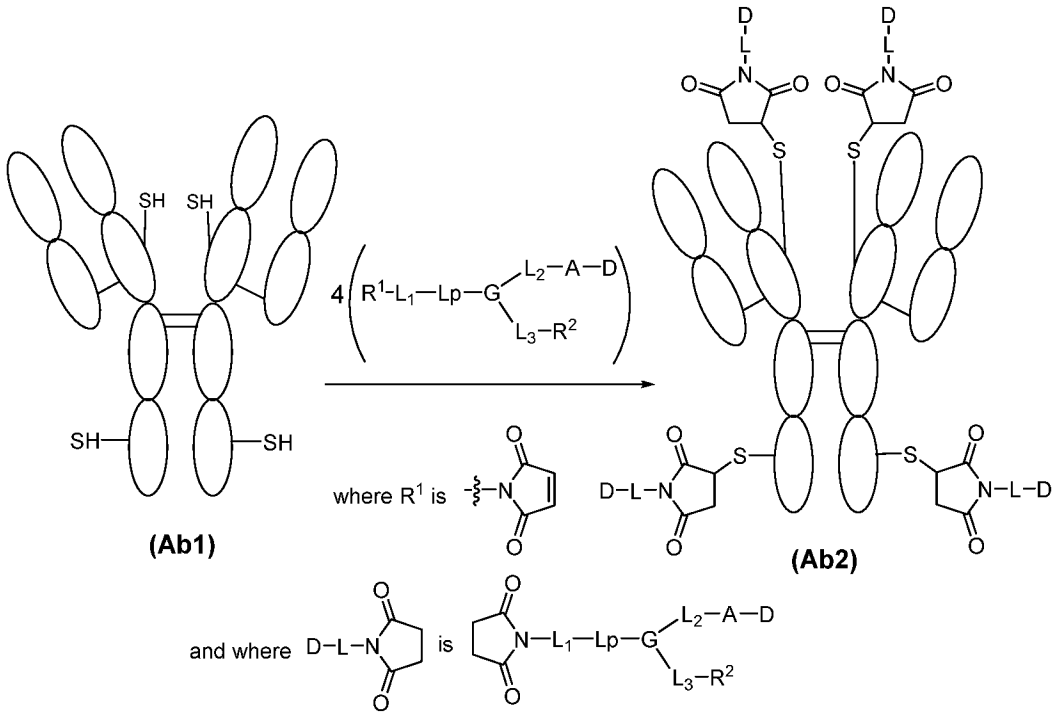
Scheme 3 further illustrates this general approach for the formation of Antibody Drug Conjugates of Formula (E'), wherein the antibody comprises reactive groups (RG_2) which react with an R^1 group (as defined herein) to covalently attach the Linker-Drug group to the antibody via an R^{100} group (as defined herein). For illustrative purposes only Scheme 3 shows the antibody having four RG_2 groups.

Scheme 3



In one aspect, Linker-Drug groups are conjugated to antibodies via modified cysteine residues in the antibodies (see for example WO2014/124316). Scheme 4 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (E') wherein a free thiol group generated from the engineered cysteine residues in the antibody react with an R^1 group (where R^1 is a maleimide) to covalently attach the Linker-Drug group to the antibody via an R^{100} group (where R^{100} is a succinimide ring). For illustrative purposes only Scheme 4 shows the antibody having four free thiol groups.

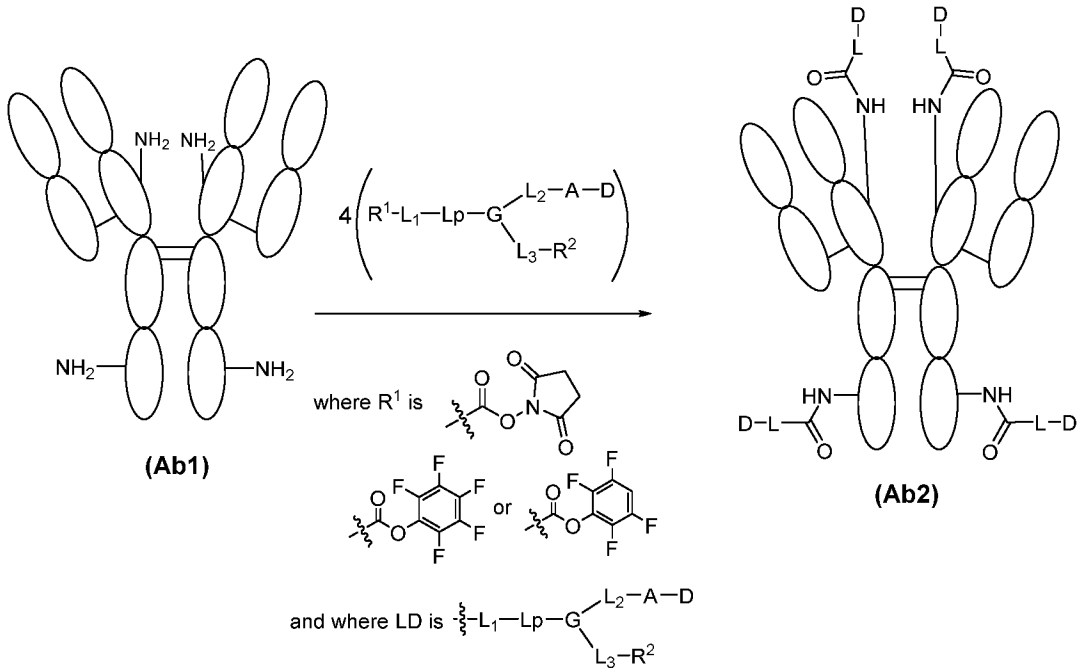
Scheme 4



In another aspect, Linker-Drug groups are conjugated to antibodies via lysine residues in the antibodies. Scheme 5 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (E') wherein a free amine group from the lysine residues in the antibody react with an R^1 group (where R^1 is an NHS ester, a pentafluorophenyl or a

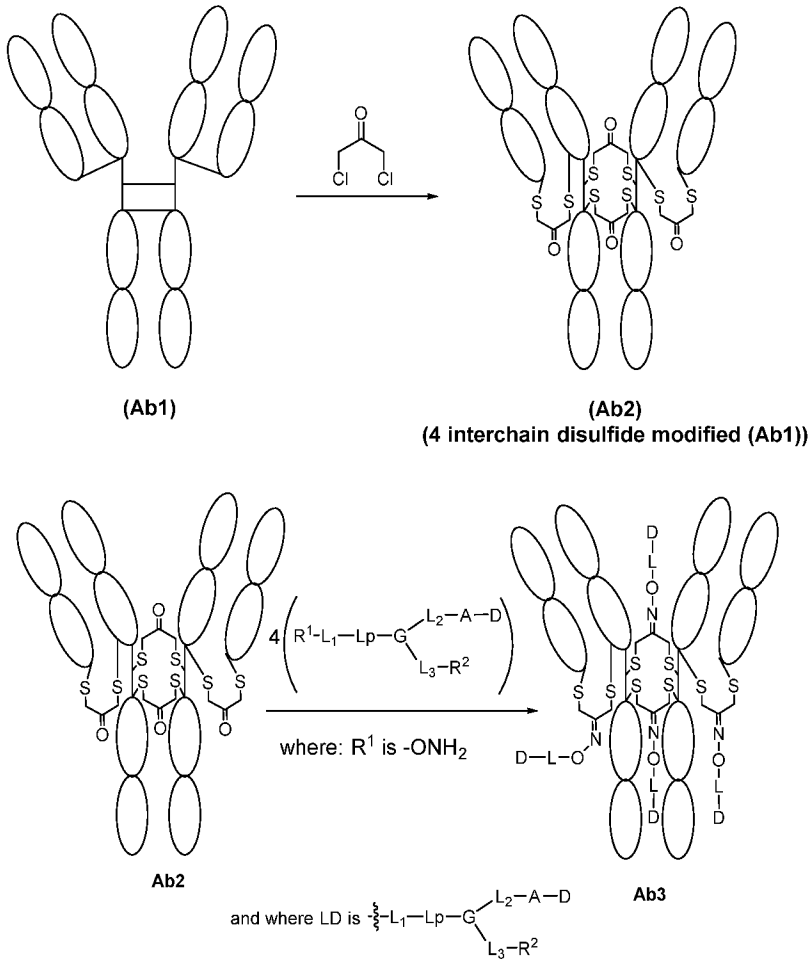
tetrafluorophenyl) to covalently attach the Linker-Drug group to the antibody via an R^{100} group (where R^{100} is an amide). For illustrative purposes only Scheme 5 shows the antibody having four amine groups.

Scheme 5



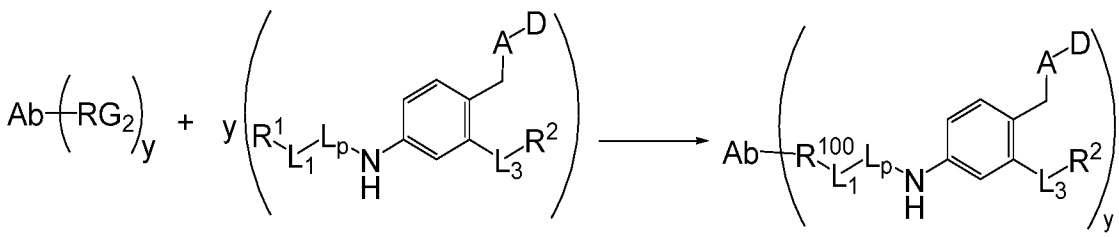
In another aspect, Linker-Drug groups are conjugated to antibodies via formation of an oxime bridge at the naturally occurring disulfide bridges of an antibody. The oxime bridge is formed by initially creating a ketone bridge by reduction of an interchain disulfide bridge of the antibody and re-bridging using a 1,3-dihaloacetone (e.g. 1,3-dichloroacetone). Subsequent reaction with a Linker-Drug group comprising a hydroxyl amine thereby form an oxime linkage (oxime bridge) which attaches the Linker-Drug group to the antibody (see for example WO2014/083505). Scheme 6 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (E').

Scheme 6



A general reaction scheme for the formation of Antibody Drug Conjugates of Formula (F') is shown in Scheme 7 below:

Scheme 7

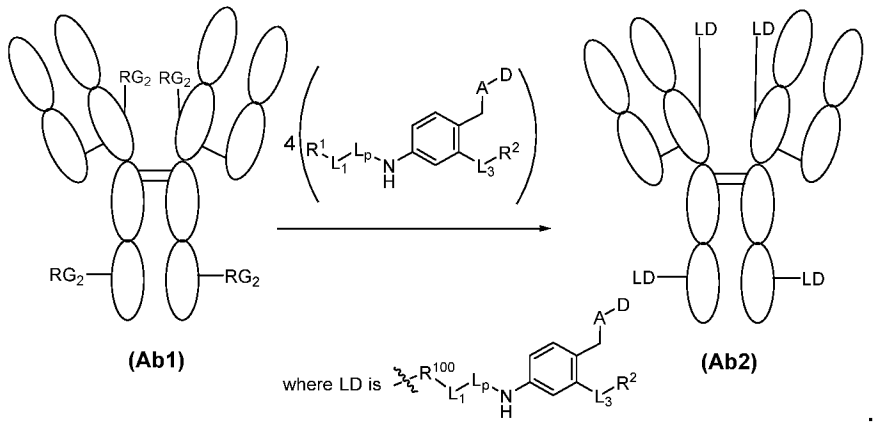


where: RG_2 is a reactive group which reacts with a compatible R^1 group to form a corresponding R^{100} group (such groups are illustrated in Table 8 and Table 9). D, R^1, L_1, Lp, Ab, y and R^{100} are as defined herein.

Scheme 8 further illustrates this general approach for the formation of Antibody Drug Conjugates of Formula (F'), wherein the antibody comprises reactive groups (RG_2) which react with an R^1 group (as defined herein) to covalently attach the Linker-Drug group to the

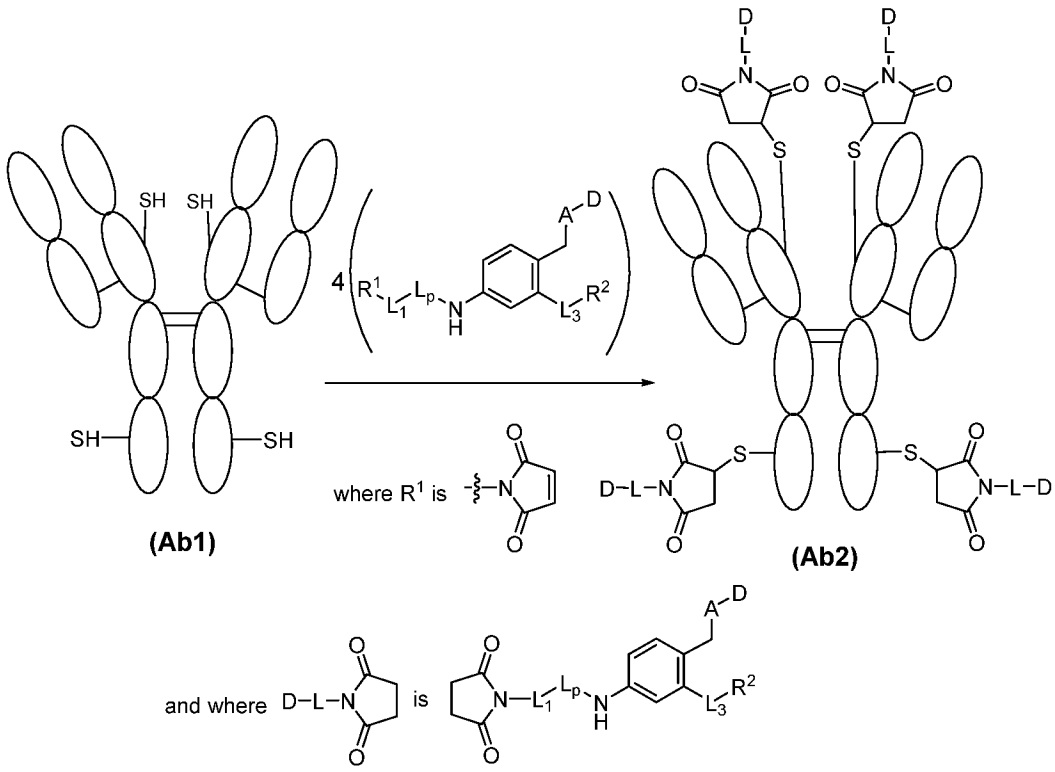
antibody via an R^{100} group (as defined herein). For illustrative purposes only Scheme 8 shows the antibody having four RG_2 groups.

Scheme 8



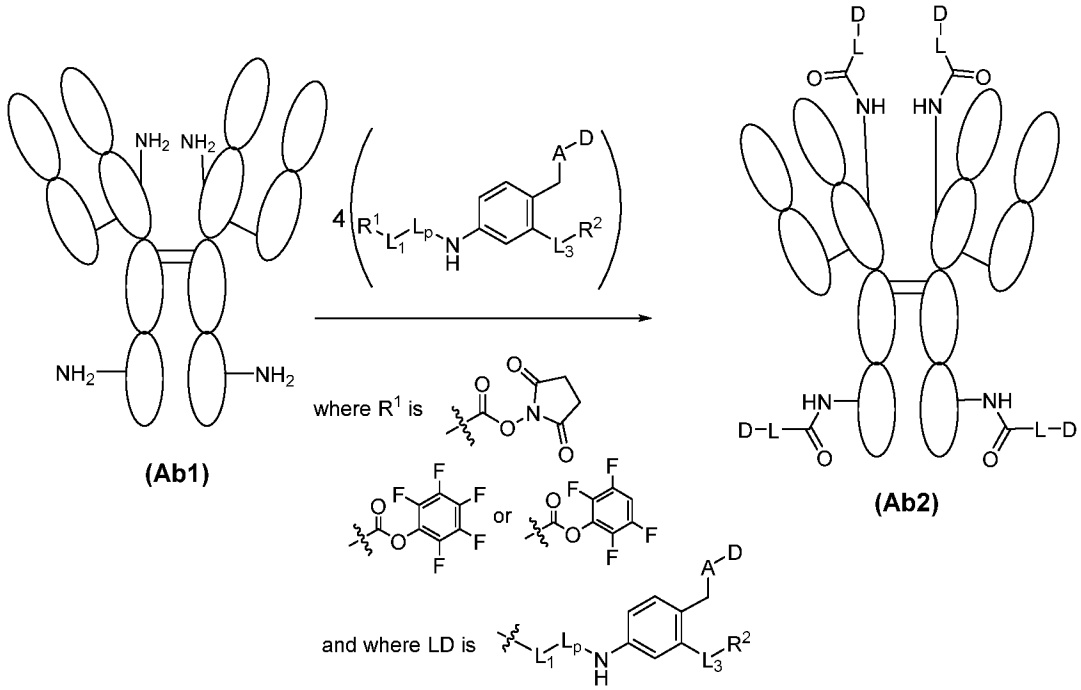
In one aspect, Linker-Drug groups are conjugated to antibodies via modified cysteine residues in the antibodies (see for example WO2014/124316). Scheme 9 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (F') wherein a free thiol group generated from the engineered cysteine residues in the antibody react with an R^1 group (where R^1 is a maleimide) to covalently attach the Linker-Drug group to the antibody via an R^{100} group (where R^{100} is a succinimide ring). For illustrative purposes only Scheme 9 shows the antibody having four free thiol groups.

Scheme 9



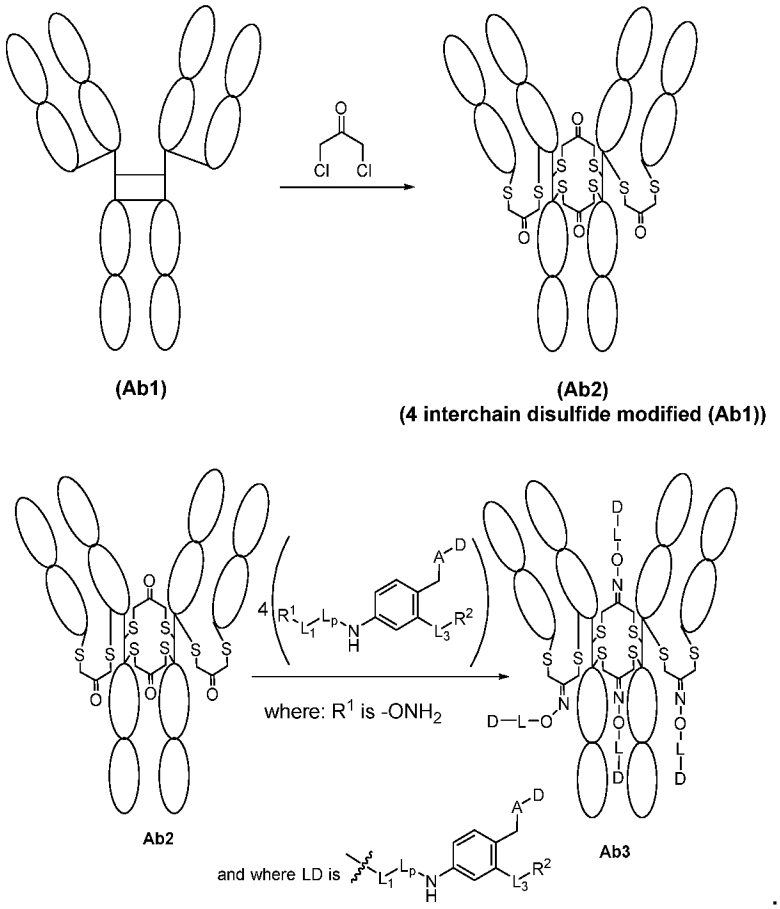
In another aspect, Linker-Drug groups are conjugated to antibodies via lysine residues in the antibodies. Scheme 10 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (F') wherein a free amine group from the lysine residues in the antibody react with an R^1 group (where R^1 is an NHS ester, a pentafluorophenyl or a tetrafluorophenyl) to covalently attach the Linker-Drug group to the antibody via an R^{100} group (where R^{100} is an amide). For illustrative purposes only Scheme 10 shows the antibody having four amine groups.

Scheme 10



In another aspect, Linker-Drug groups are conjugated to antibodies via formation of an oxime bridge at the naturally occurring disulfide bridges of an antibody. The oxime bridge is formed by initially creating a ketone bridge by reduction of an interchain disulfide bridge of the antibody and re-bridging using a 1,3-dihaloacetone (e.g. 1,3-dichloroacetone). Subsequent reaction with a Linker-Drug group comprising a hydroxyl amine thereby form an oxime linkage (oxime bridge) which attaches the Linker-Drug group to the antibody (see for example WO2014/083505). Scheme 11 illustrates this approach for the formation of Antibody Drug Conjugates of Formula (F').

Scheme 11



Provided are also protocols for some aspects of analytical methodology for evaluating antibody conjugates of the invention. Such analytical methodology and results can demonstrate that the conjugates have favorable properties, for example properties that would make them easier to manufacture, easier to administer to patients, more efficacious, and/or potentially safer for patients. One example is the determination of molecular size by size exclusion chromatography (SEC) wherein the amount of desired antibody species in a sample is determined relative to the amount of high molecular weight contaminants (e.g., dimer, multimer, or aggregated antibody) or low molecular weight contaminants (e.g., antibody fragments, degradation products, or individual antibody chains) present in the sample. In general, it is desirable to have higher amounts of monomer and lower amounts of, for example, aggregated antibody due to the impact of, for example, aggregates on other properties of the antibody sample such as but not limited to clearance rate, immunogenicity, and toxicity. A further example is the determination of the hydrophobicity by hydrophobic interaction chromatography (HIC) wherein the hydrophobicity of a sample is assessed relative to a set of standard antibodies of known properties. In general, it is desirable to have low hydrophobicity due to the impact of hydrophobicity on other properties of the antibody sample such as but not limited to aggregation, aggregation over time, adherence to

surfaces, hepatotoxicity, clearance rates, and pharmacokinetic exposure. See Damle, N.K., Nat Biotechnol. 2008; 26(8):884-885; Singh, S.K., Pharm Res. 2015; 32(11):3541-71. When measured by hydrophobic interaction chromatography, higher hydrophobicity index scores (i.e. elution from HIC column faster) reflect lower hydrophobicity of the conjugates. As shown in Examples below, a majority of the tested antibody conjugates showed a hydrophobicity index of greater than 0.8. In some embodiments, provided are antibody conjugates having a hydrophobicity index of 0.8 or greater, as determined by hydrophobic interaction chromatography.

EXAMPLES

[464] The following examples provide illustrative embodiments of the disclosure. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the disclosure. Such modifications and variations are encompassed within the scope of the disclosure. The examples provided do not in any way limit the disclosure.

Example 1. Synthesis and Charaterization of Bcl-xL Payloads

[465] Exemplary payloads were synthesized using exemplary methods described in this example. All reagents obtained from commercial sources were used without further purification. Anhydrous solvents were obtained from commercial sources and used without further drying.

[466] **Column Chromatography:** Automated flash column chromatography was performed on ISCO CombiFlash® Rf 200 or CombiFlash® Rf+ Lumen™ using RediSep® Rf Normal-phase Silica Flash Columns (35-70µm, 60 Å), RediSep Rf Gold® Normal-phase Silica High Performance Columns (20-40µm, 60 Å), RediSep® Rf Reversed-phase C18 Columns (40-63 µm, 60 Å), or RediSep Rf Gold® Reversed-phase C18 High Performance Columns (20-40 µm, 100 Å).

[467] **TLC**: Thin layer chromatography was conducted with 5 x 10 cm plates coated with Merck Type 60 F_{254} silica-gel.

[468] **Microwave Reactions:** Microwave heating was performed with a CEM Discover® SP, or with an Anton Paar Monowave Microwave Reactor.

[469] **NMR:** ^{1}H-NMR measurements were performed on a Bruker Avance III 500 MHz spectrometer, a Bruker Avance III 400 MHz spectrometer, or a Bruker DPX-400 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. ^{1}H NMR data is in the form of delta values, given in part per million (ppm), using the residual peak of the solvent (2.50 ppm for DMSO-d_6 and 7.26 ppm for $CDCl_3$) as internal standard. Splitting patterns are designated as: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septet), m (multiplet), br s

(broad singlet), dd (doublet of doublets), td (triplet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets).

[470] **Analytical LC-MS:** Certain compounds of the present invention were characterized by high performance liquid chromatography-mass spectroscopy (HPLC-MS) on Agilent HP1200 with Agilent 6140 quadrupole LC/MS, operating in positive or negative ion electrospray ionisation mode. Molecular weight scan range is 100 to 1350. Parallel UV detection was done at 210 nm and 254 nm. Samples were supplied as a 1 mM solution in ACN, or in THF/H_2O (1:1) with 5 µL loop injection. LCMS analyses were performed on two instruments, one of which was operated with basic, and the other with acidic eluents.

[471] Basic LCMS: Gemini-NX, 3 µm, C18, 50 mm × 3.00 mm i.d. column at 23 °C, at a flow rate of 1 mL min-1 using 5 mM ammonium bicarbonate (Solvent A) and acetonitrile (Solvent B) with a gradient starting from 100% Solvent A and finishing at 100% Solvent B over various/certain duration of time.

[472] Acidic LCMS: KINATEX XB-C18-100A, 2.6µm, 50 mm*2.1 mm column at 40 °C, at a flow rate of 1 mL min-1 using 0.02% v/v aqueous formic acid (Solvent A) and 0.02% v/v formic acid in acetonitrile (Solvent B) with a gradient starting from 100% Solvent A and finishing at 100% Solvent B over various/certain duration of time.

[473] Certain other compounds of the present invention were characterized HPLC-MS under specific named methods as follows. For all of these methods UV detection was by diode array detector at 230, 254, and 270 nm. Sample injection volume was 1 µL. Gradient elutions were run by defining flow rates and percentage mixtures of the following mobile phases, using HPLC-grade solvents:

Solvent A: 10 mM aqueous ammonium formate + 0.04% (v/v) formic acid

Solvent B: Acetonitrile + 5.3% (v/v) Solvent A + 0.04% (v/v) formic acid.

[474] Retention times (RT) for these named methods are reported in minutes. Ionisation is recorded in positive mode, negative mode, or positive-negative switching mode. Specific details for individual methods follow.

[475] **LCMS-V-B methods**: Using an Agilent 1200 SL series instrument linked to an Agilent MSD 6140 single quadrupole with an ESI-APCI multimode source (Methods LCMS-V-B1 and LCMS-V-B2) or using an Agilent 1290 Infinity II series instrument connected to an Agilent TOF 6230 with an ESI-jet stream source (Method LCMS-V-B1); column: Thermo Accucore 2.6 µm, C18, 50 mm x 2.1 mm at 55 ºC. Gradient details for methods LCMS-V-B1 and LCMS-V-B2 are shown in the table below:

	LCMS-V-B1	**LCMS-V-B2**	

Time (min)	Solvent A (%)	Solvent B (%)	Solvent A (%)	Solvent B (%)	Flow (mL/min)
0	95	5	60	40	1.1
0.12	95	5	60	40	1.3
1.30	5	95	2	98	1.3
1.35	5	95	2	98	1.6
1.85	5	95	2	98	1.6
1.90	5	95	2	98	1.3
1.95	95	5	95	5	1.3

[476] LCMS-V-C method: Using an Agilent 1200 SL series instrument linked to an Agilent MSD 6140 single quadrupole with an ESI-APCI multimode source; column: Agilent Zorbax Eclipse plus 3.5 µm, C18(2), 30 mm x 2.1 mm at 35 ºC. Gradient details for method LCMS-V-C are shown in the table below:

Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL/min)
0	95	5	1
0.25	95	5	1
2.50	95	5	1
2.55	5	95	1.7
3.60	5	95	1.7
3.65	5	95	1
3.70	95	5	1
3.75	95	5	1

[477] Preparative HPLC: Certain compounds of the present invention were purified by high performance liquid chromatography (HPLC) on an Armen Spot Liquid Chromatography or Teledyne EZ system with a Gemini-NX® 10 µM C18, 250 mm × 50 mm i.d. column running at a flow rate of 118 mL min-1 with UV diode array detection (210 – 400 nm) using 25 mM aqueous NH_4HCO_3 solution and MeCN or 0.1% TFA in water and MeCN as eluents.

[478] Certain other compounds of the present invention were purified by HPLC under specific named methods as follows:

[479] HPLC-V-A methods: These were performed on a Waters FractionLynx MS autopurification system, with a Gemini® 5 µm C18(2), 100 mm × 20 mm i.d. column from Phenomenex, running at a flow rate of 20 cm^3min^{-1} with UV diode array detection (210–400

nm) and mass-directed collection. The mass spectrometer was a Waters Micromass ZQ2000 spectrometer, operating in positive or negative ion electrospray ionisation modes, with a molecular weight scan range of 150 to 1000.

[480] Method HPLC-V-A1 (pH 4): Solvent A: 10 mM aqueous ammonium acetate + 0.08% (v/v) formic acid; Solvent B: acetonitrile + 5% (v/v) Solvent A + 0.08% (v/v) formic acid

[481] Method HPLC-V-A2 (pH 9): Solvent A: 10 mM aqueous ammonium acetate + 0.08% (v/v) conc. ammonia; Solvent B: acetonitrile + 5% (v/v) Solvent A + 0.08% (v/v) conc. ammonia

[482] **HPLC-V-B methods:** Performed on an AccQPrep HP125 (Teledyne ISCO) system, with a Gemini® NX 5 µm C18(2), 150 mm × 21.2 mm i.d. column from Phenomenex, running at a flow rate of 20 cm^3min^{-1} with UV (214 and 254 nm) and ELS detection.

[483] Method HPLC-V-B1 (pH 4): Solvent A: water + 0.08% (v/v) formic acid; solvent B: acetonitrile + 0.08% (v/v) formic acid.

[484] Method HPLC-V-B2 (pH 9): Solvent A: water + 0.08% (v/v) conc. ammonia; solvent B: acetonitrile + 0.08% (v/v) conc. ammonia.

[485] Method HPLC-V-B3 (neutral): Solvent A: water; Solvent B: acetonitrile.

[486] **Analytical GC-MS:** Combination gas chromatography and low resolution mass spectrometry (GC-MS) was performed on Agilent 6850 gas chromatograph and Agilent 5975C mass spectrometer using 15 m × 0.25 mm column with 0.25 µm HP-5MS coating and helium as carrier gas. Ion source: EI+, 70 eV, 230°C, quadrupole: 150°C, interface: 300°C.

[487] **High-resolution MS**: High-resolution mass spectra were acquired on an Agilent 6230 time-of-flight mass spectrometer equipped with a Jet Stream electrospray ion source in positive ion mode. Injections of 0.5µl were directed to the mass spectrometer at a flow rate 1.5 ml/min (5mM ammonium-formate in water and acetonitrile gradient program), using an Agilent 1290 Infinity HPLC system. Jet Stream parameters: drying gas (N2) flow and temperature: 8.0 l/min and 325 °C, respectively; nebulizer gas (N2) pressure: 30 psi; capillary voltage: 3000 V; sheath gas flow and temperature: 325 °C and 10.0 l/min; TOFMS parameters: fragmentor voltage: 100 V; skimmer potential: 60 V; OCT 1 RF Vpp:750 V. Full-scan mass spectra were acquired over the m/z range 105-1700 at an acquisition rate of 995.6 ms/spectrum and processed by Agilent MassHunter B.04.00 software.

[488] **Chemical naming:** IUPAC-preferred names were generated using ChemAxon's 'Structure to Name' (s2n) functionality within *MarvinSketch* or *JChem for Excel* (JChem versions 16.6.13 – 18.22.3), or with the chemical naming functionality provided by Biovia® Draw 4.2.

Abbreviations

Ahx	6-hexanoic acid monomer

Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
AgOTf	silver trifluoromethanesulfonate
^tBuOH	*tert*-butanol
cc. or conc.	concentrated
CyOH	cyclohexanol
dba	(1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one, dibenzylideneacetone
DCM	dichloromethane
DEA	diethanolamine
DIAD	diisopropylazodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPA	*N*-isopropylpropan-2-amine, diisopropylamine
DIPEA	*N*-ethyl-*N*-isopropyl-propan-2-amine, diisopropylethylamine
DMAP	4-dimethylaminopyridine
ee.	enatiomeric excess
eq.	equivalent
EtO_2	diethyl ether
EtOAc	ethyl acetate
HF×Pyr	Hydrogen fluoride pyridine
hs	*homo sapiens*
LDA	lithium diisopropylamide
MeCN	acetonitrile
MeOH	methanol
MTBE	methyl *tert*-butyl ether
NMP	*N*-methyl-2-pyrrolidone
$Pd(AtaPhos)_2Cl_2$	bis(di-tert-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II)
PPh_3	triphenylphosphine
rt	room temperature
RT	retention time (in minutes)
on	overnight
Pd\C	palladium on carbon
TBAF	tetrabutylammonium fluoride
TBAOH	tetrabutylammonium hydroxide
TBDPS-Cl	*tert*-butyl-chloro-diphenyl-silane
TBSCl	*tert*-butyl-chloro-dimethyl-silane
TEA	*N,N*-diethylethanamine

TFA	2,2,2-trifluoroacetic acid
pTSA	4-methylbenzenesulfonic acid
THF	tetrahydrofuran
TIPSCl	chloro(triisopropyl)silane
TMP-MgCl	2,2,6,6-tetramethylpiperidinylmagnesium chloride lithium chloride complex solution
DIAD	diisopropylazodicarboxylate
Xantphos	4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene
BrettPhos	2-(Dicyclohexylphosphino)-3,6-dimethoxy-2′,4′,6′-triisopropyl-1,1′-biphenyl
JosiPhos	(2*R*)-1-[(1*R*)-1-(Dicyclohexylphosphino)ethyl]-2-(diphenylphosphino)ferrocene
JosiPhos Pd G3	{(*R*)-1-[(*S*p)-2-(Dicyclohexylphosphino)ferrocenyl]ethyldi-*tert*-butylphosphine}[2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate
Xantphos Pd G3	[(4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene)-2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate
BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl
rac-BINAP Pd G3	[(2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl)-2-(2′-amino-1,1′-biphenyl)]palladium(II) methanesulfonate
$Pd(dppf)Cl_2.CH_2Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium(0)
$Pd(PPh_3)_2Cl_2$	Bis(triphenylphosphine)palladium chloride
$Pd(AtaPhos)_2Cl_2$	bis(di-*tert*-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II)

Named General Procedures

[489] The following are representative experimental procedures that are referred to by name in subsequent Preparations.

Sonogashira General Procedure

[490] The mixture of 1 eq. of aryl halogenide, 2 eq. of acetylene, 0.05 eq. of $Pd(PPh_3)_2Cl_2$, 0.05 eq. of CuI, and DIPA (1 mL/mmol) in THF (5 mL/mmol) was kept at 60°C. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash chromatography using heptane / EtOAc as eluents.

Deprotection with HFIP General Procedure

[491] Substrate in HFIP (10 mL/mmol) was kept at 100-120°C in a pressure bottle. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash chromatography using heptane / EtOAc as eluents.

Deprotection and Hydrolysis General Procedure

[492] The mixture of 1 eq. of substrate and 100 eq. of HFxPyr in MeCN (15 mL/mmol) was stirred at 60°C. After reaching an appropriate conversion, the volatiles were removed under reduced pressure, the residue was suspended in a 1:1 mixture of THF – water (30 mL/mmol), 150 eq. of LiOH x H_2O was added, and the mixture was stirred at rt. After reaching an appropriate conversion, the volatiles were removed under reduced pressure; the crude product was purified via flash chromatography using DCM and MeOH (containing 1.2% NH3) as eluents. In some alternative procedures, the 1:1 mixture of THF – water was replaced with a 1:1 mixture of 1,4-dioxane – water.

Alkylation General Procedure

[493] The mixture of 1 eq. of phenol/carbamate, 1-2 eq. of alkyl iodide/bromide, and 2-3 eq. of Cs_2CO_3 in acetone (5 mL/mmol) was stirred at rt for phenols and at 55 °C for carbamates. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash chromatography (using heptane / EtOAc as eluents for instance) or reverse phase flash column chromatography.

Alkylation with tosylate General Procedure

[494] An oven-dried vial was equipped with a *PTFE*-coated magnetic stirring bar, and was charged with 1 eq. tosylate and 5 eq. of the appropriate amine suspended in MeCN (5 mL/mmol). The reaction mixture was then warmed up to 50°C and stirred at that temperature until no further conversion was observed. The reaction mixture was diluted with DCM then it was injected onto a DCM preconditioned silica gel column. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH3) as eluents.

Buchwald General Procedure I

[495] The mixture of 1 eq. of chloro-substrate, 2 eq. of *1,3-benzothiazol-2-amine*, 0.1 eq. of $Pd_2(dba)_3$, 0.2 eq. of XantPhos, and 3 eq. of DIPEA in CyOH (5 mL/mmol) was kept at 140°C. After reaching an appropriate conversion, the reaction mixture was diluted with DCM (10 mL/mmol), injected onto a preconditioned silica gel column and was purified via flash chromatography (using heptane / EtOAc as eluents for instance).

Buchwald General Procedure II

[496] The mixture of chloro compound, 2 eq. of *1,3-benzothiazol-2-amine*, 10 mol% of JosiPhos Pd (G3) and 3 eq. of DIPE suspended in 1,4-dioxane (5 mL/mmol) were stirred at reflux until no further conversion was observed. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash

chromatography on 120 g silica gel column using heptane-EtOAc or DCM-MeOH (1.2% NH_3) as eluents.

Buchwald General Procedure III

[497] The mixture of 1 eq. of thiazol amine, 1.2-1.5 eq. of *(Z)-N-(6-chloro-4-methyl-pyridazin-3-yl)-3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-imine*, 3 eq. of Cs_2CO_3, 0.1 eq. of $Pd_2(dba)_3$, 0.2 eq. of XantPhos and 3 eq. of DIPEA in 1,4-dioxane (5 mL/mmol) was kept at reflux. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash column chromatography.

Mitsunobu General Procedure I

[498] To the mixture of 1 eq. of aliphatic alcohol, 1 eq. of carbamate/phenol, and 1 eq. triphenylphosphine in toluene (5 mL/mmol) was added 1 eq. of di-*tert*-butyl azodicarboxylate. The mixture was stirred at 50°C for the carbamate and at rt for the phenol. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash chromatography using heptane / EtOAc as eluents.

Mitsunobu General Procedure II

[499] To the mixture of 1.0-1.5 eq. of aliphatic alcohol, 1 eq. of carbamate/phenol, and 1-2 eq. *triphenylphosphine* in THF or toluene (5 mL/mmol) was added 1-3 eq. of *ditertbutyl azodicarboxylate* / *diisopropyl azodicarboxylate* in one portion. The mixture was stirred at rt or 50 °C, if necessary, for the carbamate and at rt for the phenol. After reaching an appropriate conversion the volatiles were removed under reduced pressure, the crude intermediate was purified via flash column chromatography.

Quaternary salt deprotection General Procedure

[500] To a THF (5 mL/mmol) solution of the appropriate quaternary salt 3 eq. TBAF was added, and then it was stirred at rt until no further conversion was observed. The reaction mixture was the evaporated to dry under reduced pressure. To a suspension of 1 eq. desilylated quaternary salt in dry MeCN (15 mL/mmol), 100 eq. of HF x Pyr added, and then was stirred at 60°C. After reaching an appropriate conversion, the volatiles were removed under reduced pressure, the residue was suspended in a 1:1 mixture of THF – water (30 mL/mmol), 150 eq. of LiOH x H_2O was added, and the mixture was stirred at rt. After reaching an appropriate conversion, the volatiles were removed under reduced pressure. The crude product was purified via flash chromatography using DCM and MeOH (containing 1.2% NH_3) as eluents.

Propargylic amine preparation General Procedure

[501] An oven-dried vial was equipped with a *PTFE*-coated magnetic stirring bar, it was charged with 2 eq. PPh3 and 2 eq. imidazole then DCM (5 mL/mmol) was added. To the resulting mixture 2 eq. iodine was added portionwise then stirred for 15 min at rat. To the

resulting mixture 1 eq. of the appropriate alcohol was added dissolved in DCM and stirred at rt until no further conversion was observed. To the generated iodo compound 20 eq. of the appropriate amine was added and then stirred for 30 min at rt, while full conversion was observed. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH_3) eluents.

Silver catalyzed propargylic amine preparation General Procedure

[502] A 24 ml vial was equipped with a stirring bar, and charged with 1 eq. of 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-(4-ethynyl-2-fluoro-phenoxy)propyl]thiazole-4-carboxylic acid, 20 eq. paraformaldehyde/acetone and 20 eq. of the appropriate amine were stirred in dry ethanol (5 ml/mmol) in presence of 20 mol% silver tosylate at 80°C until no further conversion was observed. Celite was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH_3) as eluents.

Hydrolysis General Procedure

[503] The appropriate methyl ester was suspended in a 1:1 mixture of THF – water (5 mL/mmol) and 10 eq. of LiOH x H_2O was added, and the mixture was stirred at 50°C. After reaching an appropriate conversion, the volatiles were removed under reduced pressure; the crude product was purified via flash chromatography using DCM and MeOH (containing 1.2% NH_3) as eluents.

Amine substitution and Hydrolysis General procedure I

[504] To the product from any of the **Preparations 12 and 13** in a 1:1 mixture of acetonitrile and *N*-methyl-2-pyrrolidone (10 ml/mmol), was added the appropriate amine (3-10 eq), and the reaction mixture was stirred at 50 °C for 2-24 h. After the purification of the substitution product by column chromatography (silica gel, using DCM and MeOH as eluents), the product was dissolved in THF (10 ml/mmol), and water (2 ml/mmol) and $LiOH \times H_2O$ (3-5 eq) was added. Then, the reaction mixture was stirred at 20-40 °C for 1-4 h. The hydrolysed product was purified by preparative HPLC (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product.

Amine substitution and Hydrolysis General procedure II

[505] To the product from **Preparation 14_01** in a 1:1 mixture of acetonitrile and *N*-methyl-2-pyrrolidone (10 ml/mmol), was added the appropriate amine (3-10 eq), and the mixture was stirred at 50 °C for 2-24 h. After the addition of 70% HF in pyridine (50-100 eq) at rt, the mixture was stirred for 4-18 h. After the purification of the substitution product by column chromatography (silica gel, using DCM and MeOH as eluents), the product was dissolved in THF (8 ml/mmol), and water (2 ml/mmol) and $LiOH \times H_2O$ (5 eq) was added, and stirred at

20-40 °C for 1-4 h. The hydrolysed product was purified by preparative HPLC (using acetonitrile and 5 mM aqueous NH_4HCO_3 solution as eluents) to give the desired product.

Amine substitution and Hydrolysis General procedure III

[506] To the product from the **Preparation 13** or **Preparation 16** in acetonitrile (13 ml/mmol) was added the appropriate amine (3 eq) and Na_2CO_3 (12 eq), and the reaction mixture was stirred at 120 °C for 1.5-3 h in a microwave reactor. After the addition of KOH (3 eq), the reaction mixture was stirred at 120 °C for 0.75-1 h. The hydrolysed product was purified by preparative HPLC or HILIC chromatography (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product.

Alkylation, Deprotection and Hydrolysis General procedure

[507] A mixture of tertiary amine (1 eq.) and alkylating agent (10 eq.) in acetonitrile (3 mL/mmol) was stirred at rt. After reaching appropriate conversion, the volatiles were removed under reduced pressure and purified via reverse phase flash column chromatography, if it was necessary, otherwise the residue was directly dissolved in acetonitrile (3 mL/mmol), HF×Pyr (100 eq.) was added and the mixture was stirred at 60°C. After reaching appropriate conversion, the volatiles were removed under reduced pressure, the residue was suspended in a 1:1 mixture of 1,4-dioxane – water (10 mL/mmol), $LiOH \times H_2O$ (150 eq.) was added and the mixture was stirred at 60°C. After reaching appropriate conversion to the desired product, the volatiles were removed under reduced pressure and the crude product was purified via reverse phase flash column chromatography.

Preparation 1a: Methyl 2-(*tert*-butoxycarbonylamino)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

Step A: methyl 2-(tert-butoxycarbonylamino)-5-iodo-thiazole-4-carboxylate

[508] 50.00 g methyl 2-(*tert*-butoxycarbonylamino)thiazole-4-carboxylate (193.55 mmol, 1 equiv) was suspended in 600 mL dry MeCN. 52.25 g *N*-iodo succinimide (232.30 mmol,) was added and the resulting mixture was stirred overnight at room temperature.
The reaction mixture was diluted with saturated brine, then it was extracted with EtOAc. The combined organic layers were extracted with 1 M $Na_2S_2O_3$, then with brine again. Then dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified via flash chromatography using heptane as eluent to obtain 60 g of the desired product (156 mmol, 80% Yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 12.03/11.06 (br s), 3.78 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 153.8, 82.5, 77.7, 52.3, 28.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{10}H_{14}IN_2O_4S$: 384.9713; found 384.9708.

Step B: methyl 2-(tert-butoxycarbonylamino)-5-(3-hydroxyprop-1-ynyl)thiazole-4-carboxylate

[509] A 500 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 9.6 g of the product from *Step A* (25 mmol, 1 equiv), 2.80 g prop-2-yn-1-ol (2.91 mL, 50 mmol, 2 equiv) and 36.10 g DIPA (50 mL, 356.8 mmol, 14.27 equiv) then 125 mL dry THF was added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 549 mg $Pd(PPh3)_2Cl_2$ (1.25 mmol, 0.05 equiv) and 238 mg CuI (1.25 mmol, 0.05 equiv) was added. The resulting mixture was then warmed up to 60°C and stirred at that temperature until no further conversion was observed. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using heptane and EtOAc as eluents to give 7.30 g of the desired product (23 mmol, 93% Yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 12.1 (br s, 1H), 5.45 (t, 1H), 4.36 (d, 2H), 3.79 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 12.1 (br s, 1H), 5.45 (t, 1H), 4.36 (d, 2H), 3.79 (s, 3H), 1.48 (s, 9H); HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{13}H_{17}N_2O_5S$: 313.0852, found 313.0866.

Step C: methyl 2-(tert-butoxycarbonylamino)-5-(3-hydroxypropyl)thiazole-4-carboxylate

[510] An 1 L oven-dried pressure bottle equipped with a *PTFE*-coated magnetic stir bar was charged with 44.75 g of the product from *Step B* (143.3 mmol, 1 equiv), 7.62 Pd/C (7.17 mmol, 0.05 equiv) in 340 mL ethanol, and then placed under a nitrogen atmosphere using hydrogenation system. After that, it was filled with 4 bar H_2 gas and stirred at rt overnight. Full conversion was observed, but only the olefin product was formed. After filtration of the catalysts through a pad of *Celite,* the whole procedure was repeated with 5 mol% new catalysts. The resulting mixtures were stirred overnight to get full conversion. *Celite* was added to the reaction mixtures and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography column using heptane and EtOAc as eluents to give 31.9 g of the desired product (101 mmol, 70.4% Yield) as light-yellow crystals. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 11.61 (br s, 1H), 4.54 (t, 1H), 3.76 (s, 3H), 3.43 (m, 2H), 3.09 (t, 2H), 1.74 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.8, 143.1, 135.4, 60.3, 51.9, 34.5, 28.3, 23.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{13}H_{21}N_2O_5S$: 317.1165, found 317.1164 (M+H).

Step D: methyl 2-(tert-butoxycarbonylamino)-5-[3-(2-fluoro-4-iodo-

phenoxy)propyl]thiazole-4-carboxylate

[511] A 250 mL oven-dried, one-necked, round-bottomed flask equipped with a *PTFE*-coated magnetic stir bar, was charged with 3.40 g 2-fluoro-4-iodo-phenol (14 mmol, 1 equiv), 5.00 g of the product from *Step C* (16 mmol, 1.1 equiv) and 4.10 g PPh_3 (16 mmol, 1.1 equiv) dissolved in 71 mL dry toluene. After 5 min stirring under nitrogen atmosphere, 3.10 mL DIAD (3.20 g, 16 mmol, 1.1 equiv) was added in one portion while the reaction mixture warmed up. Then the reaction mixture was heated up to 50°C and stirred at that temperature for 30 min, when the reaction reached complete conversion. The reaction mixture was directly injected onto a preconditioned silica gel column, and then it was purified via flash chromatography using heptane and EtOAc as eluents. The crude product was crystalized from MeOH to give 4.64 g of the desired product (9.24 mmol, 66% Yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.64 (br s, 1H), 7.59 (dd, 1H), 7.45 (dd, 1H), 6.98 (t, 1H), 4.06 (t, 2H), 3.73 (s, 3H), 3.22 (t, 2H), 2.06 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 134, 124.9, 117.6, 68.2, 51.9, 30.5, 28.3, 23.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{19}H_{23}N_2O_5FSI$: 537.0350; found 537.0348.

Preparation 1c: Methyl 2-(*tert*-butoxycarbonylamino)-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[512] A 250 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 5.36 g **Preparation 1a** (10 mmol, 1 equiv), 1.66 g *N,N*-dimethylprop-2-yn-1-amine (20 mmol, 2 equiv) and 20 mL DIPA (142.7 mmol, 14.27 equiv) then 50 mL dry THF was added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 220 mg $Pd(PPh_3)_2Cl_2$ (0.5 mmol, 0.05 equiv) and 95 CuI (0.5 mmol, 0.05 equiv) were added. The resulting mixture was then warmed up to 60°C and stirred at that temperature until no further conversion was observed. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH_3) as eluents to give 4.5 g of the desired product (7.8 mmol, 78% Yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.66 (s, 1H), 7.29 (dd, 1H), 7.19 (m, 1H), 7.12 (t, 1H), 4.09 (t, 2H), 3.73 (s, 3H), 3.44 (s, 2H), 3.23 (t, 2H), 2.24 (s, 6H), 2.07 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.8, 147.3, 129, 119.2, 115.4, 84.3, 68, 51.9, 48.1, 44.2, 30.6, 28.3, 23.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{31}FN_3O_5S$: 492.1962; found 492.1956 (M+H).

Preparation 2a: 3-(3,6-Dichloro-5-methyl-pyridazin-4-yl)propan-1-ol

Step A: [(pent-4-yn-1-yloxy)methyl]benzene

[513] To an oven-dried flask was added 4-pentyn-1-ol (11.1 mL, 119 mmol, 1 eq) in THF (100 mL) and the solution was cooled to 0 °C. Sodium hydride (60% dispersion; 7.13 g, 178 mmol, 1.5 eq) was added portionwise and the mixture was allowed to stir for 30 min at 0 °C before the dropwise addition of benzyl bromide (15.6 mL, 131 mmol, 1.1 eq). The mixture was allowed to warm to ambient temperature and was stirred for 16 h, then cooled to 0 °C, quenched with saturated aqueous ammonium chloride (30 mL) and diluted with water (30 mL). The mixture was extracted with ethyl acetate (2 x 150 mL), and the combined organic extracts were washed successively with dilute aqueous ammonium hydroxide ammonium hydroxide (150 mL) and brine (100 mL), dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 330 g RediSep™ silica cartridge) eluting with a gradient of 0 – 10% ethyl acetate in *iso*-heptane afforded the desired product as a yellow liquid (19.5 g, 112 mmol, 94%). LC/MS ($C_{12}H_{14}O$) 175 $[M+H]^+$; RT 1.28 (LCMS-V-B1). ^1H NMR (400 MHz, Chloroform-d) δ 7.37 – 7.32 (m, 4H), 7.31 – 7.27 (m, 1H), 4.52 (s, 2H), 3.58 (t, J = 6.1 Hz, 2H), 2.32 (td, J = 7.1, 2.6 Hz, 2H), 1.95 (t, J = 2.7 Hz, 1H), 1.83 (tt, J = 7.1, 6.2 Hz, 2H).

Step B: [(hex-4-yn-1-yloxy)methyl]benzene

[514] To an oven-dried flask was added the product from Step A (19.5 g, 112 mmol, 1 eq) and tetrahydrofuran (200 mL) and the solution was cooled to -78 °C. *n*-Butyllithium (66.9 mL, 135 mmol, 1.2 eq) was added dropwise over 30 min and the reaction was stirred for 1 h then iodomethane (10.5 mL, 168 mmol, 1.5 eq) was added dropwise and the mixture was allowed to warm to 0 °C over 1 h. The reaction was quenched by the addition of saturated aqueous ammonium chloride (40 mL), diluted with water (40 mL), extracted with ethyl acetate (3 x 100 mL), and the combined organic extracts were successively washed with 2M aqueous sodium thiosulfate (200 mL) and brine (200 mL), dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 330 g RediSep™ silica cartridge) eluting with a gradient of 0 – 10% ethyl acetate in *iso*-heptane afforded the desired product as a yellow liquid (19.2 g, 0.1 mol, 91%). LC/MS ($C_{13}H_{16}O$) 189 $[M+H]^+$; RT 1.34 (LCMS-V-B1). ^1H NMR (400 MHz, DMSO-d6) δ 7.41 – 7.23 (m, 5H), 4.46 (s, 2H), 3.48 (t, J = 6.3 Hz, 2H), 2.23 – 2.14 (m, 2H), 1.72 (s, 3H), 1.70 – 1.65 (m, 2H).

Step C: 4-[3-(benzyloxy)propyl]-3,6-dichloro-5-methylpyridazine

[515] A solution of 3,6-dichloro-1,2,4,5-tetrazine (5 g, 33.1 mmol, 1 eq) and the product from Step B (7.48 g, 39.8 mmol, 1.2 eq) in tetrahydrofuran (30 mL) was heated at 160 °C for 19 h in a sealed flask. The reaction was allowed to cool to ambient temperature then

concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 220 g RediSep™ silica cartridge) eluting with a gradient of 0 – 30% ethyl acetate in *iso*-heptane afforded the desired product as an orange oil (7.32 g, 23.5 mmol, 71%). LC/MS ($C_{15}H_{16}Cl_2N_2O$) 311 $[M+H]^+$; RT 1.35 (LCMS-V-B1). ^1H NMR (400 MHz, DMSO-d6) δ 7.45 – 7.18 (m, 5H), 4.48 (s, 2H), 3.53 (t, J = 5.9 Hz, 2H), 2.96 – 2.83 (m, 2H), 2.42 (s, 3H), 1.88 – 1.69 (m, 2H).

Step D: 3-(3,6-dichloro-5-methylpyridazin-4-yl)propan-1-ol

[516] To a cooled solution of the product from Step C (7.32 g, 23.5 mmol, 1 eq) in dichloromethane (100 mL) was added boron trichloride solution (1 M in dichloromethane; 58.8 mL, 58.8 mmol, 2.5 eq) dropwise and the mixture was allowed to stir at ambient temperature for 1 h. The reaction was quenched by the addition of methanol and concentrated *in vacuo*. The residue was partitioned between dichloromethane (100 mL) and saturated aqueous sodium bicarbonate (150 mL), and the organic phase was washed with brine (150 mL), dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 80% ethyl acetate in *iso*-heptane afforded the desired product as a yellow oil (4.19 g, 19 mmol, 81%). LC/MS ($C_8H_{10}Cl_2N_2O$) 221 $[M+H]^+$; RT 0.84 (LCMS-V-B1). ^1H NMR (400 MHz, DMSO-d6) δ 4.67 (t, J = 5.1 Hz, 1H), 3.49 (td, J = 6.0, 5.1 Hz, 2H), 2.91 – 2.80 (m, 2H), 2.43 (s, 3H), 1.72 – 1.59 (m, 2H).

Preparation 3a: Methyl 2-(3-chloro-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

Step A: methyl 2-{[(tert-butoxy)carbonyl][3-(3,6-dichloro-5-methylpyridazin-4-yl)propyl]amino}-5-[3-(2-fluoro-4-iodophenoxy)propyl]-1,3-thiazole-4-carboxylate

[517] Using **Mitsunobu General Procedure** I starting from 4.85 g **Preparation 1a** (9.04 mmol, 1 equiv) as the appropriate carbamate and 2 g **Preparation 2a** (9.04 mmol, 1 equiv) as the appropriate alcohol, 4.6 g of the desired product (69% Yield) was obtained. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 7.56 (dd, 1H), 7.44 (dm, 1H), 7.08 (m, 2H), 6.96 (t, 1H), 4.05 (t, 2H), 3.75 (s, 3H), 3.21 (t, 2H), 2.82 (m, 2H), 2.4 (s, 3H), 2.06 (m, 2H), 1.88 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.7, 157.6, 156.7, 156.5/153.2, 152.2, 147, 142.1, 139.8, 134, 124.9, 117.6, 84, 82.4, 68.1, 52.1, 46.1, 30.4, 28.1, 27.5, 25.8, 23.1, 16.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{27}H_{31}Cl_2FIN_4O_5S$: 739.0415, found 739.0395.

Step B: methyl 2-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

[518] Using **Deprotection with HFIPA General Procedure** starting from the product from *Step A* as the appropriate carbamate, 3.70 g the desired product (97% Yield) was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.71 (t, 1 H), 7.59 (dd, 1 H), 7.44 (dm, 1 H), 6.96 (t, 1 H), 4.03 (t, 2 H), 3.7 (s, 3 H), 3.29 (m, 2 H), 3.11 (t, 2 H), 2.84 (m, 2 H), 2.39 (s, 3 H), 2 (m, 2 H), 1.76 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.6, 163, 152.3, 147.1, 134.1, 124.8, 117.6, 82.4, 68.1, 51.9, 44, 30.7, 28, 26.9, 23.3, 16.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{23}Cl_2FIN_4O_3S$: 638.9891, found 638.9888.

Step C: methyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

[519] A suspension of 3 g of the product from *Step B* (4.69 mmol, 1 eq) and 1.81 g cesium carbonate (9.3853 mmol, 2 eq.) were stirred at 80°C for 3 h in 25 mL dry 1,4-dioxane to reach complete conversion. Reaction mixture directly was evaporated to *Celite*, and then purified by flash chromatography on using DCM-MeOH as eluents to obtain 2.67 g of the title compound (94% Yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.57 (dd, 1H), 7.43 (dm, 1H), 6.97 (t, 1H), 4.23 (t, 2 H), 4.08 (t, 2 H), 3.77 (s, 3 H), 3.22 (t, 2 H), 2.86 (t, 2 H), 2.29 (s, 3 H), 2.08 (m, 2 H), 2.03 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 155.4, 152.2, 151.6, 151.2, 147, 142.5, 136, 134.8, 134, 128.9, 124.9, 117.6, 82.3, 68.4, 51.9, 46.3, 30.7, 24.2, 23, 19.7, 15.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{22}H_{22}ClFIN_4O_3S$: 603.0124, found 603.0108.

Preparation 3c: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-(4-ethynyl-2-fluoro-phenoxy)propyl]thiazole-4-carboxylic acid

Step A: methyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-[2-fluoro-4-(2-trimethylsilylethynyl)phenoxy] propyl]thiazole-4-carboxylate

[520] A 250 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 5 g **Preparation 3a** (8.29 mmol, 1 eq.), 2.34 mL *ethynyl(trimethyl)silane* (16.58 mmol, 2 eq.) and 10 mL DIPEA, then 40 mL dry THF was added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 182 mg $Pd(PPh_3)_2Cl_2$ (0.41 mmol, 0.05 eq.) and 79 mg (0.41 mmol, 0.05 eq.) were added. The resulting mixture was then warmed up to 60°C and stirred at that temperature for 2 hours to reach complete conversion. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using Heptane-EtOAc as eluents to give 4.26 g of the desired product (89% Yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.31 (dd, 1H), 7.23 (dn, 1H), 7.13 (t, 1H), 4.25 (t, 2H), 4.12 (t, 2H), 3.77 (s, 3H), 3.24 (t, 2H), 2.87 (t, 2H), 2.31 (s,

3H), 2.1 (m, 2H), 2.03 (m, 2H), 0.21 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.0, 155.3, 151.7, 151.3, 136.1, 129.4, 129.0, 119.4, 115.3, 104.6, 93.7, 68.2, 51.9, 46.3, 30.7, 24.1, 23.0, 19.7, 15.7, 0.4; HRMS-ESI (m/z): [M]+ calcd for $C_{27}H_{30}ClFN_4O_3SSi$: 572.1481, found 572.1480.

Step B: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-(2-trimethylsilylethynyl) phenoxy]propyl]thiazole-4-carboxylate

[521] A 100 mL oven-dried, one-necked, round-bottom flask with a *PTFE*-coated magnetic stirring bar was charged with 4.25 g of the product from *Step A* (7.4 mmol, 1.0 eq.), 2.23 g 1,3-benzothiazol-2-amine (14.8 mmol, 2.0 eq.) and 3.87 mL DIPEA (2.87 mg, 22.2 mmol, 3.0 eq.) then 40 mL cyclohexanol was added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 679 mg $Pd_2(dba)_3$ (0.74 mmol, 0.10 eq.) and 858 mg XantPhos (1.48 mmol, 0.20 eq.) were added. The resulting mixture was then warmed up to 140°C and stirred at that temperature for 30 min to reach complete conversion. The reaction mixture was diluted with DCM and directly injected onto a preconditioned silica gel column, and then it was purified via flash chromatography using heptane and EtOAc as eluents. The pure fractions were combined and concentrated under reduced pressure to give 3.90 g of the desired product (77% Yield). ^{1}H NMR (500 MHz, DMSO-*d_6*) δ ppm 12.27/10.91 (brs, 1H), 8.1-7.1 (brm, 4H), 7.34 (dd, 1H), 7.24 (dm, 1H), 7.16 (t, 1H), 4.25 (t, 2H), 4.15 (t, 2H), 3.78 (s, 3H), 3.28 (t, 2H), 2.87 (t, 2H), 2.34 (s, 3H), 2.13 (m, 2H), 2.04 (m, 2H), 0.19 (s, 9H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{34}H_{36}FN_6O_3S_2Si$: 687.2038, found 687.2020.

Step C: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-(4-ethynyl-2-fluoro-phenoxy)propyl]thiazole-4-carboxylic acid

[522] A 10 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 343 mg of the product from *Step B* (0.5 mmol, 1.0 eq.) dissolved in 2.5 mL THF/H_2O (4:1). Then 105 mg LiOH x H_2O (2.50 mmol, 5.0 eq.) was added and the resulting mixture was heated to 60°C and stirred for 4 h at this temp. The reaction reached complete conversion. *Celite* gel was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH_3) as eluents to give 200 mg title compound (66% Yield). ^{1}H NMR (500 MHz, DMSO-*d_6*) δ ppm 7.88 (d, 1H), 7.49 (br., 1H), 7.37 (t, 1H), 7.36 (dd, 1H), 7.25 (dm, 1H), 7.19 (t, 1H), 7.16 (t, 1H), 4.27 (t, 2H), 4.15 (t, 2H), 4.11 (s, 1H), 3.27 (t, 2H), 2.87 (t, 2H), 2.33 (s, 3H), 2.14 (m, 2H), 2.04 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.2, 151.5, 147.9, 129.4, 126.5, 122.5,

122.3, 119.5, 115.5, 114.5, 82.9, 80.5, 68.5, 46.2, 31.0, 23.9, 23.1, 20.3, 12.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{30}H_{26}FN_6O_3S_2$: 601.1486, found 601.1498.

Preparation 3d: Methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-hydroxyprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylate

Step A: methyl 5-[3-[4-[3-[tert-butyl(dimethyl)silyl]oxyprop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)thiazole-4-carboxylate

[523] Using **Sonogashira General Procedure** starting from 4.00 g of **Preparation 3a** (6.63 mmol, 1.0 eq.) and 2.26 g *tert-butyl-dimethyl-prop-2-ynoxy-silane* (13.27 mmol, 2 eq.) as the appropriate acetylene, 2.80 g of the desired product (65% Yield) was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.27 (dd, 1H), 7.19 (dd, 1H), 7.14 (t, 1H), 4.51 (s, 1H), 4.25 (m, 2H), 4.12 (t, 2H), 3.77 (s, 3H), 3.24 (t, 2H), 2.87 (t, 2H), 2.3 (s, 3H), 2.1 (quint., 2H), 2.03 (m, 2H), 0.88 (s, 9H), 0.12 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.0, 128.9, 119.1, 115.5, 68.3, 52.1, 51.9, 46.3, 30.7, 26.2, 24.2, 23.0, 19.7, 15.7, -4.6; HRMS-ESI (m/z): [M+H]+ calcd for $C_{31}H_{39}ClFN_4O_4SSi$: 645.2128, found 645.2120.

Step B: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[tert-butyl(dimethyl)silyl]oxyprop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[524] Using **Buchwald General Procedure II** starting from 2.8 g of the product from *Step A* (4.34 mmol, 1.0 eq.) and 1.30 g *1,3-benzothiazol-2-amine* (8.67 mmol, 2.0 eq.), 2.1 g of the desired product (64% Yield) was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.25/10.91 (brs 1H), 7.88 (br, 1H), 7.51 (br, 1H), 7.37 (t, 1H), 7.29 (dd, 1H), 7.2 (t, 1H), 7.2 (dd, 1H), 7.17 (t, 1H), 4.49 (s, 2H), 4.25 (t, 2H), 4.14 (t, 2H), 3.77 (s, 3H), 3.27 (t, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.13 (qn, 2H), 2.04 (qn, 2H), 0.87 (s, 9H), 0.1 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.2, 155.7, 151.6, 148.5, 147.6, 141.5, 128.9, 127.6, 126.5, 122.5, 122.3, 119.1, 116.9, 115.5, 114.8, 88.2, 84, 68.4, 52.1, 51.9, 46.4, 31, 26.2, 24, 23.1, 20.4, 12.9, -4.6; HRMS-ESI (m/z): [M+H]+ calcd for $C_{38}H_{44}FN_6O_4S_2Si$: 759.2613, found 759.2609.

Step C: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-hydroxyprop-1-ynyl)phenoxy]propyl] thiazole-4-carboxylate

[525] A 100 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 2.10 g of

the product from *Step B* (2.76 mmol, 1.0 eq.) dissolved in 15 mL THF. Then 3.32 mL TBAF (3.32 mmol, 1.2 eq., 1 M in THF) was added dropwise via syringe over a period of 2 minutes, and stirred at that temperature for 30 min. The reaction mixture was quenched with saturated NH_4Cl, then directly evaporated to *Celite* and it was purified via flash chromatography using heptane- EtOAc as eluents to give 1.6 g of the desired product (90% Yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.14 (brs, 1H), 7.83 (brd, 1H), 7.49 (brs, 1H), 7.36 (m, 1H), 7.24 (dd, 1H), 7.19 (m, 1H), 7.18 (dm, 1H), 7.15 (t, 1H), 5.08 (t, 1H), 4.28 (m, 2H), 4.27 (d, 2H), 4.17 (t, 2H), 3.8 (s, 3H), 3.29 (m, 2H), 2.89 (m, 2H), 2.35 (s, 3H), 2.15 (m, 2H), 2.07 (m, 2H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{32}H_{30}FN_6O_4S_2$: 645.1748, found 645.1738.

Preparation 3f: Ethyl 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-5*H*,6*H*,7*H*,8*H*-pyrido[2,3-*c*]pyridazin-8-yl}-1,3-thiazole-4-carboxylate

Step A: ethyl 2-[(hex-4-yn-1-yl)amino]-1,3-thiazole-4-carboxylate

[526] To a solution of ethyl 2-bromo-1,3-thiazole-4-carboxylate (1.17 g, 4.97 mmol, 1 eq) in acetonitrile (16 mL) was added hex-4-yn-1-amine (725 mg, 7.46 mmol, 1.5 eq) and triethylamine (1.04 mL, 7.46 mmol, 1.5 eq) and the mixture was heated at 150 °C for 4 h under microwave irradiation. The reaction was partitioned between ethyl acetate and brine, and the organic phase was dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™ silica cartridge) eluting with a gradient of 0 – 60% ethyl acetate in *iso*-heptane afforded the desired product as a beige solid (741 mg, 2.94 mmol, 59%). LC/MS ($C_{12}H_{16}N_2O_2S$) 253 $[M+H]^+$; RT 2.32 (LCMS-V-C).

Step B: ethyl 2-{3-chloro-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-1,3-thiazole-4-carboxylate

[527] To a solution of 3,6-dichloro-1,2,4,5-tetrazine (443 mg, 2.94 mmol, 1 eq) in tetrahydrofuran (15 mL) was added the product from Step A (741 mg, 2.94 mmol, 1 eq) and the mixture was heated in a sealed tube at 110 °C overnight. The reaction was concentrated *in vacuo* and the residue was triturated with methanol, filtered and dried under vacuum to afford the desired product as a beige solid (607 mg, 1.79 mmol, 61%). LC/MS ($C_{14}H_{15}ClN_4O_2S$) 339 $[M+H]^+$; RT 2.41 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.06 (s, 1H), 4.38 - 4.25 (m, 4H), 2.92 (t, J = 6.3 Hz, 2H), 2.34 (s, 3H), 2.14 – 2.01(m, 2H), 1.31 (t, J = 7.1 Hz, 3H).

Step C: ethyl 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-1,3-thiazole-4-carboxylate

[528] To an oven-dried microwave vial was added the product from Step B (607 mg, 1.79 mmol, 1 eq), 2-aminobenzothiazole (404 mg, 2.69 mmol, 1.5 eq)), XantPhos (207 mg, 0.36 mmol, 0.2 eq), cesium carbonate (1.17 g, 3.58 mmol, 2 eq) and 1,4-dioxane (36 mL) and the vessel was evacuated and flushed with nitrogen then tris(dibenzylideneacetone)dipalladium(0) (164 mg, 0.18 mmol, 0.1 eq) was added and the mixture was sparged with nitrogen (10 mins) then heated at 150 °C for 4 hours under microwave irradiation. The reaction was diluted with ethyl acetate and filtered through celite, then washed with brine, dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 24 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded a solid that was triturated with diethyl ether, filtered and dried under vacuum to afford the desired product as a yellow solid (329 mg, 0.73 mmol, 41%). LC/MS ($C_{21}H_{20}N_6O_2S_2$) 453 $[M+H]^+$; RT 2.73 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.99 (br s + s, 2H), 7.65 (br s, 1H), 7.43 – 7.31 (m, 1H), 7.28 – 7.15 (m, 1H), 4.35 - 4.25 (m, 4H), 2.96 – 2.85 (m, 2H), 2.36 (s, 3H), 2.15 – 2.00 (m, 2H), 1.32 (t, J = 7.1 Hz, 3H).

Preparation 3g: Ethyl 5-(3-hydroxypropyl)-2-(4-methyl-3-{[(2*Z*)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5*H*,6*H*,7*H*,8*H*-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

Step A: ethyl 2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[529] To a solution of the product from **Preparation 3f** (11.7 g, 25.8 mmol, 1 eq) in dimethylformamide (700 mL) was added *N,N*-diisopropylethylamine (13.5 mL, 77.4 mmol, 3 eq). After 5 min the mixture was cooled to 0 °C and 4-(dimethylamino)pyridine (630 mg, 5.16 mmol, 0.2 eq) and 2-(trimethylsilyl)ethoxymethyl chloride (13.6 mL, 77.4 mmol, 3 eq) were added and the mixture was stirred at ambient temperature overnight. The reaction was concentrated *in vacuo*, then partitioned between dichloromethane and brine, and the organic phase was dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 330 g RediSep™ silica cartridge) eluting with a gradient of 0 – 40% ethyl acetate in *iso*-heptane afforded the desired product as a yellow solid (9.61 g, 16.5 mmol, 64%). LC/MS ($C_{27}H_{34}N_6O_3SiS_2$) 583 $[M+H]^+$; RT 2.90 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.99 (s, 1H), 7.82 (dd, J = 7.7, 1.1 Hz, 1H), 7.49 - 7.38 (m, 2H), 7.28 - 7.19 (m, 1H), 5.86 (s, 2H), 4.38 – 4.23 (m, 4H), 3.77 - 3.67 (m, 2H), 2.89 (t, J = 6.2 Hz, 2H), 2.38 (s, 3H), 2.13 – 2.01 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 0.91 (dd, J = 8.5, 7.4 Hz, 2H), -0.11 (s, 9H).

Step B: ethyl 5-bromo-2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[530] To a solution of the product of Step A(9.61 g, 16.5 mmol, 1 eq) in dichloromethane (400 mL) was added *N*-bromosuccinimide (3.52 g, 19.8 mmol, 1.2 eq) and the mixture was stirred at ambient temperature overnight. The reaction was partitioned between dichloromethane and water, and the organic phase was washed with brine, dried (PTFE phase separator) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 220 g RediSep™ silica cartridge) eluting with a gradient of 0 – 40% ethyl acetate in *iso*-heptane afforded the desired product as a yellow solid (9.66 g, 14.6 mmol, 89%). LC/MS ($C_{27}H_{33}BrN_6O_3SiS_2$) 663 $[M+H]^+$; RT 3.13 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.84 (dd, J = 7.5, 1.1 Hz, 1H), 7.59 - 7.38 (m, 2H), 7.24 (ddd, J = 8.3, 6.7, 1.7 Hz, 1H), 5.85 (s, 2H), 4.37 - 4.23 (m, 4H), 3.72 (dd, J = 8.5, 7.4 Hz, 2H), 2.87 (t, J = 6.2 Hz, 2H), 2.38 (s, 3H), 2.13 – 2.00 (m, 2H), 1.32 (t, 3H), 0.95 - 0.81 (m, 2H), -0.12 (s, 9H).

Step C: ethyl 5-[(1E)-3-[(tert-butyldimethylsilyl)oxy]prop-1-en-1-yl]-2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[531] To an oven-dried sealed flask was added the product from Step B (9.66 g, 14.6 mmol, 1 eq), (*E*)-3-(*tert*-butyldimethylsilyloxy)propene-1-yl-boronic acid pinacol ester (5.74 mL, 17.5 mmol, 1.2 eq), potassium carbonate (6.05 g, 43.8 mmol, 3 eq), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1.19 g, 1.46 mmol, 0.1 eq), tetrahydrofuran (360 mL) and water (120 mL), and the mixture was sparged with nitrogen (10 min) then heated at 120 °C for 2 h. The reaction was partitioned between ethyl acetate and water, and the organic layer was washed with brine, dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 220 g RediSep™ silica cartridge) eluting with a gradient of 0 – 30% ethyl acetate in *iso*-heptane afforded the desired product as a yellow solid (6.46 g, 8.58 mmol, 59%). LC/MS ($C_{36}H_{52}N_6O_4Si_2S_2$) 753 $[M+H]^+$; RT 1.62 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.80 (dd, J = 7.6, 1.0 Hz, 1H), 7.51 - 7.38 (m, 3H), 7.24 (ddd, J = 8.3, 6.8, 1.8 Hz, 1H), 6.28 (dt, J = 16.0, 4.3 Hz, 1H), 5.85 (s, 2H), 4.37 (dd, J = 4.4, 2.1 Hz, 2H), 4.35 - 4.25 (m, 4H), 3.72 (dd, J = 8.5, 7.4 Hz, 2H), 2.88 (t, J = 6.3 Hz, 2H), 2.37 (s, 3H), 2.09 – 1.99 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 0.93 (s, 9H), 0.92 - 0.83 (m, 2H), 0.11 ((s, 6H), -0.11 (s, 9H).

Step D: ethyl 5-{3-[(tert-butyldimethylsilyl)oxy]propyl}-2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[532] To a solution of the product from Step C (6.46 g, 8.58 mmol, 1 eq) in ethyl acetate (300 mL) was added platinum (IV) oxide (390 mg, 1.72 mmol, 0.2 eq) under a nitrogen atmosphere. The vessel was evacuated and backfilled with nitrogen (x3), then evacuated, placed under an atmosphere of hydrogen, and shaken for 3 days at ambient temperature. The reaction was filtered through celite, eluted with ethyl acetate and concentrated *in vacuo* to afford the desired product as a brown gum (6.72 g, 8.9 mmol, >100%). LC/MS ($C_{36}H_{54}N_6O_4Si_2S_2$) 755 $[M+H]^+$; RT 1.67 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.76 (d, 1H), 7.48 - 7.35 (m, 2H), 7.24 (ddd, J = 8.2, 6.5, 1.9 Hz, 1H), 5.84 (s, 2H), 4.33 – 4.22 (m, 4H), 3.76 - 3.62 (m, 4H), 3.15 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 6.4 Hz, 2H), 2.37 (s, 3H), 2.10 – 1.98 (m, 3H), 1.91 – 1.79 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 0.95 - 0.85 (m, 11H), 0.06 (s, 6H), -0.12 (s, 9H).

Step E: ethyl 5-(3-hydroxypropyl)-2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[533] To a solution of the product from Step D (6.72 g, 8.9 mmol, 1 eq) in 1,4-dioxane (400 mL) was added hydrochloric acid (4M in dioxane; 67 mL, 267 mmol, 30 eq) and the mixture was stirred at ambient temperature for 1 h. The reaction cooled to 0 °C and neutralised with 1N aqueous sodium hydroxide (300 mL), then partitioned between ethyl acetate and water, and the organic phase was dried (magnesium sulfate) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 120 g RediSep™ silica cartridge) eluting with a gradient of 0 – 80% ethyl acetate in *iso*-heptane gave a solid that was triturated with diethyl ether, filtered and dried under vacuum to afford the desired product as a white solid (3.87 g, 6.04 mmol, 68%). LC/MS ($C_{30}H_{40}N_6O_4SiS_2$) 641 $[M+H]^+$; RT 2.80 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.83 (dd, J = 7.6, 1.1 Hz, 1H), 7.48 - 7.37 (m, 2H), 7.23 (ddd, J = 8.3, 6.7, 1.8 Hz, 1H), 5.85 (s, 2H), 4.56 (t, J = 5.1 Hz, 1H), 4.33 – 4.22 (m, 4H), 3.72 (dd, J = 8.6, 7.3 Hz, 2H), 3.48 (td, J = 6.3, 5.1 Hz, 2H), 3.17 - 3.08 (m, 2H), 2.88 (t, J = 6.4 Hz, 2H), 2.38 (s, 3H), 2.11 – 1.99 (m, 2H), 1.87 - 1.75 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 0.96 – 0.86 (m, 2H), -0.11 (s, 9H).

Preparation 4c: *tert*-Butyl *N*-[3-(3-fluoro-4-hydroxy-phenyl)prop-2-ynyl]-*N*-methyl-carbamate

[534] Using **Sonogashira General Procedure** starting from 10.00 g of 2-fluoro-4-iodo-phenol (42.0 mmol, 1 eq.) as the appropriate phenol and 10.67 g of *tert*-butyl *N*-methyl-*N*-prop-2-ynyl-carbamate (63.1 mmol, 1.5 eq.) as the alkyne, 10.8 g (92%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.32 (s, 1 H), 7.22 (brd, 1H), 7.08 (dm, 1H), 6.92 (dd, 1H), 4.21 (s, 2H), 2.85 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 150.8, 146.4, 129.0, 119.6, 118.4, 113.2, 84.4, 82.7, 38.5, 33.8, 28.5; HRMS-ESI (m/z): $[M-C_4H_8+H]^+$ calcd for $C_{11}H_{11}FNO_3$: 224.0717, found 224.0720.

Preparation 7: *tert*-butyl-diphenyl-[2-[[3,5-dimethyl-7-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]oxy]ethoxy]silane

Step A*: *3-bromo-5,7-dimethyladamantane-1-carboxylic acid

[535] After stirring iron (6.7 g, 120 mmol) in bromine (30.7 mL, 600 mmol, 5 eq) at 0 °C for 1 h, 3,5-dimethyladamantane-1-carboxylic acid (25 g, 1 eq) was added and the reaction mixture was stirred at rt for 2 days. After the addition of EtOAc, the reaction mixture was treated carefully with a saturated solution of sodium-thiosulfate at 0 °C and stirred for 15 min. After filtration through a pad of Celite and rinsing with EtOAc, the organic phase was separated, washed with a saturated solution of sodium-thiosulfate and brine, dried, concentrated to give the desired product (34.28 g, 74.6%), which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 12.33 (br., 1H), 2.21 (s, 2H), 1.96/1.91 (d+d, 4H), 1.50/1.43 (d+d, 4H), 1.21/1.14 (dm+dm, 2H), 0.86 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 176.8, 66.8, 54.0, 48.7, 48.5, 45.7, 43.3, 35.5, 29.4; HRMS-ESI (m/z): [M-H]- calcd for $C_{13}H_{18}BrO_2$: 285.0496; found 285.0498.

Step B*: *3-bromo-5,7-dimethyl-1-adamantyl-methanol

[536] To the product from Step A (34.3 g, 119 mmol) in THF (77.6 mL) was added slowly a 1 M solution of BH_3-THF in THF (358 mL, 3 eq) and the reaction mixture was stirred for 18 h. After the addition of methanol and stirring for 30 min, purificationby column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (16.19 g, 49.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.51 (t, 1H), 3.05 (d, 2H), 1.91 (s, 2H), 1.91 (s, 4H), 1.19/1.09 (d+d, 2H), 1.19/1.05 (d+d, 4H), 0.85 (s, 6H) ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 70.4, 68.9, 54.9, 49.8, 49.3, 43.8, 41.4, 35.7, 29.7; HRMS-ESI (m/z): [M-Br]- calcd for $C_{13}H_{21}O$: 193.1598 found: 193.1589.

Step C*: *1-[3-bromo-5,7-dimethyl-1-adamantyl]methyl]pyrazole

[537] To the product from Step B (16.19 g, 59.26 mmol) and 1*H*-pyrazole (4.841 g, 1.2 eq) in toluene (178 mL) was added cyanomethylenetributylphosphorane (18.64 mL, 1.2 eq) in one portion and the reaction mixture was stirred at 90 °C for 2 h. Purificationby column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (17.88 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.63 (d, 1H), 7.43 (d, 1H), 6.23 (t, 1H), 3.90 (s, 2H), 1.92-1.02 (m, 12H), 0.83 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.0, 131.8, 105.2, 67.7, 61.4, 54.4/48.8/44.6, 50.4, 35.7, 29.6; HRMS-ESI (m/z): [M]+ calcd for $C_{16}H_{23}BrN_2$: 322.1045 found: 322.1014.

Step D*: *5-methyl-1-[[-3-bromo-5,7-dimethyl-1-adamantyl]methyl]pyrazole

[538] To the solution of the product from Step C (17.88 g, 55.3 mmol) in THF (277 mL) was added butyllithium (2.5 M in THF, 66 mL, 3 eq) at -78 °C, then after 1 h, iodomethane (17.2 mL, 5 eq) was added. After 10 min, the reaction mixture was quenched with a saturated solution of NH_4Cl, extracted with EtOAc and the combined organic layers were dried and concentrated to give the desired product (18.7 g, 100%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.31 (d, 1H), 6.00 (d, 1H), 3.79 (s, 2H), 2.23 (s, 3H), 2.01 (s, 2H), 1.89/1.85 (d+d, 4H), 1.23/1.15 (d+d, 4H), 1.16/1.05 (d+d, 2H), 0.83 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.2, 138.0, 105.2, 67.8, 57.8, 54.4, 50.6, 48.8, 44.8, 41.5, 35.7, 29.6, 11.8; HRMS-ESI (m/z): [M+H]+ calcd for $C_{17}H_{26}BrN_2$: 337.1279 found: 337.1289.

Step E: 2-[[-3,5-dimethyl-7-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]oxy]ethanol

[539] The mixture of the product from Step D (18.7 g, 55.3 mmol), ethylene glycol (123 mL, 40 eq), and DIPEA (48.2 mL, 5 eq) was stirred at 120 °C for 6 h. After the reaction mixture was diluted with water and extracted with EtOAc, the combined organic layers were dried and concentrated to give the desired product (18.5 g, 105%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.29 (d, 1H), 5.99 (d, 1H), 4.45 (t, 1H), 3.78 (s, 2H), 3.39 (q, 2H), 3.32 (t, 2H), 2.23 (s, 3H), 1.34 (s, 2H), 1.27/1.21 (d+d, 4H), 1.13/1.07 (d+d, 4H), 1.04/0.97 (d+d, 2H), 0.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.0, 137.8, 105.1, 74.0, 62.1, 61.5, 58.5, 50.1, 47.0, 46.1, 43.3, 39.7, 33.5, 30.2, 11.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{19}H_{31}N_2O_2$: 319.2386 found: 319.2387.

Step F: tert-butyl-diphenyl-[2-[[-3,5-dimethyl-7-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]oxy]ethoxy]silane

[540] To the mixture of the product from Step E (17.6 g, 55.3 mmol) and imidazole (5.65 g, 1.5 eq) in DCM (150 ml) was added *tert*-butyl-chloro-diphenyl-silane (18.6 g, 1.2 eq) and the

reaction mixture was stirred for 1 h. Purificationby column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (27.0 g, 87.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.72-7.34 (m, 10H), 7.29 (d, 1H), 5.99 (br., 1H), 3.78 (s, 2H), 3.67 (t, 2H), 3.44 (t, 2H), 2.21 (s, 3H), 1.33 (s, 2H), 1.26/1.18 (d+d, 4H), 1.12/1.06 (d+d, 4H), 1.03/0.96 (d+d, 2H), 0.98 (s, 9H), 0.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.0, 137.8, 105.1, 74.2, 64.4, 61.7, 58.5, 50.0, 46.9, 46.0, 43.4, 39.6, 33.5, 30.1, 27.1, 19.3, 11.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{35}H_{49}N_2O_2Si$: 557.3563 found: 557.3564.

Step G: tert-butyl-diphenyl-[2-[[3-[(4-iodo-5-methyl-pyrazol-1-yl)methyl]-5,7-dimethyl-1-adamantyl]oxy]ethoxy]silane

[541] To the solution of the product from Step F (27.0 g, 48.56 mmol) in DMF (243 mL) was added *N*-iodosuccinimide (13.6 g, 1.25 eq) and the reaction mixture was stirred for 2 h. After the dilution with water, the mixture was extracted with DCM. The combined organic layers were washed with saturated solution of sodium-thiosulphate and brine, dried, and concentrated to afford the desired product (30.1g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.68-7.37 (m, 10H), 7.45 (s, 1H), 3.89 (s, 2H), 3.67 (t, 2H), 3.44 (t, 2H), 2.23 (s, 3H), 1.30 (s, 2H), 1.26/1.17 (d+d, 4H), 1.12/1.05 (d+d, 4H), 1.00/0.96 (d+d, 2H), 0.98 (s, 9H), 0.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 142.5, 140.8, 133.7, 64.4, 61.7, 60.3, 59.9, 49.9, 46.8, 45.9, 43.2, 39.7, 33.5, 30.1, 27.1, 19.3, 12.2; HRMS-ESI (m/z): [M+H]+ calcd for $C_{35}H_{48}IN_2O_2Si$: 683.2530 found: 683.2533.

Step H: tert-butyl-diphenyl-[2-[[3,5-dimethyl-7-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]oxy]ethoxy]silane

[542] To the product from Step G (17.5 g, 25.6 mmol) in THF (128 mL) was added chloro(isopropyl)magnesium-LiCl (1.3 M in THF, 24 mL, 1.2 eq) at 0 °C, stirred for 40 min, treated with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (15.7 mL, 3 eq), and the reaction mixture was stirred for 10 min. After dilution with a saturated solution NH_4Cl and extraction with EtOAc, the combined organic phases were concentrated and was purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (15.2g, 86.9%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.65 (dm, 4H), 7.47 (s, 1H), 7.45 (tm, 2H), 7.40 (tm, 4H), 3.80 (s, 2H), 3.66 (t, 2 H), 3.44 (t, 2H), 2.35 (s, 3H), 1.35-0.94 (m, 12H), 1.24 (s, 12H), 0.97 (s, 9H), 0.83 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 146.9, 144.3, 135.6, 130.2, 128.2, 104.7, 83.0, 74.2, 64.4, 61.7, 58.4, 30.1, 27.1, 25.2, 19.3, 12.0; HRMS-ESI (m/z): [M+H]+ calcd for $C_{41}H_{60}BN_2O_4Si$: 683.4415 found: 683.4423.

Preparation 8: *tert*-butyl- [3-[3,5-dimethyl-7-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]propoxy]-diphenyl-silane

Step A*: *1-[[3-allyl-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazole

[543] To the product of Step D of **Preparation 7** (15.66 g, 46.43 mmol) and AgOTf (597 mg, 0.05 eq) in THF (232 mL) was added a 2 M solution of allyl-Mg-Cl in THF (46.4 mL, 2 eq) and the reaction mixture was stirred for 0.5 h. After quenching with a saturated solution of NH_4Cl and extracting with EtOAc, the combined organic phases were concentrated and purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (11.32 g, 81.7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.27 (d, 1H), 5.98 (m, 1H), 5.76 (m, 1H), 5.01/4.96 (dm+dm, 2H), 3.73 (s, 2H), 2.22 (s, 3H), 1.83 (d, 2H), 1.15-0.93 (m, 12H), 0.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.0, 137.7, 135.0, 117.7, 105.0, 59.0, 47.8, 44.2, 35.0, 31.8, 30.6, 11.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{20}H_{31}N_2$: 299.2487 found: 299.2485.

Step B: 3-[3,5-dimethyl-7-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]propan-1-ol

[544] To the product of Step A (10.2 g, 34.17 mmol), in THF (85 mL) was added a 1 M solution of BH_3-THF in THF (85.4 mL, 2 eq) and the reaction mixture was stirred for 1 h. After treatment with a 10 M solution of NaOH (24 mL, 7 eq) and a 33 % solution of hydrogen peroxide (73 mL, 25 eq) at 0 °C, the reaction wasstirred at rt for 1 h. Then, it was quenched with aqueous HCl solution, extracted with EtOAc, and purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (9.75 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.28 (d, 1H), 5.98 (m, 1H), 4.33 (t, 1H), 3.73 (s, 2H), 3.32 (m, 2H), 2.22 (brs, 3H), 1.32 (m, 2H), 1.12-0.92 (m, 12H), 1.06 (m, 2H), 0.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 137.7, 105.0, 62.1, 59.1, 39.7, 30.7, 26.5, 11.9, HRMS-ESI (m/z): [M+H]+ calcd for $C_{20}H_{33}N_2O$: 317.2593 found: 317.2590

Step C: tert-butyl-[3-[3,5-dimethyl-7-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]propoxy]-diphenyl-silane

[545] To the product of Step B (9.75 g, 30.8 mmol) and imidazole (3.1 g, 1.5 eq) in DCM (92 ml) was added *tert-butyl*-chloro-diphenyl-silane (9.45 mL, 1.2 eq) and the reaction mixture was stirred for 1 h. Purificationby column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (12.5 g, 73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.63-7.39 (m, 10H), 7.27 (d, 1H), 5.98 (d, 1H), 3.72 (s, 2H), 3.59 (t, 2H), 2.21 (s, 3H), 1.42 (m, 2H), 1.1-0.92 (br., 12H), 1.09 (m, 2H), 0.98 (s, 9H), 0.77 (s, 6H); ^{13}C NMR (100

MHz, DMSO-d_6) δ ppm 137.7, 105.0, 64.8, 59.1, 39.3, 38.0, 34.2, 31.8, 30.6, 27.2, 26.1, 19.2, 11.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{36}H_{51}N_2OSi$: 555.3771 found: 555.3770.

Step D*: *tert-butyl-[3-[3-[(4-iodo-5-methyl-pyrazol-1-yl)methyl]-5,7-dimethyl-1-adamantyl] propoxy]-diphenyl-silane

[546] To the product of Step C (12.5 g, 22.54 mmol) in DMF (112 mL) was added *N*-iodosuccinimide (6.34 g, 1.25 eq) and the reaction mixture was stirred for 2 h. After quenching with a saturated solution of sodium thiosulfate and extraction with DCM, the combined organic phases were washed with saturated sodium thiosulphate and brine, dried, and evaporated to afford the desired product (16.3 g, 105%). LC/MS ($C_{36}H_{50}IN_2OSi$) 681 $[M+H]^+$.

Step E: tert-butyl-[3-[3,5-dimethyl-7-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]propoxy]-diphenyl-silane

[547] To the product of Step D (16.25 g, 23.9 mmol) in THF (119 mL) was added chloro(isopropyl)magnesium-LiCl (1.3 M in THF, 22 mL, 1.2 eq.) at 0 °C, the mixture was stirred for 40 min, treated with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (14.6 mL, 3 eq), and stirred for 10 min. After dilution with a saturated solution NH_4Cl and extraction with EtOAc, the combined organic phases were concentrated and was purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (11.4 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.59 (d, 4H), 7.46 (s, 1H), 7.45 (t, 2H), 7.43 (t, 4H), 3.74 (s, 2H), 3.59 (t, 2H), 2.35 (s, 3H), 1.41 (qn, 2H), 1.24 (s, 12H), 1.09 (m, 2H), 1.08 (s, 4H), 1.05 (s, 2H), 0.98 (s, 9H), 0.98 (s, 2H), 0.94 (s, 4H), 0.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 146.9, 144.2, 135.5, 133.8, 130.3, 128.3, 104.6, 83.0, 64.7, 64.7, 59.0, 50.6, 48.2, 46.5, 44.1, 39.2, 37.9, 31.8, 30.7, 27.2, 26.1, 25.2, 19.2, 12.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{42}H_{62}BN_2O_3Si$: 681.4623 found: 681.4631.

Preparation 10: methyl 3-bromo-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-l)propylamino]pyridine-2-carboxylate

Step A*: *methyl 6-[bis(tert-butoxycarbonyl)amino]-3-bromo-pyridine-2-carboxylate

[548] To methyl 6-amino-3-bromo-pyridine-2-carboxylate (25.0 g, 108.2 mmol) and DMAP (1.3 g, 0.1 eq) in DCM (541 mL) was added Boc2O (59.0 g, 2.5 eq) at 0 °C and the reaction mixture was stirred for 2.5 h. After the addition of a saturated solution of $NaHCO_3$ and extraction with DCM, the combined organic phases were dried and concentrated to afford the desired product (45.0 g, 72.3%). LC/MS ($C_{17}H_{23}BrN_2O_6Na$) 453 $[M+Na]^+$.

Step B*: *methyl 3-bromo-6-(tert-butoxycarbonylamino)pyridine-2-carboxylate

[549] To the product from Step A (42.7 g, 74.34 mmol) in DCM (370 mL) was added TFA (17.1 mL, 3 eq) at 0 °C and the reaction mixture was stirred for 18 h. After washing with a saturated solution of $NaHCO_3$ and brine, the combined organic phases were dried, concentrated, and purified by column chromatography (silica gel, heptane and EtOAc as eluents) to give the desired product (28.3 g, 115.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.29 (s, 1H), 8.11 (d, 1H), 7.88 (d, 1H), 3.87 (s, 3H), 1.46 (s, 9H) ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.6, 153.1, 151.8/148.3, 143.5, 116.3, 109.2, 53.2, 28.4. LC/MS ($C_{12}H_{15}BrN_2O_4Na$) 353 $[M+Na]^+$.

Step C*: *methyl 3-bromo-6-[tert-butoxycarbonyl-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propyl]amino]pyridine-2-carboxylate

[550] To the product from Step B (10.0 g, 30.1967 mmol) in acetone (150 mL), were added Cs_2CO_3 (29.5 g, 3 eq) and 3,6-dichloro-4-(3-iodopropyl)-5-methyl-pyridazine (9.9 g, 1 eq) and the reaction mixture was stirred for 18 h. After dilution with water and extraction with EtOAc, the combined organic phases were washed with brine, dried and concentrated to give the desired product (17.5 g, 108%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.13 (d, 1H), 7.78 (d, 1H), 3.91 (t, 2H), 3.89 (s, 3H), 2.79 (m, 2H), 2.38 (s, 3H), 1.82 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.3, 157.6, 156.6, 153.2, 152.9, 147.2, 143.1, 142.2, 139.7, 122.6, 111.8, 82.2, 53.3, 46.4, 28.1, 27.7, 26.5, 16.3; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{20}H_{23}BrCl_2N_4NaO_4$: 555.0177 found: 555.0172.

Step D: methyl 3-bromo-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-l)propylamino]pyridine-2-carboxylate

[551] The product from Step C (17.5 g, 32.7 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (330 mL) was stirred at 110 °C for 18 h. Purificationby column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (9.9 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.63 (d, 1H), 7.22 (t, 1H), 6.57 (d, 1H), 3.83 (s, 3H), 3.30 (m, 2H), 2.83 (m, 2H), 2.37 (s, 3H), 1.74 (m, 2H) ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.5, 141.5, 112.6, 52.9, 40.9, 28.0, 27.0, 16.4.

Preparation 11: (4-methoxyphenyl)methyl 3-bromo-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylate

Step A: 3-bromo-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylic acid

[552] The mixture of the product from **Preparation 10** (35.39 g, 81.52 mmol) and $LiOH \times H_2O$ (13.68 g, 4 eq) in 1,4-dioxane (408 mL) and water (82 mL) was stirred at 60 °C for 1 h. After quenching with a 1 M solution of HCl and extraction with EtOAc, the combined organic phases were dried, concentrated, and purified by flash chromatography (silica gel, using DCM and MeOH as eluents) to give the desired product (27.74 g, 81%). LC/MS ($C_{14}H_{14}BrCl_2N_4O_2$) 421 $[M+H]^+$.

Step B: (4-methoxyphenyl)methyl 3-bromo-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylate

[553] To the product of Step A (27.7 g, 65.9 mmol), (4-methoxyphenyl)methanol (16.4 mL, 2 eq), and PPh_3 (34.6 g, 2 eq) in toluene (660 mL) and THF (20 ml) was added dropwise diisopropyl azodicarboxylate (26 mL, 2 eq) and the reaction mixture was stirred at 50 °C for 1 h. Purificationby flash chromatography (silica gel, using heptane and EtOAc as eluents) afforded the desired product (23.65 g, 66.4%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.62 (d, 1H), 7.37 (dn, 2H), 7.21 (t, 1H), 6.91 (dm, 2H), 6.56 (d, 1H), 5.25 (s, 2H), 3.74 (s, 3H), 3.30 (q, 2H), 2.81 (m, 2H), 2.33 (s, 3H), 1.73 (m, 2H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 165.9, 159.7, 157.6, 157.5, 156.8, 148.0, 142.7, 141.5, 139.7, 130.6, 127.8, 114.3, 112.6, 101.6, 67.0, 55.6, 40.9, 28.0, 27.1, 16.4; HRMS-ESI (m/z): [M+H]+ calcd for $C_{22}H_{22}BrCl_2N_4O_3$: 539.0252, found: 539.0246.

Preparation 12: methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(*p*-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

Step A: methyl 6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]-3-[5-methyl-1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylate

[554] The mixture of the product from **Preparation 10** (15.0 g, 34.55 mmol), the product from **Preparation 7** (30.7 g, 1.3 eq), Cs_2CO_3 (33.8 g, 3.0 eq), and $Pd(AtaPhos)_2Cl_2$ (1.53 g, 0.1 eq) in 1,4-dioxane (207 mL) and H_2O (34.5 mL) was stirred at 80 °C for 1.5 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (18.5 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.69-7.37 (m, 10H), 7.32 (d, 1H), 7.23 (s, 1H), 6.98 (t, 1H), 6.63 (d, 1H), 3.82 (s, 2H), 3.67 (t, 2H), 3.58 (s, 3H), 3.46 (t, 2H), 3.35 (m, 2H), 2.86 (m, 2H), 2.40 (s, 3H), 2.06 (s, 3H), 1.78 (m, 2H), 1.35 (s, 2H), 1.27/1.2 (m+m, 4H), 1.15/1.09 (m+m, 4H), 1.05/0.97 (m+m, 2H), 0.97 (s, 9H), 0.84 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{50}H_{63}Cl_2N_6O_4Si$: 909.4057 found: 909.4053.

Step B*: *methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[5-methyl-1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylate

[555] The mixture of the product from Step A (18.5 g, 20.3 mmol), Cs_2CO_3 (13.2 g, 2 eq), DIPEA (7.1 mL, 2 eq), and $Pd(Ataphos)_2Cl_2$ (900 mg, 0.1 eq) in 1,4-dioxane (102 mL) was stirred at 110 °C for 18 h. After filtration and concentration, the residue was taken up with DCM, washed with water, and purified by column chromatography (silica gel, DCM and EtOAc as eluents) to give the desired product (12.6 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.85 (d, 1H), 7.69 (d, 1H), 7.66 (dm, 4H), 7.47-7.36 (m, 6H), 7.38 (s, 1H), 3.97 (t, 2H), 3.87 (s, 2H), 3.68 (t, 2H), 3.66 (s, 3H), 3.47 (t, 2H), 2.87 (t, 2H), 2.30 (s, 3H), 2.14 (s, 3H), 1.99 (br., 2H), 1.38 (s, 2H), 1.32-0.96 (br., 10H), 0.98 (s, 9H), 0.85 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.9, 137.6, 120.5, 64.4, 61.7, 58.9, 52.3, 46.0, 43.4, 30.2, 27.1, 24.6, 21.0, 15.5, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{50}H_{62}ClN_6O_4Si$: 873.4290 found: 873.4291.

Step C*: *methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[556] To the product from Step B (8.46 g, 9.68 mmol) in THF (95 mL) was added a 1 M solution of TBAF in THF (10.6 mL, 1.1 eq) at 0 °C and the reaction mixture was stirred for 2 h. After quenching with a saturated solution of NH_4Cl and extraction with EtOAc, the combined organic phases were washed with brine, dried, and purified by column chromatography (silica gel, DCM and MeOH as eluents) to give the desired product (5.38g, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.86 (d, 1H), 7.71 (d, 1H), 7.38 (s, 1H), 4.46 (t, 1H), 3.97 (t, 2H), 3.87 (s, 2H), 3.70 (s, 3H), 3.40 (m, 2H), 3.35 (t, 2H), 2.87 (t, 2H), 2.30 (s, 3H), 2.15 (s, 3H), 1.99 (m, 2H), 1.42-0.95 (m, 12H), 0.87 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{34}H_{44}ClN_6O_4$: 635.3113 found: 635.3112.

Step D*: *methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[557] Using **Buchwald General Procedure I** at 130 °C for 1 h, starting from 3.7 g of the product from Step C (5.78 mmol) and 1.74 g of 1,3-benzothiazol-2-amine (2 eq), 3.1 g of the desired product (72% Yield) were obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.96 (d, 1H), 7.82 (br., 1H), 7.70 (d, 1H), 7.50 (br., 1H), 7.38 (s, 1H), 7.35 (t, 1H), 7.17 (t, 1H), 4.46

(br., 1H), 4.00 (t, 2H), 3.88 (s, 2H), 3.70 (s, 3H), 3.40 (brt., 2H), 3.35 (t, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.16 (s, 3H), 2.03-1.94 (m, 2H), 1.42-0.96 (m, 12H), 0.87 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.8, 137.5, 126.4, 122.4, 122.1, 119.0, 62.1, 61.5, 59.0, 52.6, 45.4, 30.2, 24.3, 21.7, 12.6, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{41}H_{49}N_8O_4S$: 749.3597 found: 749.3595.

Step E*: *methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[558] To the product from Step D (3.85 g, 5.14 mmol) and triethylamine (2.15 mL, 3 eq) in DCM (50 mL) was added *p*-tolylsulfonyl 4-methylbenzenesulfonate (2.51 g, 1.5 eq) and the reaction mixture was stirred for 1 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (3.2 g, 69%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.96 (d, 1H), 7.81 (br., 1H), 7.77 (d, 2H), 7.70 (d, 1H), 7.50 (br., 1H), 7.46 (d, 2H), 7.39 (s, 1H), 7.35 (t, 1H), 7.17 (t, 1H), 4.06 (t, 2H), 4.00 (t, 2H), 3.85 (s, 2H), 3.69 (s, 3H), 3.49 (t, 2H), 2.86 (t, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.15 (s, 3H), 1.99 (m, 2H), 1.32-0.93 (m, 12H), 0.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 139.8, 137.6, 130.6, 128.1, 126.4, 122.4, 122.1, 119, 71.5, 58.8, 58.4, 52.6, 45.4, 30.1, 24.3, 21.7, 21.6, 12.6, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{48}H_{55}N_8O_6S_2$: 903.3686 found: 903.3685.

Preparation 13: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[3-(p-tolylsulfonyloxy)propyl]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

Step A*: (*4-methoxyphenyl)methyl 3-[1-[[3-[3-[tert-butyl(diphenyl)silyl]oxypropyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylate

[559] The mixture of the product from **Preparation 11** (3.67 g, 6.79 mmol), the product from **Preparation 8** (5.09 g, 1.1 eq), $Pd(AtaPhos)_2Cl_2$ (301 mg, 0.1 eq), and Cs_2CO_3 (6.64 g, 3 eq) in 1,4-dioxane (41 mL) and H_2O (6.8 mL) was stirred at 80 °C for 18 h. Purificationby column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (4.43 g, 64%).^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.62-7.38 (m, 10H), 7.32 (d, 1H), 7.26 (s, 1H), 7.10 (m, 2H), 6.98 (t, 1H), 6.83 (m, 2H), 6.63 (d, 1H), 4.98 (s, 2H), 3.74 (s, 2H), 3.70 (s, 3H), 3.58 (t, 2H), 3.35 (m, 2H), 2.84 (m, 2H), 2.34 (s, 3H), 2.02 (s, 3H), 1.77 (m, 2H), 1.43 (m, 2H), 1.18-0.85 (m, 12H), 1.09 (t, 2H), 0.97 (s, 9H), 0.77 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{58}H_{71}Cl_2N_6O_4Si$: 1013.4683 found: 1013.4683;

Step B*: *(4-methoxyphenyl)methyl 3-[1-[[3-[3-[tert-butyl(diphenyl)silyl]oxypropyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)pyridine-2-carboxylate

[560] The mixture of the product from Step A (4.43 g, 4.37 mmol), Cs_2CO_3 (2.84 g, 2 eq), DIPEA (1.5 mL, 2 eq) and $Pd(Ataphos)_2Cl_2$ (193 mg, 0.1 eq) in 1,4-dioxane (22 mL) was stirred at 110 °C for 18 h. After quenching with water and extracting with EtOAc, the combined organic phases were dried, concentrated, and purified by column chromatography (silica gel, DCM and EtOAc as eluents) to give the desired product (2.83 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.84 (d, 1H), 7.68 (d, 1H), 7.59 (d, 4H), 7.44 (t, 2H), 7.42 (t, 4H), 7.38 (s, 1H), 7.14 (d, 2H), 6.87 (d, 2H), 5.07 (s, 2H), 3.96 (t, 2H), 3.78 (s, 2H), 3.71 (s, 3H), 3.59 (t, 2H), 2.86 (t, 2H), 2.29 (s, 3H), 2.08 (s, 3H), 1.97 (qn, 2H), 1.43 (qn, 2H), 1.12 (s, 4H), 1.10 (s, 2H), 1.09 (t, 2H), 0.97 (s, 9H), 0.95 (s, 2H), 0.94/0.91 (d+d, 4H), 0.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.9, 159.6, 156.3, 153.6, 150.8, 147.7, 140.1, 137.5, 137.3, 136.0, 135.5, 133.8, 130.3, 130.1, 129.1, 128.3, 127.6, 123.1, 120.5, 115.5, 114.3, 66.8, 64.8, 64.8, 59.6, 55.6, 50.5, 48.1, 46.4, 46.0, 44.2, 39.3, 38.1, 31.7, 30.6, 27.2, 26.1, 24.6, 21.0, 19.3, 15.5, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{58}H_{70}ClN_6O_4Si$: 977.4916 found: 977.4915.

Step C: (4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3-(3-hydroxypropyl)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[561] To the product from Step B (2.83 g, 2.89 mmol) in THF (95 mL) was added a 1 M solution of TBAF in THF (3.2 mL, 1.1 eq) at 0 °C and the reaction mixture was stirred for 2 h. After quenching with a saturated solution of NH_4Cl and extracted with EtOAc, the combined organic phases were washed with brine, dried, concentrated, and purified by column chromatography (silica gel, DCM and MeOH as eluents) to give the desired product (2.21 g, 103%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.85 (d, 1H), 7.70 (d, 1H), 7.39 (s, 1H), 7.17 (d, 2H), 6.90 (d, 2H), 5.09 (s, 2H), 4.34 (t, 1H), 3.96 (t, 2H), 3.79 (s, 2H), 3.74 (s, 3H), 3.32 (q, 2H), 2.86 (t, 2H), 2.29 (s, 3H), 2.09 (s, 3H), 1.98 (qn, 2H), 1.34 (qn, 2H), 1.13 (s, 2H), 1.13 (s, 4H), 1.06 (t, 2H), 0.99/0.95 (d+d, 4H), 0.97 (s, 2H), 0.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.9, 159.7, 156.4, 153.6, 150.8, 147.7, 140.2, 137.5, 137.3, 136.0, 130.2, 129.1, 127.6, 123.1, 120.4, 115.5, 114.3, 66.8, 66.8, 62.1, 59.7, 55.6, 50.6, 48.2, 46.5, 46.0, 44.3, 39.7, 38.1, 31.8, 30.6, 26.5, 24.6, 21.0, 15.5, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{42}H_{52}ClN_6O_4$: 739.3739 found: 739.3739.

Step D*: *(4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(3-hydroxypropyl)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[562] The mixture of the product from Step C (1.71 g, 2.31 mmol), 1,3-benzothiazol-2-amine (695 mg, 2 eq), Pd_2dba_3 (212 mg, 0.1 eq), XantPhos (268 mg, 0.2 eq), and DIPEA (1.2 mL, 3 eq) in cyclohexanol (14 mL) was stirred at 130 °C for 1 h. Purification by column chromatography (silica gel, heptane, DCM and MeCN as eluents) afforded the desired product (1.25g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 12.08/10.87 (brs/brs, 1H), 7.95 (d, 1H), 7.81 (br, 1H), 7.68 (d, 1H), 7.50 (br, 1H), 7.39 (s, 1H), 7.35 (t, 1H), 7.18 (d, 2H), 7.17 (t, 1H), 6.90 (d, 2H), 5.10 (s, 2H), 4.34 (t, 1H), 3.99 (t, 2H), 3.79 (s, 2H), 3.74 (s, 3H), 3.33 (q, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 2.11 (s, 3H), 1.98 (qn, 2H), 1.34 (qn, 2H), 1.14 (s, 4H), 1.14 (s, 2H), 1.07 (t, 2H), 1.00/0.95 (d+d, 2H), 0.99/0.95 (d+d, 4H), 0.79 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 140.0, 137.6, 130.2, 126.4, 122.4, 122.0, 119.0, 114.3, 66.7, 62.1, 59.6, 55.6, 50.6, 48.2, 46.5, 45.4, 44.3, 39.7, 30.6, 26.5, 24.3, 21.7, 12.6, 11.0; HRMS-ESI (m/z): [M+H]+ calcd for $C_{49}H_{57}N_8O_4S$: 853.4223 found: 853.4229.

Step E: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[3-(p-tolylsulfonyloxy)propyl]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[563] To the product from Step D (1.25 g, 1.47 mmol) and triethylamine (0.61 mL, 3 eq) in DCM (15 mL) was added *p*-tolylsulfonyl 4-methylbenzenesulfonate (717 mg, 1.5 eq) and the reaction mixture was stirred for 1 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded 800 mg (54%) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.95 (d, 1H), 7.88 (brs, 1H), 7.77 (m, 2H), 7.68 (d, 1H), 7.62 (brs, 1H), 7.47 (m, 2H), 7.39 (s, 1H), 7.35 (brs, 1H), 7.17 (brs, 1H), 7.10 (m, 2H), 6.90 (m, 2H), 5.09 (s, 2H), 4.00 (m, 2H), 3.98 (t, 2H), 3.77 (s, 2H), 3.74 (s, 3H), 2.85 (t, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.09 (s, 3H), 1.98 (m, 2H), 1.45 (m, 2H), 1.17-0.8 (m, 12H), 0.98 (m, 2H), 0.77 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{56}H_{63}N_8O_6S_2$: 1007.4312 found: 1007.4318.

Preparation 15: ethyl 2-(3-chloro-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-c]pyridazin-8-yl)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

Step A*: *2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylic acid

[564] The mixture of the product from **Preparation 3a** (35.39 g, 81.52 mmol) and $LiOH \times H_2O$ (4 eq) in 1,4-dioxane (408 mL) and water (82 mL) was stirred at 60 °C for 1 h. After quenching with a 1 M solution of HCl and extraction with EtOAc, the combined organic phases were dried, concentrated, and purified by flash chromatography (silica gel, using DCM and MeOH as eluents) to give the desired product (27.7 g, 81%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.56 (dd, 1H), 7.43 (brd., 1H), 6.96 (t, 1H), 4.18 (t, 2H), 4.05 (t, 2H), 3.28 (t, 2H), 2.84 (t, 2H), 2.29 (s, 3H), 2.07 (m, 2H), 1.97 (m, 2H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 166.4, 154.8, 152.1, 151.8, 151.1, 147.1, 143.9, 135.7, 134.0, 133.8, 129.0, 124.9, 117.6, 82.3, 68.8, 46.3, 31.0, 24.0, 22.5, 19.8, 15.7; HRMS-ESI (m/z): [M+H]+ calcd for $C_{21}H_{20}ClFIN_4O_3S$: 588.9973 found: 588.9969.

Step B*: *ethyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

[565] To the mixture of the product of Step A (27.7 g, 65.9 mmol), ethanol (2 eq) and PPh_3 (2 eq) in toluene (660 mL) and THF (20 ml) was added dropwise diisopropyl azodicarboxylate (2 eq) and the reaction was stirred at 50 °C 1 h. Purification by flash chromatography (silica gel, using heptane and EtOAc as eluents) afforded the desired product (23.65 g, 66.4%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.59 (dd, 1H), 7.44 (dm, 1H), 6.98 (t, 1H), 4.29 (m, 2H), 4.25 (q, 2H), 4.08 (t, 2H), 3.24 (t, 2H), 2.89 (t, 2H), 2.32 (s, 3H), 2.09 (m, 2H), 2.04 (m, 2H), 1.28 (t, 3H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 162.6, 155.4, 152.2, 151.7, 151.3, 147.0, 134.0, 124.9, 117.6, 82.4, 68.3, 60.7, 46.3, 30.8, 24.1, 23.1, 19.7, 15.7, 14.6; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{23}H_{24}ClFIN_4O_3S$: 617.0286, found: 617.0282.

<u>Preparation 16:</u> (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

<u>Step A:</u> 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)pyridine-2-carboxylic acid

[566] The mixture of 1.5 g (1.72 mmol) of the product of **Preparation 12, *Step B***, 290 mg (4 eq) of LiOH in 17 mL of a 4:1 mixture of THF and water was stirred at 60 °C to reach complete conversion. After the reaction was quenched by the addition of 1M aqueous HCl solution, the mixture was extracted with EtOAc and the organic phases were dried, concentrated, and purified by column chromatography (silica gel, using DCM and MeOH as

eluents) to give 1.23 g (83%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 13.11 (s, 1H), 7.80 (d, 1H), 7.66 (d, 4H), 7.65 (d, 1H), 7.44 (t, 2H), 7.41 (s, 1H), 7.40 (t, 4H), 3.99 (t, 2H), 3.86 (s, 2H), 3.68 (t, 2H), 3.47 (t, 2H), 2.87 (t, 2H), 2.29 (s, 3H), 2.17 (s, 3H), 1.99 (qn, 2H), 1.39 (s, 2H), 1.27/1.22 (d+d, 4H), 1.17/1.12 (d+d, 4H), 1.05/0.99 (d+d, 2H), 0.98 (s, 9H), 0.85 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 168.5, 156.5, 153.2, 150.7, 148.9, 139.8, 137.7, 137.3, 136.0, 135.6, 133.8, 130.2, 129.0, 128.3, 122.1, 119.9, 115.7, 74.3, 64.4, 61.7, 59.0, 50.1, 46.9, 46.0, 46.0, 43.4, 39.7, 33.6, 30.2, 27.1, 24.6, 21.0, 19.2, 15.5, 11.1; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{49}H_{60}ClN_6O_4Si$: 859.4134 found: 859.4130.

Step B:* *(4-methoxyphenyl)methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)pyridine-2-carboxylate

[567] To 1.23 g (1.43 mmol) of the product from *Step A*, 0.35 mL (2 eq) of (4-methoxyphenyl)methanol, 748 mg (2 eq) of PPh_3 in 7 mL of toluene was added 0.56 mL (2 eq) of DIAD dropwise, and the mixture was stirred at 50 °C until complete conversion. The product was purified by column chromatography (silica gel, using DCM and EtOAc as eluents) to give 1.11 g (79%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.84 (d, 1H), 7.67 (d, 1H), 7.65 (d, 4H), 7.44 (t, 2H), 7.41 (s, 1H), 7.40 (t, 4H), 7.15 (d, 2H), 6.87 (d, 2H), 5.07 (s, 2H), 3.96 (t, 2H), 3.83 (s, 2H), 3.71 (s, 3H), 3.66 (t, 2H), 3.45 (t, 2H), 2.86 (t, 2H), 2.29 (s, 3H), 2.08 (s, 3H), 1.97 (qn, 2H), 1.38 (s, 2H), 1.25/1.18 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.01/0.93 (d+d, 2H), 0.97 (s, 9H), 0.82 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 166.8, 159.7, 156.3, 153.6, 150.8, 147.7, 140.1, 137.6, 137.3, 136.0, 135.6, 133.8, 130.2, 130.2, 129.1, 128.2, 127.7, 123.0, 120.4, 115.6, 114.3, 74.2, 66.8, 64.4, 61.7, 59.3, 55.6, 49.9, 46.8, 46.0, 46.0, 43.3, 39.7, 33.6, 30.1, 27.1, 24.6, 21.0, 19.3, 15.5, 10.8; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{57}H_{68}ClN_6O_5Si$: 979.4709 found: 979.4710.

Step C:* *(4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[568] To 45.4 g (46.3 mmol) of the product from *Step B* in 470 mL of THF was added 51 mL (1.1 eq) of a 1 M solution of TBAF in THF and mixture was stirred for 2 h. After quenching with a saturated NH_4Cl solution, the mixture was extracted with EtOAc and the organic phase was dried and purified by column chromatography (silica gel, using DCM and MeOH as eluents) to give 21.6 g (63%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.85 (d, 1H), 7.70 (d, 1H), 7.39 (s, 1H), 7.18 (d, 2H), 6.90 (d, 2H), 5.10 (s, 2H), 4.45 (t, 1H), 3.96 (t, 2H), 3.84 (s, 2H), 3.74 (s, 3H), 3.40 (q, 2H), 3.33 (t, 2H), 2.86 (t, 2H), 2.29 (s,

3H), 2.09 (s, 3H), 1.98 (qn, 2H), 1.39 (s, 2H), 1.27/1.21 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.03/0.94 (d+d, 2H), 0.84 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 166.8, 159.7, 156.3, 153.6, 150.8, 147.8, 140.2, 137.6, 137.3, 136, 130.2, 129.1, 127.7, 123.0, 120.4, 115.6, 114.3, 74.0, 66.8, 62.2, 61.5, 59.0, 55.6, 50.0, 46.9, 46.0, 46.0, 43.3, 39.7, 33.5, 30.1, 24.6, 21.0, 15.5, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{41}H_{50}ClN_6O_5$: 741.3531 found: 741.3530.

Step D:* *(4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[569] The mixture of 7.1 g (9.6 mmol) of the product from *Step C*, 2.8 g (19 mmol) of 1,3-benzothiazol-2-amine, 4.8 mL (28 mmol) of *N*-ethyl-*N*-isopropyl-propan-2-amine, 861 mg (0.94 mmol) of $Pd_2(dba)_3$ and 1.1 g (1.9 mmol) of XantPhos in 66 mL of cyclohexanol was stirred at 130 °C for 2 h. The product was purified by column chromatography (silica gel, using DCM and MeOH as eluents) to give 5.71 g (63%) of desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.95 (d, 1H), 7.81 (brd, 1H), 7.69 (d, 1H), 7.49 (brs, 1H), 7.39 (s, 1H), 7.35 (m, 1H), 7.19 (m, 2H), 7.16 (m, 1H), 6.91 (m, 2H), 5.10 (s, 2H), 4.46 (t, 1H), 3.99 (m, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.40 (m, 2H), 3.34 (t, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 2.11 (s, 3H), 1.99 (m, 2H), 1.45-0.9 (m, 12H), 0.84 (s, 6H); **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{48}H_{55}N_8O_5S$: 855.4016 found: 855.4011.

Step E:* *(4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[570] To 5.0 g (5.8 mmol) of the product from *Step D* in 50 mL of dichloromethane were added 2.5 mL (3.1 eq.) of *N,N*-diethylethanamine and 2.9 g (1.5 eq) of p-tolylsulfonyl 4-methylbenzenesulfonate, then the mixture was stirred for 18 h. The product was purified by column chromatography (silica gel, using DCM and EtOAc as eluents) to give 2.95 g (50%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.95 (d, 1H), 7.81 (brs, 1H), 7.76 (m, 2H), 7.45 (brs, 1H), 7.45 (m, 2H), 7.40 (s, 1H), 7.35 (m, 1H), 7.18 (m, 2H), 7.17 (m, 1H), 6.97 (d, 1H), 6.90 (m, 2H), 5.10 (s, 2H), 4.05 (m, 2H), 4.00 (m, 2H), 3.82 (s, 2H), 3.74 (s, 3H), 3.47 (m, 2H), 2.85 (m, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.10 (s, 3H), 1.98 (m, 2H), 1.87-1.34 (m, 12H), 0.81 (s, 6H); **HRMS-ESI** (m/z): [M+Na]+ calcd for $C_{55}H_{61}N_8O_7S_2$: 1009.4105 found: 1009.4102.

Preparation 17: ***tert*-butyl-[2-[[3-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]oxy]ethoxy]-diphenyl-silane**

Step A*: *(3-bromo-1-adamantyl)methanol

[571] To 3-bromoadamantane-1-carboxylic acid (10.0 g, 38.6 mmol) in THF (25 mL) was added slowly a 1 M solution of BH3-THF in THF (115 mL, 3 eq), and the mixture was stirred for 48 h. After the addition of methanol and stirring for 30 min, purification by column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (8.37 g, 88%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 4.50 (t, 1H), 3.02 (d, 2H), 2.28/2.21 (dm+dm, 4H), 2.11 (m, 2H), 2.07 (s, 2H), 1.66/1.56 (dm+dm, 2H), 1.48/1.39 (dm+dm, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 70.9, 69.3, 51.3, 49.0, 40.6, 37.3, 35.1, 32.3.

Step B:* *1-[(3-bromo-1-adamantyl)methyl]pyrazole

[572] To the product from *Step A* (8.37 g, 34.1 mmol), 1*H*-pyrazole (2.79 g, 1.2 eq) in toluene (100 mL) was added (cyanomethylene)tributylphosphorane (10.7 mL, 1.2 eq) and the reaction mixture was stirred at 90 °C for 2 h. Purification by column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (8.50 g, 84%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.63 (dd, 1H), 7.43 (dd, 1H), 6.23 (t, 1H), 3.87 (s, 2H), 2.24/2.13 (m+m, 4H), 2.10 (m, 2H), 2.07 (s, 2H), 1.63/1.50 (m+m, 2H), 1.47/1.43 (m+m, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 138.9, 131.7, 105.1, 68.0, 61.8, 51.8, 48.5, 39.8, 38.3, 34.6, 32.1; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{14}H_{20}BrN_2$: 295.0810 found: 295.0804.

Step C*: *1-[(3-bromo-1-adamantyl)methyl]-5-methyl-pyrazole

[573] To the product from *Step B* (1.70 g, 5.76 mmol) in THF (30 mL) was added butyllithium (2.5 M in THF, 12 mL, 5 eq) at -78 °C. After 1 h, iodomethane (7.2 mL, 5 eq) was added to the mixture. After 10 min, the reaction mixture was quenched with a saturated solution of NH_4Cl, extracted with EtOAc and the combined organic layers were dried and concentrated to give the desired product (2.0 g, 112%), which was used in the next step without further purification. **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.31 (d, 1H), 6.01 (d, 1H), 3.76 (s, 2H), 2.25/2.15 (d+d, 4H), 2.24 (s, 3H), 2.16 (s, 2H), 2.10 (m, 2H), 1.63/1.52 (d+d, 2H), 1.52/1.49 (d+d, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 139.2, 138.0, 105.2, 68.2, 58.3, 52.1, 48.5, 40.5, 38.4, 34.5, 32.2, 11.8; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{15}H_{22}BrN_2$: 309.0966 found: 309.0962.

Step D*: *2-[[3-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]oxy]ethanol

[574] The mixture of the product from *Step C* (2.00 g, 6.47 mmol), ethylene glycol (14.4 mL, 40 eq), and DIPEA (5.6 mL, 5 eq) was stirred at 120 °C for 6 h. After diluting with water and extracting with EtOAc, the combined organic phases were purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (1.62 g, 86.6%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.28 (d, 1H), 5.99 (m, 1H), 4.46 (t, 1H), 3.75 (s, 2H), 3.40 (m, 2H), 3.32 (m, 2H), 2.23 (brs, 3H), 2.13 (m, 2H), 1.61/1.52 (m+m, 4H), 1.47/1.43 (m+m, 2H), 1.45 (s, 2H), 1.44-1.35 (m, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 137.8, 105.1, 61.8, 61.5, 59.0, 44.6, 40.8, 39.6, 35.7, 30.0, 11.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{17}H_{27}N_2O_2$: 291.2073 found: 291.2069.

***Step E:* tert-butyl-[2-[[3-[(5-methylpyrazol-1-yl)methyl]-1-adamantyl]oxy]ethoxy]-diphenyl-silane**

[575] To the product from *Step D* (6.52 g, 22.5 mmol) and imidazole (2.29 g, 1.5 eq) in DCM (67 ml) was added *tert*-butyl-chloro-diphenyl-silane (6.9 mL, 1.2 eq) and the reaction mixture was stirred for 1 h. Purification by column chromatography (silica gel, heptane and MTBE as eluents) afforded the desired product (11.0 g, 92.7%). **LC/MS** ($C_{33}H_{45}N_2O_2Si$) 529 $[M+H]^+$.

***Step F:* tert-butyl-[2-[[3-[(4-iodo-5-methyl-pyrazol-1-yl)methyl]-1-adamantyl]oxy]ethoxy]-diphenyl-silane**

[576] To the product from *Step E* (11.0 g, 20.8 mmol) in DMF (105 mL) was added *N*-iodosuccinimide (5.85 g, 1.25 eq.) and the reaction mixture was stirred for 3 h. After the reaction mixture was diluted with water and extracted with DCM, the combined organic phases were washed with saturated sodium thiosulphate and brine, dried, and evaporated to get the desired product (11.0 g, 81%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.70-7.36 (m, 10H), 7.44 (s, 1H), 3.86 (s, 2H), 3.67 (t, 2H), 3.45 (t, 2H), 2.24 (s, 3H), 2.12 (m, 2H), 1.66-1.32 (m, 12H), 0.98 (s, 9H) **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 142.4, 140.9, 64.4, 61.4, 60.4, 60.3, 30.0, 27.1, 12.2; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{33}H_{44}IN_2O_2Si$: 655.2217 found: 655.2217.

***Step G:* tert-butyl-[2-[[3-[[5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazol-1-yl]methyl]-1-adamantyl]oxy]ethoxy]-diphenyl-silane**

[577] To the product from *Step F* (11.0 g, 16.8 mmol) in THF (84 mL) was added chloro(isopropyl)magnesium-LiCl (1.3 M in THF, 17 mL, 1.2 eq) at 0 °C, and the reaction mixture was stirred for 40 min, treated with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-

dioxaborolane (10.3 mL, 3 eq), and stirred for 10 min. After dilution with a saturated solution NH_4Cl and extraction with EtOAc, the combined organic phases were concentrated and purified by column chromatography (silica gel, heptane and MTBE as eluents) to give the desired product (9.0 g, 82%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.66 (d, 4H), 7.47 (s, 1H), 7.45 (t, 2H), 7.40 (t, 4H), 3.77 (s, 2H), 3.67 (t, 2H), 3.44 (t, 2H), 2.36 (s, 3H), 2.11 (br, 2H), 1.60/1.48 (d+d, 4H), 1.44 (d, 2H), 1.44 (s, 2H), 1.40 (d, 4H), 1.23 (s, 12H), 0.97 (s, 9H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 146.9, 144.2, 133.8, 130.2, 128.3, 125.7, 104.6, 83.0, 72.5, 64.4, 61.4, 58.9, 44.6, 40.7, 39.6, 38.7, 35.6, 30.0, 27.1, 25.2, 19.3, 12.1; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{39}H_{56}BN_2O_4Si$: 655.4102 found: 655.4108.

Preparation 18: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-c]pyridazin-8-yl]-3-[5-methyl-1-[[3-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylate

Step A: (4-methoxyphenyl)methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylate

[578] The mixture of the product from **Preparation 11** (3.67 g, 6.79 mmol), the product from **Preparation 17** (4.89 g, 1.1 eq), $Pd(AtaPhos)_2Cl_2$ (301 mg, 0.1 eq), and Cs_2CO_3 (6.64 g, 3 eq) in 1,4-dioxane (41 mL) and H_2O (6.8 mL) was stirred at 80 °C for 12 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (3.0 g, 45%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.69-7.37 (m, 10H), 7.31 (d, 1H), 7.24 (s, 1H), 7.12 (m, 2H), 6.98 (t, 1H), 6.83 (m, 2H), 6.62 (d, 1H), 4.99 (s, 2H), 3.76 (s, 2H), 3.70 (s, 3H), 3.66 (t, 2H), 3.45 (t, 2H), 3.35 (m, 2H), 2.85 (m, 2H), 2.34 (s, 3H), 2.12 (m, 2H), 2.02 (s, 3H), 1.77 (m, 2H), 1.65-1.33 (m, 12H), 0.97 (s, 9H); **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{55}H_{65}Cl_2N_6O_5Si$: 987.4163 found: 987.4158.

Step B: (4-methoxyphenyl)methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)pyridine-2-carboxylate

[579] The mixture of the product from *Step A* (3.00 g, 3.00 mmol), Cs_2CO_3 (1.95 g, 2 eq), DIPEA (1.0 mL, 2 eq), and $Pd(Ataphos)_2Cl_2$ (212 mg, 0.1 eq) in 1,4-dioxane (15 mL) was stirred at 110 °C for 18 h. Purification by column chromatography (silica gel, DCM and MeOH as eluents) afforded the desired product (1.74 g, 60%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.84 (d, 1H), 7.68 (d, 1H), 7.68-7.37 (m, 10H), 7.36 (s, 1H), 7.16 (m, 2H), 6.87 (m, 2H), 5.08 (s, 2H), 3.96 (m, 2H), 3.81 (s, 2H), 3.72 (s, 3H), 3.67 (t, 2H), 3.46 (t, 2H), 2.87

(t, 2H), 2.29 (s, 3H), 2.13 (m, 2H), 2.09 (s, 3H), 1.98 (m, 2H), 1.65-1.37 (m, 12H), 0.97 (s, 9H); **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{55}H_{64}ClN_6O_5Si$: 951.4396 found: 951.4397.

***Step C*: (4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3-(2-hydroxyethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate**

[580] To the product from *Step B* (1.73 g, 1.82 mmol) in THF (20 mL) was added a 1 M solution of TBAF in THF (2.0 mL, 1.1 eq) at 0 °C and the reaction mixture was stirred for 2 h. Purification by column chromatography (silica gel, DCM and MeOH as eluents) afforded the desired product (1.06 g, 82%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 7.85 (d, 1H), 7.71 (d, 1H), 7.36 (s, 1H), 7.19 (m, 2H), 6.90 (m, 2H), 5.10 (s, 2H), 4.47 (t, 1H), 3.96 (m, 2H), 3.81 (s, 2H), 3.75 (s, 3H), 3.40 (m, 2H), 3.34 (t, 2H), 2.87 (t, 2H), 2.29 (s, 3H), 2.14 (m, 2H), 2.10 (s, 3H), 1.98 (m, 2H), 1.67-1.36 (m, 12H); **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{39}H_{46}ClN_6O_5$: 713.3218 found: 713.3217.

***Step D*: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(2-hydroxyethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate**

[581] The mixture of the product from *Step C* (1.00 g, 1.40 mmol), 1,3-benzothiazol-2-amine (421 mg, 2 eq), $Pd_2(dba)_3$ (128 mg, 0.1 eq), XantPhos (162 mg, 0.2 eq), and DIPEA (0.72 mL, 3 eq) in cyclohexanol (10 mL) was stirred at 130 °C for 1 h. Purification by column chromatography (silica gel, heptane, then DCM and MeOH as eluents) afforded the desired product (600 mg, 53%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ ppm 12.18/10.84 (brs/brs, 1H), 7.94 (d, 1H), 7.83 (br, 1H), 7.69 (d, 1H), 7.57 (br, 1H), 7.36 (s, 1H), 7.35 (brt, 1H), 7.20 (d, 2H), 7.17 (brt, 1H), 6.91 (d, 2H), 5.11 (s, 2H), 4.47 (brt, 1H), 4.00 (t, 2H), 3.81 (s, 2H), 3.75 (s, 3H), 3.41 (brq, 2H), 3.35 (t, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 2.14 (m, 2H), 2.12 (s, 3H), 1.99 (qn, 2H), 1.62/1.53 (d+d, 4H), 1.53 (s, 2H), 1.49/1.44 (d+d, 2H), 1.44 (s, 4H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ ppm 139.9, 137.6, 130.1, 126.4, 122.4, 122.0, 118.9, 114.2, 66.7, 61.9, 61.5, 59.5, 55.6, 45.4, 44.7, 40.8, 39.5, 35.6, 30.1, 24.3, 21.7, 12.6, 10.8; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{46}H_{51}N_8O_5S$: 827.3703 found: 827.3709.

***Step E*: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[5-methyl-1-[[3-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylate**

To the product from *Step D* (600 mg, 0.726 mmol) and *N,N*-diethylethanamine (0.31 mL, 3 eq) in dichloromethane (7 mL) was added *p*-tolylsulfonyl 4-

methylbenzenesulfonate (357 mg, 1.5 eq) and the reaction mixture was stirred for 18 h. Purification by flash chromatography (silica gel, using DCM and MeOH as eluents) afforded 354 mg (50%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 12.22/10.85 (brs/brs, 1H), 7.94 (d, 1H), 7.81 (br, 1H), 7.77 (d, 2H), 7.70 (d, 1H), 7.52 (br, 1H), 7.45 (d, 2H), 7.37 (s, 1H), 7.35 (t, 1H), 7.19 (d, 2H), 7.17 (t, 1H), 6.89 (d, 2H), 5.10 (s, 2H), 4.05 (t, 2 H), 4.00 (t, 2H), 3.79 (s, 2H), 3.74 (s, 3H), 3.49 (t, 2H), 2.86 (t, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.11 (m, 2H), 2.11 (s, 3H), 1.99 (qn, 2H), 1.55-1.36 (m, 12H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 139.9, 137.6, 130.5, 130.3, 128.1, 126.4, 122.4, 122.0, 118.9, 114.2, 71.4, 66.8, 59.4, 58.2, 55.6, 45.4, 30.0, 24.2, 21.6, 21.6, 12.6, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{53}H_{57}N_8O_7S_2$: 981.3792 found: 981.3795.

Preparation 1b_01: Methyl 2-(*tert*-butoxycarbonylamino)-5-[3-[4-[3-[*tert*-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[582] A 500 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 13.41 g of **Preparation 1a** (25 mmol, 1 eq.), 8.46 g of *tert-butyl N-methyl-N-prop-2-ynyl-carbamate* (50 mmol, 2 eq.) and 50 mL of DIPA (36.10 g, 50 mL, 356.8 mmol, 14.27 eq.) then 125 mL of dry THF was added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 549 mg of $Pd(PPh_3)_2Cl_2$ (1.25 mmol, 0.05 eq.) and 238 mg of CuI (1.25 mmol, 0.05 eq.) were added. The resulting mixture was then warmed up to 60°C and stirred at that temperature until no further conversion was observed. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash column chromatography using heptane and EtOAc as eluents to give 10.5 g (18.2 mmol, 73%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.65 (br s, 1H), 7.31 (br d, 1H), 7.21 (br d, 1H), 7.14 (t, 1H), 4.23 (s, 2H), 4.10 (t, 2H), 3.73 (s, 3H), 3.23 (t, 2H), 2.86 (s, 3H), 2.07 (m, 2H), 1.46/1.41 (s, 18H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 129.1, 119.2, 115.4, 68.1, 51.9, 38.6, 33.8, 30.5, 23.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{28}H_{37}FN_3O_7S$: 578.2331, found 578.2331.

Preparation 2a_01: 5-[*tert*-Butyl(dimethyl)silyl]oxy-4-[*tert*-butyl(diphenyl)silyl]oxy-pentan-1-ol

Step A: pent-4-enyl benzoate

[583] 30.00 g of *pent-4-en-1-ol* (0.35 mol, 1 eq.) and 58.5 mL of *N,N-diethylethanamine* (0.42 mol, 1.2 eq.) were mixed in 200 mL of DCM then cooled to 0°C. 48.5 mL of *benzoyl*

chloride (0.42 mol, 1.2 eq.) was added to the mixture at 0°C via dropping funnel under inert atmosphere. After the addition the mixture was further stirred at 0°C for 30 min then at rt for on. The mixture was diluted with 100 mL of DCM then the organic phase was washed with water, 1 M NaOH, 1 M HCl, brine, respectively. The organic phase was dried over $MgSO_4$, filtered, concentrated and purified via flash column chromatography using heptane and EtOAc as eluents to give 63.19 g (95%) of the desired product as colorless liquid. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ ppm 7.97 (dd, 2H), 7.66 (t, 1H), 7.53 (t, 2H), 5.91-5.81 (m, 1H), 5.09-4.97 (m, 2H), 4.27 (t, 2H), 2.17 (q, 2H), 1.81 (qv, 2H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ ppm 166.2, 138.2, 133.8, 130.3, 129.6, 129.2, 115.8, 64.5, 30.1, 27.8; GC-MS-EI (m/z): $[M]^+$ calcd for $C_{12}H_{14}O_2$: 190.1, found 190.

Step B: 4,5-dihydroxypentyl benzoate

[584] 42.22 g of the product from *Step A* (0.26 mol, 1.0 eq.), 50.40 g of *4-methyl-4-oxido-morpholin-4-ium;hydrate* (0.37 mol, 1.7 eq) were mixed in 360 mL of *2-methylpropan-2-ol* and 40 mL of water then 6.57 g of *tetraoxoosmium* (2.5 w% *in 2-methylpropan-2-ol*, 0.64 mmol, 0.002 eq.) was added and the mixture was stirred at 60°C for 24 h. Full conversion was observed. The mixture was cooled down to rt and 1 M $Na_2S_2O_3$ was added then stirred for further 10 min at rt. DCM was added and the organic phase was separated, washed with water, brine, respectively. The solution was dried over over $MgSO_4$, filtered, concentrated and purified via flash column chromatography using heptane and EtOAc as eluents to give 36.9 g (63%) of the desired product as white solid. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ ppm 7.99-7.50 (m, 5H), 4.50 (m, 2H), 4.28 (m, 2H), 3.45 (m, 1H), 3.30-3.24 (m+m, 2H), 1.85-1.72 (m+m, 2H), 1.59-1.33 (m+m, 2H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ ppm 166.2, 133.8-129.1, 71.2, 66.3, 65.5, 30.3, 25.2; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{12}H_{16}NaO_4$: 247.0941, found 247.0941.

Step C: 5-[tert-butyl(dimethyl)silyl]oxy-4-hydroxy-pentyl] benzoate

[585] 24.86 g of the product from *Step B* (0.11 mol, 1 eq) and 15.09 g of *imidazole* (0.22 mol, 2 eq.) were mixed in 120 mL of *N,N-dimethylformamide* then cooled to -20°C under inert atmosphere. 16.71 g of *tert-butyl-chloro-dimethyl-silane* (0.11 mol, 1 eq.) in 40 mL of *N,N-dimethylformamide* was added in slow rate over a period of 30 min, supported with 10 mL of DCM then left to warm up to rt and further stirred for on. Full conversion was observed. Quenched with cc. NH_4Cl then evaporated most of the volatiles. EtOAc and water were added to the residue, the organic phase was separated then washed with water and brine, dried over $MgSO_4$, filtered, concentrated and purified via flash column chromatography using heptane and EtOAc as eluents to give 33.71 g (90%) of the desired

product as colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.95 (m, 2H), 7.66 (m, 1H), 7.52 (m, 2H), 4.58 (d, 1H), 4.29 (m, 2H), 3.51-3.35 (dd+dd, 2H), 3.48 (m, 1H), 1.86-1.74 (m+m, 2H), 1.67-1.34 (m+m, 2H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 166.2, 133.7, 130.4, 129.5, 129.2, 70.6, 67.7, 65.3, 30.2, 26.3, 24.9, -4.9.

Step D: [5-[tert-butyl(dimethyl)silyl]oxy-4-[tert-butyl(diphenyl)silyl]oxy-pentyl] benzoate

[586] 33.51 g of the product from *Step C* (0.10 mol, 1 eq), 16.85 g of *imidazole* (0.25 mol, 2.5 eq.) and 1.21 g of *N,N-dimethylpyridin-4-amine* (0.01, 0.1 eq.) were mixed in 230 mL of *N,N-dimethylformamide* then 38 mL of *tert-butyl-chloro-diphenyl-silane* (0.15 mol, 1.5 eq.) was added in slow rate, supported with 20 mL of *N,N-dimethylformamide* then stirred at 50°C for overnight. Full conversion was observed. The mixture was cooled to rt, quenched with cc. NH_4Cl then evaporated most of the volatiles. EtOAc and water were added to the residue, the organic phase was separated then washed with water and brine, dried over $MgSO_4$, filtered, concentrated and purified via flash column chromatography using heptane and EtOAc as eluents to give 56.43 g (99%) of the desired product as colorless thick oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.91-7.37 (m, 15H), 4.17 (m, 2 H), 3.76 (m, 1 H), 3.45 (m, 2H), 1.72 (m, 2H), 1.66-1.57 (m+m, 2H), 0.99 (s, 9H), 0.74 (s, 9H), -0.12/-0.16 (s+s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 166.1, 136.0-128.0, 73.3, 66.0, 65.1, 30.3, 27.3, 26.1, 24.0, -5.1; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{34}H_{48}NaO_4Si_2$: 599.2983, found 599.2981.

Step E: 5-[tert-butyl(dimethyl)silyl]oxy-4-[tert-butyl(diphenyl)silyl]oxy-pentan-1-ol

[587] 46.10 g of the product from *Step D* (0.08 mol, 1 eq) was dissolved in 227 mL of MeOH and 117 mL of THF then 12.79 g of NaOH (0.32 mol, 4.0 eq.) in 85 mL of water was added slowly while the mixture was cooled with ice. After the addition the mixture left to stir at rt until full conversion was observed (ca. 4 h). EtOAc and water were added then separated and the organic phase was washed with brine, dried over $MgSO_4$, filtered, concentrated and purified via flash column chromatography using heptane and EtOAc as eluents to give 29.32 g (78%) of the desired product as colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.65-7.37 (m, 10H), 4.34 (t, 1H), 3.71 (m, 1H), 3.42 (m, 2H), 3.26 (m, 2H), 1.52 (m, 2H), 1.42 (m, 2H), 0.99 (s, 9H), 0.77 (s, 9H), -0.13 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 135.8, 135.8, 134.3, 134.0, 130.3, 130.2, 128.2, 128.0, 74.0, 66.4, 61.4, 30.4, 28.3, 27.3, 26.2, -5.1; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{27}H_{44}NaO_3Si_2$: 495.2721, found 495.2706.

Preparation 3a_01: Methyl 5-[3-[4-[3-[*tert*-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[5-[*tert*-butyl(dimethyl)silyl]oxy-4-[*tert*-butyl(diphenyl)silyl]oxy-pentyl]amino]thiazole-4-carboxylate

Step A: methyl 2-[tert-butoxycarbonyl-[5-[tert-butyl(dimethyl)silyl]oxy-4-[tert-butyl(diphenyl)silyl]oxy-pentyl]amino]-5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[588] Using **Mitsunobu General Procedure II** starting from **Preparation 1b_01** as the appropriate carbamate and **Preparation 2a_01** as the appropriate alcohol, 2.5 g (61%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.60-7.33 (m, 10H), 7.28 (dd, 1H), 7.17 (m, 1H), 7.1 (t, 1H), 4.22 (s, 2H), 4.09 (t, 2H), 3.94 (m, 2H), 3.71 (s, 3H), 3.67 (m, 1H), 3.38 (m, 2H), 3.22 (t, 2H), 2.85 (s, 3H), 2.07 (m, 2H), 1.65 (m, 2H), 1.48 (m, 2H), 1.45/1.40 (s+s, 18H), 0.93 (s, 9H), 0.71 (s, 9H), -0.17/-0.22 (s+s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 147.4, 129, 119.3, 115.4, 85.1, 82.3, 73.3, 68.1, 65.6, 51.9, 46.5, 38.4, 33.8, 30.5, 30.5, 28.5/28, 27.2, 26.0, 23.1, 23.0, -5.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{55}H_{79}FN_3O_9SSi_2$: 1032.5054, found 1032.5060.

Step B: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[5-[tert-butyl(dimethyl)silyl]oxy-4-[tert-butyl(diphenyl)silyl]oxy-pentyl]amino]thiazole-4-carboxylate

[589] Using **Deprotection with HFIP General Procedure** starting from the product from *Step A* as the appropriate carbamate, 1.2 g (53%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.68-7.35 (m, 10H), 7.56 (t, 1H), 7.30 (d, 1H), 7.20 (d, 1H), 7.11 (t, 1H), 4.22 (br., 2H), 4.07 (t, 2H), 3.70 (m, 1H), 3.68 (s, 3H), 3.42/3.38 (dd+dd, 2H), 3.11 (t, 2H), 3.04 (brq., 2H), 2.86 (br., 3H), 1.99 (quint., 2H), 1.54 (m, 2H), 1.53/1.45 (m+m, 2H), 1.41 (s, 9H), 0.97 (s, 9H), 0.74 (s, 9H), -0.14/-0.18 (s+s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.6, 163.0, 154.9, 151.4, 147.5, 136.9, 136.0, 129.1, 119.3, 115.4, 114.8, 85.2, 82.3, 79.8, 73.6, 68.0, 66.2, 51.7, 44.7, 38.5, 33.8, 31.1, 30.6, 28.5, 27.2, 26.2, 24.3, 23.3, 19.4, 18.3, -5.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{50}H_{71}FN_3O_7SSi_2$: 932.4530, found 932.4526.

Preparation 3e_01: Ethyl 5-(3-chloropropyl)-2-(methylamino)thiazole-4-carboxylate

[590] A suspension of 2.25 g of *methylthiourea* (25.0 mmol, 1 eq.) in 100 mL of ethanol was cooled to 0°C, and then 7.46 g of *ethyl 3-bromo-6-chloro-2-oxo-hexanoate* (27.5 mmol, 1.1 eq.) was added dropwise at this temperature. After 15 min stirring at 0°C, 7 mL of TEA (5.06 g, 50 mmol, 2 eq.) was added. The resulting mixture was stirred overnight at rt. Full

conversion was observed. The volatiles were removed in vacuo, then the resultant residue was portioned between EtOAc and water. The layers were separated then the organic layer was washed with water then followed with brine. The combined organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. Then it was purified via flash column chromatography using heptane and EtOAc as eluents to give 5 g (76%) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (q, 1H), 4.21 (q, 2H), 3.65 (t, 2H), 3.09 (m, 2H), 2.78 (d, 3H), 1.98 (m, 2H), 1.26 (t, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 165.6, 162.5, 137.4, 135.5, 60.5, 45.0, 34.1, 31.2, 24.4, 14.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{10}H_{16}ClN_2O_2S$: 263.0616, found 263.0615.

Preparation 3h_01: Methyl 5-[3-[4-[3-[*tert*-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethylamino]thiazole-4-carboxylate

Step A: methyl 2-[tert-butoxycarbonyl-[2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl]amino]-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

[591] Using **Mitsunobu General Procedure II** starting from 2.68 g of **Preparation 1a** (5 mmol, 1 eq.) and 1.46 g of *2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanol* (1.42 mL, 10 mmol, 2 eq.) as the appropriate alcohol, 2.8 g (84%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.57 (dd, 1H), 7.44 (dm, 1H), 6.96 (t, 1H), 4.12/4.02 (m+m, 2H), 4.07 (m, 1H), 4.05 (t, 2H), 4.02/3.54 (dd+dd, 2H), 3.75 (s, 3H), 3.21 (t, 2H), 2.06 (m, 2H), 1.86/1.82 (m+m, 2H), 1.51 (s, 9H), 1.29 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 134.0, 124.9, 117.6, 73.8, 68.9, 68.1, 52.0, 44.0, 32.2, 30.5, 28.1, 27.3, 25.9, 23.1; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{35}FIN_2O_7S$: 665.1188, found 665.1175.

Step B: methyl 2-[2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethylamino]-5-[3-(2-fluoro-4-iodo-phenoxy)propyl]thiazole-4-carboxylate

[592] Using **Deprotection with HFIP General Procedure** starting from 2.5 g of the product from *Step A* (3.80 mmol) as the appropriate carbamate, 1.6 g (75%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.6 (t, 1H), 7.59 (dd, 1H), 7.45 (dm, 1H), 6.97 (dd, 1H), 4.10 (m, 1H), 4.03 (t, 2H), 4.01/3.48 (dd+dd, 2H), 3.69 (s, 3H), 3.27/3.19 (m+m, 2H), 3.11 (t, 2H), 1.99 (m, 2H), 1.76/1.72 (m+m, 2H), 1.31 (s, 3H), 1.25 (s, 3 H); HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{21}H_{27}FIN_2O_5S$: 565.0663, found 565.0642.

Step C: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethylamino]thiazole-4-carboxylate

[593] Using **Sonogashira General Procedure** starting from 400 mg of the product from *Step B* (0.71 mmol, 1 eq.) and 240 mg of *tert-butyl N-methyl-N-prop-2-ynyl-carbamate* (1.42 mmol, 2 eq.) as the appropriate acetylene, 300 mg (70%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.60 (t, 1H), 7.31 (brd, 1H), 7.21 (dd, 1H), 7.13 (t, 1H), 4.23 (brs, 2H), 4.09 (m, 1H), 4.07 (t, 2H), 4.00/3.48 (dd+dd, 2H), 3.69 (s, 3H), 3.27/3.19 (m+m, 2H), 3.12 (t, 2H), 2.86 (brs, 3H), 2.00 (m, 2H), 1.74 (m, 2H), 1.41 (s, 9H), 1.31 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.5, 136.9, 136.4, 129.1, 119.3, 115.4, 85.2, 82.3, 73.8, 69.0, 68.0, 51.7, 41.4, 38.4, 33.8, 33.2, 30.6, 28.5, 27.3, 26.1, 23.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{30}H_{41}FN_3O_7S$: 606.2644, found 606.2650.

Preparation 3n_01: Methyl 5-[3-[4-[3-[*tert*-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-[*tert*-butyl(dimethyl)silyl]oxypropylamino]thiazole-4-carboxylate

Step A: methyl 2-[tert-butoxycarbonyl-[3-[tert-butyl(dimethyl)silyl]oxypropyl]amino]-5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino] prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[594] Using **Mitsunobu General Procedure II** starting from 577 mg of **Preparation 1b_01** (1 mmol, 1 eq.) as the appropriate carbamate and 380 mg of *3-[tert-butyl(dimethyl)silyl]oxypropan-1-ol* (2 mmol, 2 eq.) as the appropriate alcohol, 600 mg (80%) of the desired product was obtained.

Step B: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-[tert-butyl(dimethyl)silyl]oxypropylamino]thiazole-4-carboxylate

[595] Using **Deprotection with HFIP General Procedure** starting from the product from *Step A* as the appropriate carbamate, 310 mg (47%) of the desired product was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.50 (t, 1H), 7.30 (d, 1H), 7.20 (d, 1H), 7.11 (t, 1H), 4.21 (bs, 2H), 4.05 (t, 2H), 3.62 (t, 2H), 3.67 (s, 3H), 3.19 (q, 2H), 3.10 (t, 2H), 2.84 (brs, 3H), 2.04-1.94 (m, 2H), 1.74-1.63 (m, 2H), 1.40 (s, 9H), 0.84 (s, 9H), 0.00 (s, 6H).

Preparation 4a_01: *N*-(6-Chloro-4-methyl-pyridazin-3-yl)-3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-imine

Step A: N-(6-chloro-4-methyl-pyridazin-3-yl)-1,3-benzothiazol-2-amine

[596] A 2 L oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 34.0 g of *6-chloro-4-methyl-pyridazin-3-amine* (237 mmol, 1 eq.), 34 mL of *2-chloro-1,3-benzothiazole* (44.2 g, 260 mmol, 1.1 eq.), 124 mL of DIPEA (91.8 g, 710 mmol, 3 eq.) and 137 g of

Cs_2CO_3 (710 mmol, 3 eq.), then 1 L of DMF were added and the system was flushed with argon. After 5 minutes stirring under inert atmosphere 2.01 g of $Pd_2(dba)_3$ (5.9 mmol, 0.025 eq.) and 6.85 g of XantPhos (11.8 mmol, 0.05 eq.) were added. The resulting mixture was then warmed up to 75°C and stirred at that temperature for 4 hours to reach complete conversion. Reaction mixture was left to cool down to rt, then poured into 3 L of water while it was intensively stirred. After 30 min the precipitated product was removed by filtration, and then it was washed with water for 2 times (2×2 L). The product was dried overnight on high vacuum. The dried crude product was stirred in 1 L of heptane : Et_2O (3:2) for 30 min then filtered off to give 64.5 g (98%) of the desired product as green powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.96 (brs, 1H), 7.86 (d, 1H), 7.65 (s, 1H), 7.51 (d, 1H), 7.38 (t, 1H), 7.21 (t, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 130.3, 129.5, 126.6, 122.8, 122.3, 17.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{12}H_{10}ClN_4S$: 277.0309, found 277.0305.

Step B: N-(6-chloro-4-methyl-pyridazin-3-yl)-3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-imine

[597] A 2 L oven-dried, one-necked, round-bottomed flask equipped with a PTFE-coated magnetic stirring bar was charged with 64.5 g of the product from *Step A* (236 mmol, 1 eq.), 123 mL of DIPEA (9.16 g, 708 mmol, 3 eq.), 14.43 g of *N,N-dimethylpyridin-4-amine* (11.81 mmol, 0.05 eq.) in 1 L of dry DCM were cooled down to 0 °C under N_2. And during intensive mechanical stirring 46.00 mL of *2-(chloromethoxy)ethyl-trimethyl-silane* (43.32 g, 259 mmol, 1.1 eq.) was added to the mixture dropwise over 5 min period of time. It was stirred at 0°C for 30 min when the reaction reached complete conversion. 24.5 mL of water was added to the reaction mixture then *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. It was purified via flash column chromatography using heptane and EtOAc as eluents to obtain 46.62 g (48%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.85 (dm, 1H), 7.72 (q, 1H), 7.53 (dm, 1H), 7.47 (m, 1H), 7.29 (m, 1H), 5.89 (s, 2H), 3.70 (m, 2H), 2.39 (d, 3H), 0.90 (m, 2H), -0.12 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d6) δ ppm 159.5, 158.5, 150.0, 138.1, 137.4, 129.5, 127.4, 125.5, 123.8, 123.2, 112.4, 73.0, 66.8, 17.7, 17.1, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{18}H_{24}ClN_4OSSi$: 407.1123, found 407.1120.

Preparation 5a_01: Methyl 5-[3-[4-[3-[*tert*-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[4-[*tert*-butyl(diphenyl)silyl]oxy-5-(*p*-tolylsulfonyloxy)pentyl]-[5-methyl-6-[(*Z*)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[5-[tert-butyl(dimethyl)silyl]oxy-4-[tert-butyl(diphenylsilyl]oxy-pentyl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[598] Using **Buchwald General Procedure III** starting from 12 g of **Preparation 3a_01** (13 mmol) and 6.30 g of **Preparation 4a_01** (15.6 mmol) as the appropriate halide, 14 g (83%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.85-7.23 (m, 14H), 7.58 (s, 1H), 7.31 (t, 1H), 7.19 (m, 1H), 7.14 (t, 1H), 5.86 (s, 2H), 4.37 (t, 2H), 4.20 (s, 2H), 4.15 (t, 2H), 3.73 (s, 3H), 3.71 (t, 2H), 3.67 (m, 1H), 3.39 (m, 2H), 3.27 (t, 2H), 2.83 (s, 3H), 2.41 (s, 3H), 2.12 (m, 2H), 1.72 (m, 2H), 1.52 (m, 2H), 1.40 (s, 9H), 0.90 (t, 2H), 0.89 (s, 9H), 0.69 (s, 9H), -0.14 (s, 9H), -0.19/-0.23 (s+s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 147.5, 129.1, 119.3, 117.5, 115.4, 73.4, 72.3, 68.4, 66.8, 65.8, 51.8, 46.6, 38.5, 33.8, 31.0, 30.5, 28.5, 27.1, 26.1, 23.0, 22.6, 17.9, 17.8, -1.0, -5.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{68}H_{93}FN_7O_8S_2Si_3$: 1302.5813, found 1302.5819.

Step B: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[4-[tert-butyl(diphenyl)silyl]oxy-5-hydroxy-pentyl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[599] A 100 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 1.40 g of the product from *Step A* (1.1 mmol, 1 eq.) and 12 mg of camphor sulfonic acid (0.054 mmol, 0.05 eq.), 5 mL of DCM and 1 mL of MeOH. The resulting mixture was stirred overnight at rt to reach complete conversion. Reaction mixture was concentrated directly to *Celite* then purified by flash column chromatography using heptane and EtOAc as eluents to give 700 mg (55%) of the desired product as yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.85-7.14 (m, 14H), 7.56 (s, 1H), 7.32 (dd, 1H), 7.20 (m, 1H), 7.15 (t, 1H), 5.86 (s, 2H), 4.56 (t, 1H), 4.33 (m, 2H), 4.20 (s, 2H), 4.15 (t, 2H), 3.74 (s, 3H), 3.72 (t, 2H), 3.65 (m, 1H), 3.27 (t, 2H), 3.27 (t, 2H), 2.83 (s, 3H), 2.41 (s, 3H), 2.13 (m, 2H), 1.73/1.64 (m+m, 2H), 1.52 (m, 2H), 1.40 (s, 9H), 0.90 (t, 2H), 0.86 (s, 9H), -0.13 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 154.9, 147.6, 129.1, 119.4, 117.5, 115.4, 82.4, 73.7, 72.9, 68.4, 66.8, 64.5, 51.9, 46.8, 38.5, 33.8, 31.0, 30.6, 28.5, 27.2, 23.1, 22.5, 17.9, 17.8, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{62}H_{79}FN_7O_8S_2Si_2$: 1188.4949, found 1188.4938.

Step C: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[4-[tert-butyl(diphenyl)silyl]oxy-5-(p-tolylsulfonyloxy)pentyl]-[5-

methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[600] A 100 mL oven-dried, one-necked, round-bottom flask was equipped with a *PTFE*-coated magnetic stirring bar was charged with 700 mg of the product from *Step B* (0.58 mmol, 1 eq.) and 907 mg of *N,N-dimethyl-1-(p-tolylsulfonyl)pyridin-1-ium-4-amine chloride* (2.9 mmol, 5 eq.; see, e.g., *Tetrahedron Lett.* 2016, *57*, 4620) were dissolved in 35 mL of DCM and stirred overnight at rt. Reaction reached complete conversion. Reaction mixture directly was concentrated onto *Celite*, and then purified by flash column chromatography using heptane and EtOAc as eluents to give 450 mg (56%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.88-7.23 (m, 14H), 7.58 (m, 2H), 7.53 (s, 1H), 7.31 (m, 2H), 7.31 (dd, 1H), 7.19 (m, 1H), 7.15 (t, 1H), 5.86 (s, 2H), 4.20 (s, 2H), 4.16 (t, 2H), 4.15 (t, 2H), 3.92 (m, 2H), 3.84 (m, 1H), 3.72 (t, 2H), 3.70 (s, 3H), 3.27 (t, 2H), 2.83 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 2.13 (m, 2H), 1.47 (m, 2H), 1.47 (m, 2H), 1.40 (s, 9H), 0.91 (t, 2H), 0.86 (s, 9H), -0.13 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 147.5, 145.3, 130.4, 129.1, 128.0, 119.3, 117.4, 115.5, 72.9, 72.6, 70.4, 68.4, 66.8, 51.8, 46.2, 38.6, 33.8, 31.0, 30.1, 28.5, 27.0, 23.1, 22.4, 21.5, 17.8, 17.8, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{69}H_{85}FN_7O_{10}S_3Si_2$: 1342.5037, found 1342.5039.

Preparation 5g_01: Ethyl 5-(3-iodopropyl)-2-[methyl-[5-methyl-6-[(*Z*)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

Step A: ethyl 5-(3-chloropropyl)-2-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[601] Using **Buchwald General Procedure III** starting from 3.15 g of **Preparation 3e_01** (12 mmol, 1.2 eq.) and 4.07 g of **Preparation 4a_01** (10 mmol, 1 eq.) as the appropriate halide, 2.6 g (41%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.84 (d, 1H), 7.65 (s, 1H), 7.45 (d, 1H), 7.43 (tm, 1H), 7.25 (tm, 1H), 5.85 (s, 2H), 4.30 (q, 2H), 3.77 (s, 3H), 3.71 (t, 2H), 3.71 (t, 2H), 3.22 (t, 2H), 2.48 (s, 3H), 2.10 (quin, 2H), 1.31 (t, 3H), 0.92 (t, 2H), -0.11 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.6, 157.4, 156.8, 155.1, 151.7, 140.5, 137.6, 137.1, 135.3, 125.6, 123.5, 123.2, 123.1, 117.6, 111.9, 72.9, 66.7, 60.7, 45.3, 35.4, 34.4, 24.3, 18.0, 17.8, 14.7, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{28}H_{38}ClN_6O_3S_2Si$: 633.1899, found 633.1891.

Step B: ethyl 5-(3-iodopropyl)-2-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-

yl]amino]thiazole-4-carboxylate

[602] A 100 mL one-necked, round-bottomed flask was equipped with a *PTFE*-coated magnetic stirring bar and fitted with a reflux condenser. It was charged with 2.6 g of the product from *Step A* (4.10 mmol, 1 eq.), 1.23 g of NaI (8.2 mmol, 2 eq.) and 20 mL of dry acetone. The reaction mixture was warmed up to 60°C and stirred at that temperature for 3 days, when the reaction reached complete conversion. The reaction mixture was diluted with the addition of water then the precipitated product was collected by filtration, washed with water, and then dried on high vacuum to obtain 2.5 g (84%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.82 (d, 1H), 7.61 (s, 1H), 7.47-7.39 (m, 1H), 7.47-7.39 (m, 1H), 7.23 (t, 1H), 5.83 (s, 2H), 4.29 (q, 2H), 3.75 (s, 3H), 3.71 (t, 2H), 3.33 (t, 2H), 3.16 (t, 2H), 2.42 (s, 3H), 2.13 (quint., 2H), 1.33 (t, 3H), 0.91 (t, 2H), -0.12 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.6, 157.3, 156.7, 155.1, 151.6, 140.2, 137.6, 137.1, 135.2, 127.1, 125.4, 123.4, 123.2, 117.5, 111.9, 72.8, 66.7, 60.7, 35.2, 35.2, 27.6, 17.8, 17.8, 14.8, 7.8, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{28}H_{38}I\ N_6O_3S_2Si$: 725.1255, found 725.1248.

Preparation 5j_01: Ethyl 5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-2-[methyl(5-methyl-6-{[(2*Z*)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}pyridazin-3-yl)amino]-1,3-thiazole-4-carboxylate

Step A: ethyl 5-{3-[4-(3-{[(tert-butoxy)carbonyl](methyl)amino}prop-1-yn-1-yl)-2-fluorophenoxy]propyl}-2-[methyl(5-methyl-6-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}pyridazin-3-yl)amino]-1,3-thiazole-4-carboxylate

[603] To the product from **Preparation 5g_01** (1.75 g, 2.41 mmol, 1 eq) in dimethylformamide (50 mL) was added the product from **Preparation 6a_01** (877 mg, 3.14 mmol, 1.3 eq) in dimethylformamide (10 mL) and cesium carbonate (2.36 g, 7.24 mmol, 3 eq) and the mixture was heated at 80 ºC for 16 h. The reaction was concentrated *in vacuo* then partitioned between ethyl acetate and brine, and the organic phase was dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™ silica cartridge) eluting with a gradient of 0 – 50% ethyl acetate in *iso*-heptane afforded the desired product as a yellow oil (1.75 g, 2 mmol, 83%). LC/MS ($C_{43}H_{54}FN_7O_6SiS_2$) 876 $[M+H]^+$; RT 1.46 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.83 (dd, 1H), 7.65 (d, J = 1.1 Hz, 1H), 7.49 - 7.39 (m, 2H), 7.35 - 7.28 (m, 1H), 7.27 - 7.12 (m, 3H), 5.86 (s, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.19 (s, 2H), 4.14 (t, J = 6.1 Hz, 2H), 3.77 (s, 3H), 3.76 – 3.68 (m, 2H), 3.26 (t, J = 7.7 Hz, 2H), 2.84 (s, 3H), 2.45 (s, 3H), 2.19 – 2.05 (m, 1H), 1.41 (s, 9H), 1.30 (t, 3H), 0.97 - 0.88 (m, 2H), -0.12 (s, 9H).

Step B: ethyl 5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-2-[methyl(5-methyl-6-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}pyridazin-3-yl)amino]-1,3-thiazole-4-carboxylate

[604] Trifluoroacetic acid (20 mL) was added to a stirred solution of the product from Step A (1.5 g, 1.71 mmol, 1 eq) in dichloromethane (60 mL) and the mixture was stirred at ambient temperature for 5 h. The reaction was diluted with dichloromethane, cooled to 0 °C and basified by the addition of 2N aqueous sodium hydroxide. The organic phase was dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™ silica cartridge) eluting with a gradient of 0 – 10% methanol in dichloromethane afforded the desired product as a yellow gum (329 mg, 0.42 mmol, 25%). LC/MS ($C_{38}H_{46}FN_7O_4SiS_2$) 776 $[M+H]^+$; RT 2.58 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.84 (dd, 1H), 7.67 (d, J = 1.0 Hz, 1H), 7.49 - 7.40 (m, 2H), 7.31 - 7.22 (m, 2H), 7.21 - 7.11 (m, 2H), 5.86 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 4.15 (t, J = 6.1 Hz, 2H), 3.76 (s, 3H), 3.76 – 3.67 (m, 2H), 3.45 (s, 2H), 3.33 - 3.22 (m, 2H), 2.46 (d, J = 1.0 Hz, 3H), 2.30 (s, 3H), 2.18 – 2.06 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H), 0.97 - 0.88 (m, 2H), -0.11 (s, 9H).

Preparation 6a_01: *tert*-Butyl *N*-[3-(3-fluoro-4-hydroxy-phenyl)prop-2-ynyl]-*N*-methyl-carbamate

[605] Using **Sonogashira General Procedure** starting from 10.00 g of 2-fluoro-4-iodo-phenol (42.0 mmol, 1 eq.) as the appropriate phenol and 10.67 g of *tert*-butyl *N*-methyl-*N*-prop-2-ynyl-carbamate (63.1 mmol, 1.5 eq.) as alkyne reactant, 10.8 g (92%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.32 (s, 1 H), 7.22 (brd, 1H), 7.08 (dm, 1H), 6.92 (dd, 1H), 4.21 (s, 2H), 2.85 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 150.8, 146.4, 129.0, 119.6, 118.4, 113.2, 84.4, 82.7, 38.5, 33.8, 28.5; HRMS-ESI (m/z): $[M-C_4H_8+H]^+$ calcd for $C_{11}H_{11}FNO_3$: 224.0717, found 224.0720.

Preparation 6b_01: 4-[3-(Dimethylamino)prop-1-ynyl]-2-fluoro-phenol

[606] Using **Sonogashira General Procedure** starting from 10.00 g of 2-fluoro-4-iodo-phenol (42.0 mmol, 1 eq.) as the appropriate phenol and 5.24 g of *N,N*-dimethylprop-2-yn-1-amine (63 mmol, 1.5 eq.) as alkyne reactant, 7.30 g (90%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.20 (dd, 1H), 7.07 (dm, 1H), 6.91 (m, 1H), 3.39 (m, 2H), 2.21 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 150.9, 146.2, 128.9, 119.5, 118.4, 113.6, 84.5, 84.2, 48.2, 44.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{11}H_{13}FNO$: 194.0976, found 194.0981.

Preparation 6f_01: 4-[3-(Dimethylamino)but-1-ynyl]-2-fluoro-phenol

Step A: 4-(3-fluoro-4-triisopropylsilyloxy-phenyl)but-3-yn-2-ol

[607] A 500 mL oven-dried, one-necked, round-bottomed flask equipped with a *PTFE*-coated magnetic stirring bar. It was charged with 4.76 g of *2-fluoro-4-iodo-phenol* (20 mmol, 1 eq.) and 3.96 g of K_2CO_3 (40 mmol, 2 eq.) then 100 mL of dry MeCN was added. To the resulting mixture 5.13 mL of *TIPSCl* (4.62 g, 24 mmol, 1.2 eq.) was added dropwise near intensive stirring at rt. The resulting mixture was stirred at room temperature for 30 min, while the reaction reached complete conversion. The reaction mixture was filtered through a pad of *Celite* to remove the solid particles then to the filtrate 3.10 mL of *but-3-yn-2-ol* (2.81 g, 40 mmol, 2 eq.) and 20 mL of *DIPA* were added and placed under a nitrogen atmosphere through a gas inlet. After addition of 702 mg of $Pd(PPh_3)_2Cl_2$ (1 mmol, 0.05 eq.) and 190 mg of CuI (1 mmol, 0.05 eq.) the resulting mixture was stirred at room temperature for 30 min, while the reaction reached complete conversion. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash column chromatography using heptane and EtOAc as eluents to give 6.2 g (92%) of the desired product as yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26 (dd, 1H), 7.12 (dm, 1H), 6.98 (t, 1H), 5.44 (d, 1H), 4.55 (m, 1H), 1.36 (d, 3H), 1.24 (sp, 1H), 1.05 (d, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 153.2, 144.1, 128.8, 122.3, 119.6, 116.5, 93.4, 81.4, 57.1, 25.0, 18.0, 12.5; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{19}H_{30}FO_2Si$: 337.1994, found 337.1994.

Step B: 4-(3-fluoro-4-triisopropylsilyloxy-phenyl)-N,N-dimethyl-but-3-yn-2-amine

[608] Using **Alkylation with *in situ* generated iodine General Procedure** starting from 644 mg of the product from *Step A* (2 mmol, 1 eq.) as the appropriate alcohol and 5 mL of *N*-methylmethanamine (10 mmol, 5 eq., 2 M solution in MeOH), 360 mg (50%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.28 (dd, 1H), 7.14 (dm, 1H), 6.97 (t, 1H), 3.67 (q, 1H), 2.19 (s, 6H), 1.27 (d, 3H), 1.25 (m, 3H), 1.05 (d, 18H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 153.1, 144.0, 129.0, 122.3, 119.8, 116.6, 88.2, 84.1, 52.3, 41.3, 20.1, 18.0, 12.5; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{21}H_{35}FNOSi$: 364.2466, found 364.2470.

Step C: 4-[3-(dimethylamino)but-1-ynyl]-2-fluoro-phenol

[609] A 4 mL oven-dried vial equipped with a *PTFE*-coated magnetic stirring bar was charged with 200 mg of the product from *Step B* (0.55 mmol, 1 eq.) dissolved in 3.0 mL of dry THF, and then 660 uL of TBAF (1 M in THF, 0.66 mmol, 1.1 eq.) was added dropwise at rt. The resulting mixture was stirred at rt for 15 min, when the reaction reached complete conversion. The reaction mixture was quenched with the addition of 200 uL of cc. NH_4Cl, then *Celite* was added to the reaction mixture and the volatiles were removed under reduced

pressure. Then it was purified via flash column chromatography using DCM and MeOH (1.2% NH_3) as eluents to give 80 mg (70%) of the desired product.

Preparation 13_01: methyl 3-bromo-6-(methylamino)pyridine-2-carboxylate

Step A: methyl 6-[bis(tert-butoxycarbonyl)amino]-3-bromo-pyridine-2-carboxylate

[610] To methyl 6-amino-3-bromo-pyridine-2-carboxylate (25.0 g, 108.2 mmol) and DMAP (1.3 g, 0.1 eq) in DCM (541 mL) was added Boc_2O (59.0 g, 2.5 eq) at 0 °C and the reaction mixture was stirred for 2.5 h. After the addition of a saturated solution of $NaHCO_3$ and the extraction with DCM, the combined organic phases were dried and concentrated to get the desired product (45.0 g, 72.3%). LC/MS ($C_{17}H_{23}BrN_2O_6Na$) 453 $[M+H]^+$.

Step B: methyl 3-bromo-6-(tert-butoxycarbonylamino)pyridine-2-carboxylate

[611] To the product from Step A (42.7 g, 74.34 mmol) in DCM (370 mL) was added TFA (17.1 mL, 3 eq) at 0 °C and the reaction mixture was stirred for 18 h. After washing with a saturated solution of $NaHCO_3$ and brine, the combined organic phases were dried, concentrated, and purified by column chromatography (silica gel, heptane and EtOAc as eluents) to give the desired product (28.3 g, 115.2%). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 10.29 (s, 1H), 8.11 (d, 1H), 7.88 (d, 1H), 3.87 (s, 3H), 1.46 (s, 9H) ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.6, 153.1, 151.8/148.3, 143.5, 116.3, 109.2, 53.2, 28.4. LC/MS ($C_{12}H_{15}BrN_2O_4Na$) 353 $[M+H]^+$.

Step C: methyl 3-bromo-6-[tert-butoxycarbonyl(methyl)amino]pyridine-2-carboxylate

[612] To the product from Step B (2.96 g, 8.93 mmol) in acetone (45 mL) was added Cs_2CO_3 (8.7 g, 3 eq) and iodomethane (0.67 mL, 1.2 eq) and the reaction mixture was stirred for 3 h. After dilution with water and extraction with EtOAc, the combined organic phases were washed with brine, dried and concentrated to give the desired product (3.5 g, 112%). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 8.13 (d, 1H), 7.78 (d, 1H), 3.90 (s, 3H), 3.27 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.5, 153.6, 153.6, 147.5, 142.8, 122.5, 111.3, 82.0, 53.3, 34.3, 28.2; HRMS-ESI (m/z): [M+H]+ calcd for $C_{13}H_{18}BrN_2O_4$: 345.0450 found: 345.0429.

Step D: methyl 3-bromo-6-(methylamino)pyridine-2-carboxylate

[613] The product from Step C (3.0 g, 8.9 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (90 mL) was stirred at 100 °C for 18 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (2.1 g, 96%). ^1H NMR (400

MHz, DMSO-d_6): δ ppm 7.63 (d, 1H), 7.04 (q, 1H), 6.53 (d, 1H), 3.83 (s, 3H), 2.73 (d, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.6, 158.2, 148.2, 141.3, 112.1, 101.3, 52.9, 28.3; HRMS-ESI (m/z): [M]+ calcd for $C_8H_9BrN_2O_2$: 243.9847 found: 243.9843.

Preparation 14_01: methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]pyridine-2-carboxylate

Step A: methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(methylamino)pyridine-2-carboxylate

[614] The mixture of the product from **Preparation 13_01** (2.07 g, 8.45 mmol), the product from **Preparation 7** (6.9 g, 1.2 eq), Cs_2CO_3 (8.26 g, 3 eq), and $Pd(AtaPhos)_2Cl_2$ (374 mg, 0.1 eq) in 1,4-dioxane (51 mL) and water (8.5 mL) was stirred at 80 °C for 1 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (4.5 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.66 (dm, 4H), 7.47-7.38 (m, 6H), 7.31 (d, 1H), 7.23 (s, 1H), 6.78 (q, 1H), 6.59 (d, 1H), 3.82 (s, 2H), 3.67 (t, 2H), 3.58 (s, 3H), 3.46 (t, 2H), 2.77 (d, 3H), 2.06 (s, 3H), 1.35 (s, 2H), 1.27/1.20 (d+d, 4H), 1.14/1.09 (d+d, 4H), 1.05/0.97 (d+d, 2H), 0.98 (s, 9H), 0.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 140.1, 137.4, 135.6, 130.2/128.3, 109.8, 74.2, 64.4, 61.7, 58.9, 52.2, 50.0, 46.9, 46.0, 43.4, 39.8, 33.5, 30.1, 28.4, 27.1, 10.8; HRMS-ESI (m/z): [M+H]+ calcd for $C_{43}H_{57}N_4O_4Si$: 721.4149 found: 721.4148.

Step B: methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]pyridine-2-carboxylate

[615] Using **Buchwald General Procedure III** starting from the product from Step A at reflux for 18 h, 4.7 g (86%) of the desired product was obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.78 (dm, 1H), 7.69-7.36 (m, 10H), 7.63 (q, 1H), 7.63 (d, 1H), 7.47 (dm, 1H), 7.44 (m, 1H), 7.35 (s, 1H), 7.31 (d, 1H), 7.24 (m, 1H), 5.86 (s, 2H), 3.86 (s, 2H), 3.72 (m, 2H), 3.67 (t, 2H), 3.64 (s, 3H), 3.61 (s, 3H), 3.46 (t, 2H), 2.36 (d, 3H), 2.13 (s, 3H), 1.40-0.94 (m, 12H), 0.97 (s, 9H), 0.92 (m, 2H), 0.85 (s, 6H), -0.11 (s, 9H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{61}H_{79}N_8O_5SSi_2$: 1091.5433 found: 1091.5426.

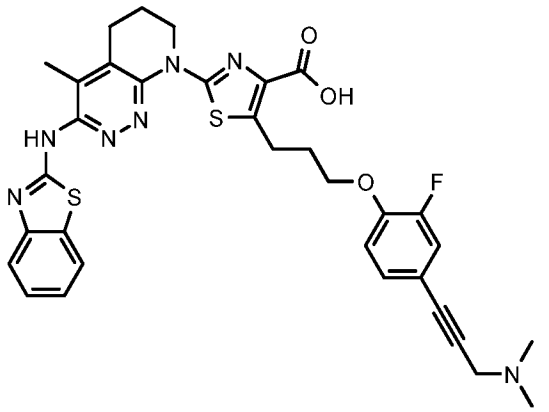
Step C: methyl 3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]pyridine-2-carboxylate

[616] To the product from Step B (1.0 g, 0.916 mmol) in THF (9 mL) was added a 1 M solution of TBAF in THF (1.0 mL, 1.1 eq) at 0 °C and the reaction mixture was stirred for 1 h. After quenching with a saturated solution of NH_4Cl and extraction with EtOAc, the combined organic phases were dried, concentrated, and purified by column chromatography (silica gel, DCM and MeOH as eluents) to give the desired product (752 mg, 96%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.79 (dm, 1H), 7.66 (d, 1H), 7.64 (s, 1H), 7.47 (dm, 1H), 7.43 (m, 1H), 7.36 (s, 1H), 7.33 (d, 1H), 7.25 (m, 1H), 5.87 (s, 2H), 4.46 (t, 1H), 3.86 (s, 2H), 3.73 (m, 2H), 3.68 (s, 3H), 3.62 (s, 3H), 3.40 (m, 2H), 3.35 (t, 2H), 2.37 (s, 3H), 2.14 (s, 3H), 1.42-0.96 (m, 12H), 0.92 (m, 2H), 0.86 (s, 6H), -0.10 (s, 9H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{61}N_8O_5SSi$: 853.4255 found: 853.4256.

Step D: methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]pyridine-2-carboxylate

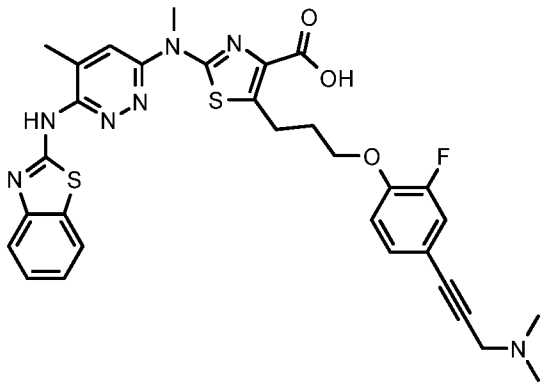
[617] To the product from Step C (752 mg, 0.88 mmol) and triethylamine (0.5 mL, 4 eq) in DCM (4.4 mL) was added *p*-tolylsulfonyl-4-methylbenzenesulfonate (575.4 mg, 1.76 mmol, 2 eq) and the reaction mixture was stirred for 1 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded the desired product (722 mg, 81%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.79 (dm, 1H), 7.76 (dm, 2H), 7.68 (d, 1H), 7.64 (s, 1H), 7.47 (m, 1H), 7.46 (dm, 2H), 7.43 (td, 1H), 7.36 (s, 1H), 7.33 (d, 1H), 7.25 (td, 1H), 5.87 (s, 2H), 4.06 (m, 2H), 3.84 (s, 2H), 3.73 (t, 2H), 3.66 (s, 3H), 3.62 (s, 3H), 3.48 (m, 2H), 2.40 (s, 3H), 2.37 (s, 3H), 2.13 (s, 3H), 1.31-0.94 (m, 12H), 0.92 (t, 2H), 0.83 (s, 6H), -0.10 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 141.2, 137.5, 130.6, 128.1, 127.2, 123.4, 123.4, 123.1, 114.7, 112.0, 72.9, 71.5, 66.7, 58.8, 58.4, 52.6, 36.6, 30.1, 21.6, 17.8, 17.4, 10.8, -0.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{52}H_{67}N_8O_7S_2Si$: 1007.4343 found: 1007.4344.

Preparation of P1: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



[618] Using **Propargylic amine preparation General Procedure** starting from **Preparation 3d** and dimethylamine as the appropriate amine. Then **Hydrolysis General Procedure** starting from the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{34}H_{35}FN_7O_3S_2$: 672.2221, found 672.2205.

Preparation of P2: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



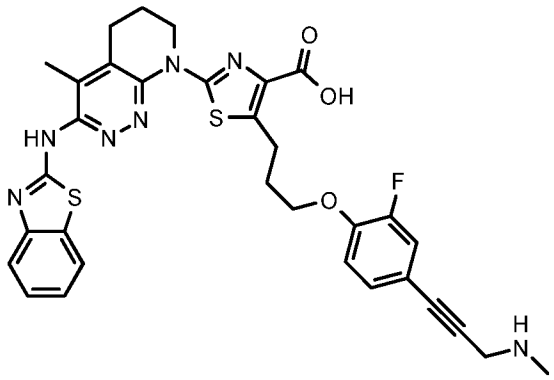
Step A: ethyl 5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[619] Using **Alkylation General Procedure** starting from **Preparation 5g_01** and **Preparation 6b_01** as the appropriate phenol, the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.84 (d, 1H), 7.67 (s, 1H), 7.47 (d, 1H), 7.44 (t, 1H), 7.33 (dd, 1H), 7.25 (t, 1H), 7.22 (dd, 1H), 7.16 (t, 1H), 5.86 (s, 2H), 4.26 (q, 2H), 4.15 (t, 2H), 3.77 (s, 3H), 3.72 (t, 2H), 3.49 (brs, 2H), 3.27 (t, 2H), 2.46 (s, 3H), 2.27 (s, 6H), 2.13 (qn, 2H), 1.29 (t, 3H), 0.92 (t, 2H), -0.11 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 129.0, 127.2, 123.5, 123.2, 119.2, 117.7, 115.5, 111.9, 72.8, 68.5, 66.7, 60.7, 48.2, 44.0, 35.3, 31.1, 23.2, 17.9, 17.8, 14.6, -0.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{39}H_{49}FN_7O_4S_2Si$: 790.3035, found 790.3023.

Step B: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[620] Using **Deprotection and Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate ethyl ester, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{31}H_{31}FN_7O_3S_2$: 632.1908, found 632.1913.

Preparation of P3: 2-{3-[(1,3-Benzothiazol-2-yl)amino]-4-methyl-5*H*,6*H*,7*H*,8*H*-pyrido[2,3-*c*]pyridazin-8-yl}-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylic acid



Step A: ethyl 5-{3-[4-(3-{[(tert-butoxy)carbonyl](methyl)amino}prop-1-yn-1-yl)-2-fluorophenoxy]propyl}-2-(4-methyl-3-{[(2Z)-3-{[2-(trimethylsilyl)ethoxy]methyl}-2,3-dihydro-1,3-benzothiazol-2-ylidene]amino}-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl)-1,3-thiazole-4-carboxylate

[621] To a solution of the product from **Preparation 3g** (500 mg, 0.78 mmol, 1 eq) in toluene (15 mL) was added the product from **Preparation 4c** (327 mg, 1.17 mmol, 1.5 eq), followed by triphenylphosphine (307 mg, 1.17 mmol, 1.5 eq) and diisopropyl azodicarboxylate (230 µL, 1.17 mmol, 1.5 eq) and he mixture was heated at reflux overnight. The reaction was partitioned between dichloromethane and water, and the organic phase was dried (PTFE phase separator) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 24 g RediSep™ silica cartridge) eluting with a gradient of 0 – 50% ethyl acetate in *iso*-heptane afforded the desired product as an off-white foam (715 mg, 0.79 mmol, >100%). LC/MS ($C_{45}H_{56}FN_7O_6SiS_2$) 902 $[M+H]^+$; RT 1.46 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.82 (dt, J = 7.6, 0.9 Hz, 1H), 7.48 - 7.37 (m, 2H), 7.33 (d, J = 11.6 Hz, 1H), 7.28 - 7.13 (m, 3H), 5.84 (s, 2H), 4.32 - 4.17 (m, 6H), 4.15 (t, J = 6.1 Hz, 2H), 3.72 (dd, J = 8.5, 7.4 Hz, 2H), 3.27 (d, J = 15.4 Hz, 2H), 2.93 - 2.75 (m, 5H), 2.36 (s, 3H), 2.19 – 2.10 (m, 2H), 2.10 – 1.98 (m, 2H), 1.40 (s, 9H), 1.28 (t, 3H), 0.96 – 0.89 (m, 2H), -0.11 (s, 9H).

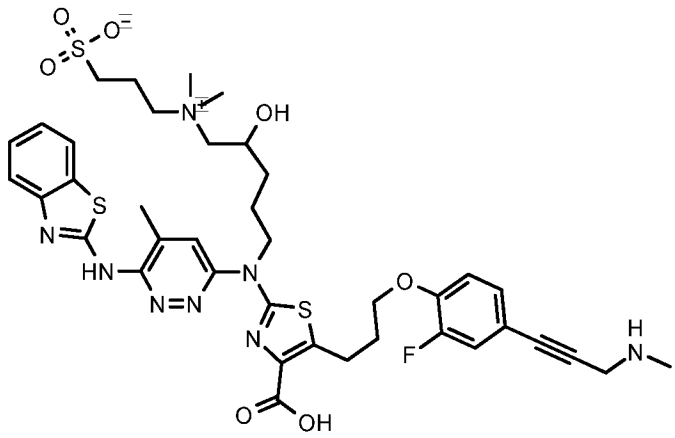
Step B: ethyl 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylate

[622] To a solution of the product from Step A (1.67 g, 1.85 mmol, 1 eq) in acetonitrile (17 mL) was added hydrogen fluoride-pyridine (3.22 mL, 37 mmol, 20 eq) and the mixture was heated at 60 °C for 2 h. The reaction was partitioned between 3:1 dichloromethane / isopropanol and 2N aqueous sodium hydroxide, and the organic phase was washed with brine, dried (PTFE phase separator) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 7% methanol in dichloromethane afforded the desired product as a yellow solid (1.02 g, 1.52 mmol, 82%). LC/MS ($C_{34}H_{34}FN_7O_3S_2$) 672 $[M+H]^+$; RT 2.06 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.89 (dd, J = 7.8, 1.2 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.38 (ddd, J = 8.2, 7.3, 1.2 Hz, 1H), 7.32 - 7.25 (m, 1H), 7.23 – 7.12 (m, 3H), 4.32 – 4.21 (m, 4H), 4.15 (t, J = 6.1 Hz, 2H), 3.45 (s, 2H), 3.32 – 3.23 (m, 2H), 2.89 (t, J = 6.4 Hz, 2H), 2.35 (s, 3H), 2.31 (s, 3H), 2.20 – 2.10 (m, 2H), 2.09 - 1.97 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H).

Step C: 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylic acid

[623] To a solution of the product from Step B (1.02 g, 1.52 mmol, 1 eq) in 1,4-dioxane (50 mL) was lithium hydroxide monohydrate (637 mg, 15.2 mmol, 10 eq) and the mixture was heated at 110 °C overnight. Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 70% 0.7N methanolic ammonia in dichloromethane gave a solid that was triturated with acetonitrile, filtered and dried under vacuum to afford the desired product as a yellow solid (657 mg, 1.02 mmol, 67%). HRMS-ESI (m/z) [M+H]+ calcd for $C_{32}H_{31}FN_7O_3S_2$: 644.1914, found 644.1930.

Preparation of P4: 3-[[5-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[4-carboxy-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazol-2-yl]amino]-2-hydroxy-pentyl]-dimethyl-ammonio]propane-1-sulfonate



Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[4-[tert-butyl(diphenyl)silyl]oxy-5-(dimethylamino)pentyl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

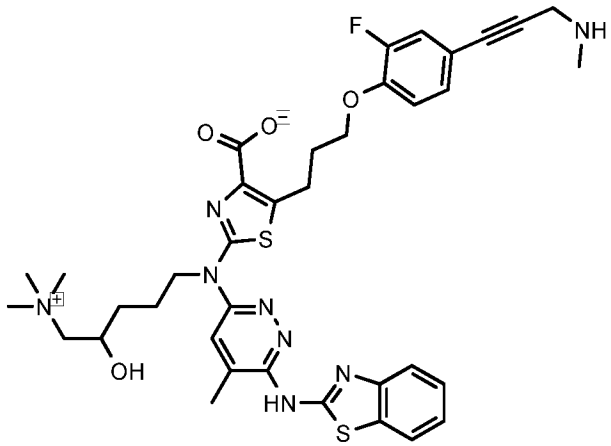
[624] Using **Alkylation with tosylate General Procedure** starting from **Preparation 5a_01** and *N-methylmethanamine* as the appropriate amine, the desired product was obtained.

HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{64}H_{84}FN_8O_7S_2Si_2$: 1215.5421, found 1215.5389.

Step B: 3-[[5-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[4-carboxy-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazol-2-yl]amino]-2-hydroxy-pentyl]-dimethyl-ammonio]propane-1-sulfonate

[625] The product from *Step A* was suspended in MeCN (5 mL/mmol) then *oxathiolane 2,2-dioxide* (10 eq.) was added and stirred at 60°C for on (full conversion was observed). The reaction mixture was concentrated. The crude mixture which contained *3-[[5-[[5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-4-methoxycarbonyl-thiazol-2-yl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]-2-[tert-butyl(diphenyl)silyl]oxy-pentyl]-dimethyl-ammonio]propane-1-sulfonate* (LC-MS-ESI (m/z): $[M+H]^+$ calcd for $C_{67}H_{90}FN_8O_{10}S_3Si_2$: 1337.5, found 1337.6) was transferred directly to the next reaction using **Quaternary salt deprotection General Procedure**, to afford the desired product. **HRMS-ESI (m/z):** $[M+H]^+$ calcd for $C_{39}H_{48}FN_8O_7S_3$: 855.2787, found 855.2786.

Preparation of P5: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[4-hydroxy-5-(trimethylammonio)pentyl]amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate



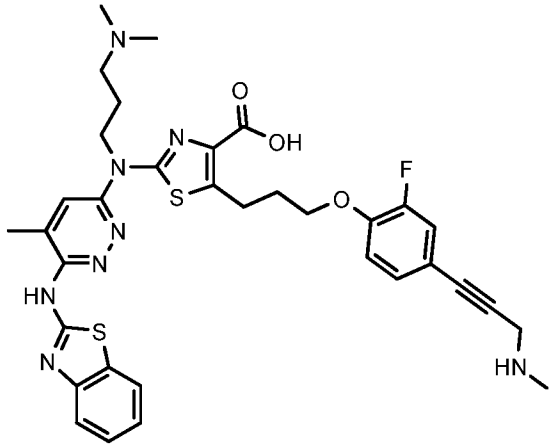
Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[4-[tert-butyl(diphenyl)silyl]oxy-5-(dimethylamino)pentyl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[626] Using **Alkylation with tosylate General Procedure** starting from **Preparation 5a_01** and *N-methylmethanamine* as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{64}H_{84}FN_8O_7S_2Si_2$: 1215.5421, found 1215.5389.

Step B: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[4-hydroxy-5-(trimethylammonio)pentyl]amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[627] The product from *Step A* was dissolved in the mixture of *acetonitrile* (4 mL/mmol) and *N,N-dimethylformamide* (1 mL/mmol) then *iodomethane* (5 eq.) was added and stirred at rt until full conversion was observed (ca. 1 h). The reaction mixture was concentrated. The crude mixture which contained *[5-[[5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-4-methoxycarbonyl-thiazol-2-yl]-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]-2-[tert-butyl(diphenyl)silyl]oxy-pentyl]-trimethyl-ammonium* (LC-MS-ESI (m/z): $[M]^+$ calcd for $C_{65}H_{86}FN_8O_7S_2Si_2$: 1229.6, found 1229.4) was transferred to the next reaction using **Quaternary salt deprotection General Procedure**, to afford the desired product. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{37}H_{44}FN_8O_4S_2$: 747.2905, found 747.2900.

Preparation of P6: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[3-(dimethylamino)propyl]amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



Step A: methyl 2-[tert-butoxycarbonyl-[3-(dimethylamino)propyl]amino]-5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[628] Using **Mitsunobu General Procedure II** starting from **Preparation 1b_01** and *3-(dimethylamino)propan-1-ol*, 1.40 g (quant., the sample contained aprox. 35 n/n% DIAD-2H) of the desired product was produced. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.30 (dd, 1H), 7.21 (dm, 1H), 7.13 (t, 1H), 4.23 (s, 2H), 4.10 (t, 2H), 4.01 (t, 2H), 3.74 (s, 3H), 3.22 (t, 2H), 2.86 (s, 3H), 2.24 (t, 2H), 2.12 (s, 6H), 2.08 (m, 2H), 1.74 (m, 2H), 1.51/1.41 (s, 18H); HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{33}H_{48}FN_4O_7S$: 663.3228, found 663.3218.

Step B: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-(dimethylamino)propylamino]thiazole-4-carboxylate

[629] Using **Deprotection with HFIP General Procedure** starting from the product from *Step A*, 0.95 g (80%) of the desired product was produced. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.57 (t, 1H), 7.31 (d, 1H), 7.21 (d, 1H), 7.13 (t, 1H), 4.23 (br., 2H), 4.07 (t, 2H), 3.69 (s, 3H), 3.17 (q, 2H), 3.12 (t, 2H), 2.86 (br., 3H), 2.24 (t, 2H), 2.11 (s, 6H), 2.00 (quint., 2H), 1.63 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 129.1, 119.3, 115.4, 68, 57.0, 51.7, 45.6, 42.8, 38.6, 33.8, 30.6, 28.5, 27.0, 23.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{28}H_{40}FN_4O_5S$: 563.2703, found 563.2694.

Step C: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-(dimethylamino)propyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

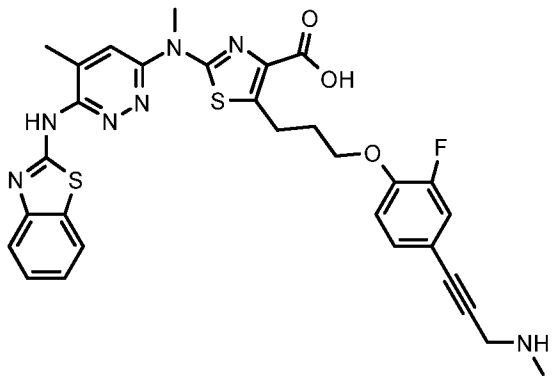
[630] Using **Buchwald General Procedure III** starting from the product from *Step B* and **Preparation 4a_01**, 0.79 g (51%) of the desired product was produced. ^{1}H NMR (500 MHz,

DMSO-d_6) δ ppm 7.84 (d, 1H), 7.73 (s, 1H), 7.46 (dd, 1H), 7.43 (td, 1H), 7.31 (brd., 1H), 7.25 (td, 1H), 7.21 (d, 1H), 7.16 (t, 1H), 5.86 (s, 2H), 4.35 (t, 2H), 4.20 (br., 2H), 4.15 (t, 2H), 3.76 (s, 3H), 3.72 (t, 2H), 3.27 (t, 2H), 2.84 (br., 3H), 2.45 (s, 3H), 2.32 (t, 2H), 2.18 (s, 6H), 2.13 (m, 2H), 1.86 (m, 2H), 1.40 (s, 9H), 0.92 (t, 2H), -0.11 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 129.1, 127.2, 123.4, 123.2, 119.3, 117.6, 115.4, 111.9, 72.8, 68.4, 66.7, 56.4, 51.9, 45.7, 45.5, 38.5, 33.8, 31.0, 28.5, 25.0, 23.1, 17.9, 17.8, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{46}H_{62}FN_8O_6S_2Si$: 933.3987, found 933.3990.

Step D: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-[3-(dimethylamino)propyl]amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[631] Using **Deprotection and Hydrolysis General Procedure** followed by repurification via reverse phase preparative chromatography (C18, 0.1% TFA in water : MeCN) starting from the product from *Step C*, the TFA-salt of the desired product was obtained. HRMS-ESI (m/z): $[M+2H]^{2+}$ calcd for $C_{34}H_{39}FN_8O_3S_2$: 345.1280, found 345.1265.

Preparation of P7: 2-({6-[(1,3-Benzothiazol-2-yl)amino]-5-methylpyridazin-3-yl}(methyl)amino)-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylic acid



Step A: ethyl 2-({6-[(1,3-benzothiazol-2-yl)amino]-5-methylpyridazin-3-yl}(methyl)amino)-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylate

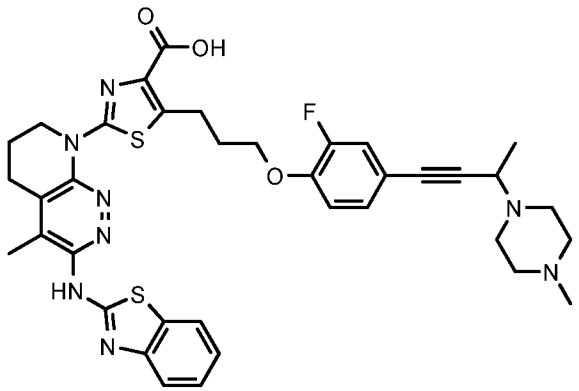
[632] Trifluoroacetic acid (20 mL) was added to a stirred solution of the product from **Preparation 5j_01**, Step A (1.5 g, 1.71 mmol, 1 eq) in dichloromethane (60 mL) and the mixture was stirred at ambient temperature overnight. The reaction was diluted with dichloromethane, cooled to 0 °C then basified by the addition of 2N aqueous sodium hydroxide, and the organic phase was dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™

silica cartridge) eluting with a gradient of 0 – 10% methanol in dichloromethane afforded the desired product as a yellow solid (361 mg, 0.56 mmol, 33%). LC/MS ($C_{32}H_{32}FN_7O_3S_2$) 646 $[M+H]^+$; RT 1.98 (LCMS-V-C). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.91 (d, 1H), 7.68 (d, J = 1.2 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.39 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 7.32 - 7.11 (m, 4H), 4.25 (q, J = 7.1 Hz, 2H), 4.15 (t, J = 6.2 Hz, 2H), 3.77 (s, 3H), 3.46 (s, 2H), 3.27 (t, J = 7.7 Hz, 2H), 2.47 (d, J = 1.0 Hz, 3H), 2.31 (s, 3H), 2.19 - 2.07 (m, 2H), 2.23 (s, 1H), 1.30 (t, J = 7.1 Hz, 3H).

Step B: 2-({6-[(1,3-benzothiazol-2-yl)amino]-5-methylpyridazin-3-yl}(methyl)amino)-5-(3-{2-fluoro-4-[3-(methylamino)prop-1-yn-1-yl]phenoxy}propyl)-1,3-thiazole-4-carboxylic acid

[633] To a solution of the product from Step B (361 mg, 0.56 mmol, 1 eq) in 1,4-dioxane (15 mL) was added lithium hydroxide monohydrate (352 mg, 8.39 mmol, 15 eq) and the mixture was heated at 100 °C overnight. The reaction was allowed to cool to ambient temperature and concentrated *in vacuo*. The residue was triturated with water, filtered, washed with water then diethyl ether, and dried under vacuum to afford the desired product as a yellow solid (286 mg, 0.46 mmol, 83%) [as a lithium salt]. HRMS-ESI (m/z) [M+H]+ calcd for $C_{30}H_{29}FN_7O_3S_2$: 618.1752, found 618.1767.

Preparation of P8: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)but-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



Step A: methyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)but-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[634] A 24 mL oven-dried vial was equipped with a *PTFE*-coated magnetic stirring bar, and was charged with 250 mg 1-methylpiperazine (2.5 mmol, 5.0 eq.) dissolved in 2.5 mL dry THF Then 133 mg 3-bromobut-1-yne (1.0 mmol, 2.0 equiv) was added dropwise via syringe over a period of 5 minutes, and stirred at that temperature for 30 min. To the resulting mixture 301 mg of **Preparation 3a** (0.50 mmol, 1.0 eq.), 18.15 mg $Pd(PPh_3)_2Cl_2$ (0.025

mmol, 0.05 eq.) and 4.76 CuI (0.025 mmol, 0.05 eq.) were added, then it was heated to 60°C and stirred for 2h at that temperature. The reaction reached complete conversion. *Celite* was added to the reaction mixture and the volatiles were removed under reduced pressure. Then it was purified via flash chromatography using DCM and MeOH (1.2% NH_3) as eluents to give 300 mg (95% Yield) of the desired product.

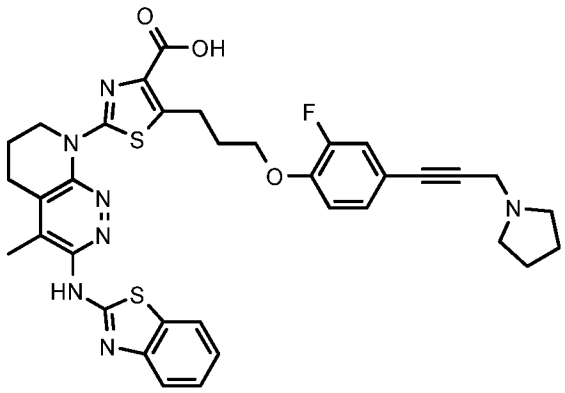
Step B: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)but-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[635] Using **Buchwald General Procedure II** starting from 300 mg of the product from *Step A* (0.47 mmol, 1.0 eq.) and 140 mg *1,3-benzothiazol-2-amine* (0.94 mmol, 2.0 eq.), 150 mg (42%) mg of the desired product was obtained.

Step C: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)but-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[636] Using **Hydrolysis General Procedure** starting from the product from *Step B* as the appropriate methyl ester, the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.87 (d, 1H), 7.49 (d, 1H), 7.36 (t, 1H), 7.26 (dd, 1H), 7.2 (t, 1H), 7.16 (dd, 1H), 7.13 (t, 1H), 4.27 (t, 2H), 4.12 (t, 2H), 3.65 (q, 1H), 3.27 (t, 2H), 2.87 (t, 2H), 2.62-2.21 (brm, 8H), 2.14 (s, 3H), 2.13 (qn, 2H), 2.04 (qn, 2H), 1.33 (s, 3H), 1.25 (d, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 164.3, 155.4, 151.5, 151.4, 148.6, 147.2, 145.1, 140.2, 136.3, 130.2, 129.0, 129.0, 127.6, 126.5, 122.5, 122.3, 119.2, 116.4, 115.5, 115.4, 88.4, 84.1, 68.5, 51.7, 46.3, 46.1, 31, 23.9, 23.0, 20.3, 19.6, 12.9; HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{37}H_{40}FN_8O_3S_2$: 727.2649, found 727.2630

Preparation of P9: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-pyrrolidin-1-ylprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylic acid



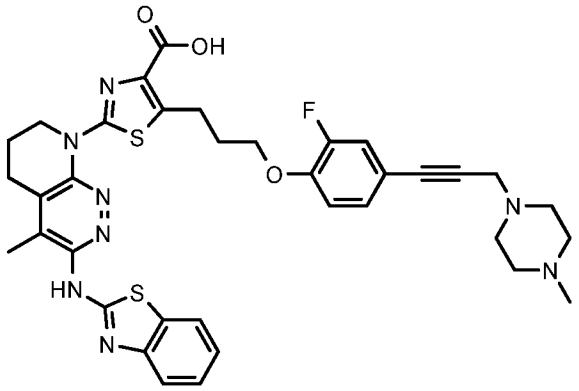
Step A: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-pyrrolidin-1-ylprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylate

[637] Using **Propargylic amine preparation General Procedure** starting from 258 mg of **Preparation 3d** (0.40 mmol, 1eq.) as the appropriate propargylic alcohol and pyrrolidine (20 eq, 670 mg), 120 mg of the desired product (43%) was obtained.

Step B: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-pyrrolidin-1-ylprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylic acid

[638] Using **Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate methyl ester, the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.88 (d, 1H), 7.49 (d, 1H), 7.37 (t, 1H), 7.29 (dd, 1H), 7.2 (dd, 1H), 7.19 (t, 1H), 7.14 (t, 1H), 4.27 (t, 2H), 4.14 (t, 2H), 3.52 (s, 2H), 3.27 (t, 2H), 2.88 (t, 2H), 2.52 (t, 4H), 2.34 (s, 3H), 2.13 (qn, 2H), 2.04 (qn, 2H), 1.69 (t, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 151.5, 151.4, 148.6, 147.3, 145.1, 140.1, 136.7, 130.2, 129.0, 129.0, 127.5, 126.5, 122.5, 122.3, 119.2, 116.5, 115.5, 115.4, 85.9, 83.3, 68.6, 52.3, 46.3, 43.3, 31.1, 23.8, 23.8, 23.0, 20.4, 12.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{35}H_{35}FN_7O_3S_2$: 684.2221, found 684.2209.

Preparation of P10: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)prop-1-ynyl]phenoxy]propyl] thiazole-4-carboxylic acid



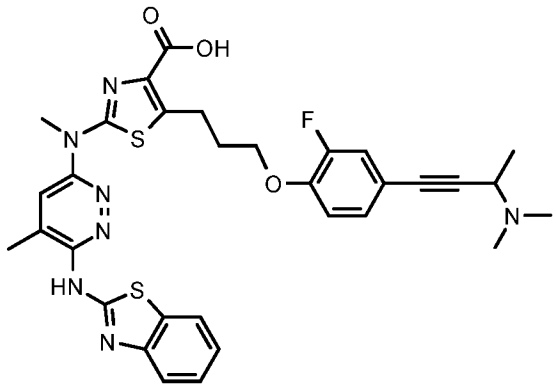
Step A: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[639] Using **Propargylic amine preparation General Procedure** starting from 100 mg of **Preparation 3d** (0.155 mmol, 1eq.) as the appropriate propargylic alcohol and 1-methylpiperazine (310.7 mg, 20 eq.), 150 mg of the desired product (79%) was obtained.

Step B: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(4-methylpiperazin-1-yl)prop-1-ynyl]phenoxy]propyl] thiazole-4-carboxylic acid

[640] Using **Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{36}H_{38}FN_8O_3S_2$: 713.2486, found 713.2474.

Preparation of P11: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)but-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



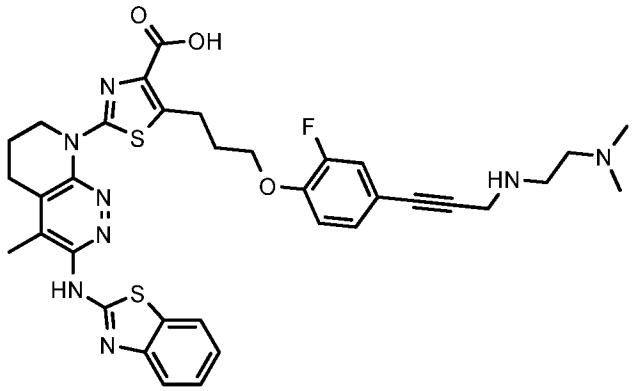
Step A: ethyl 5-[3-[4-[3-(dimethylamino)but-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[methyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

[641] Using **Alkylation General Procedure** starting from **Preparation 5g_01** and **Preparation 6f_01** as the appropriate phenol, the desired product was obtained.

Step B: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)but-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[642] Using **Deprotection and Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate ethyl ester, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{32}H_{33}FN_7O_3S_2$: 646.2065, found 646.2057.

Preparation of P12: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-[2-(dimethylamino)ethylamino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl-[2-(dimethylamino)ethyl]amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)thiazole-4-carboxylate

[643] Using **Sonogashira General Procedure** starting from 1.00 g of **Preparation 3a** (1.66 mmol, 1 eq.) and 413 mg of *tert-butyl N-[2-(dimethylamino)ethyl]-N-prop-2-ynyl-carbamate* (1.83 mmol, 1.1 eq.) as the appropriate alkyne, the desired product was isolated as yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.30 (d, 1H), 7.21 (d, 1H), 7.15 (t, 1H), 4.27 (brt, 2H), 4.26 (t, 2H), 4.12 (t, 2H), 3.77 (s, 3H), 3.47 (brt, 2H), 3.26 (t, 2H), 2.89 (t, 2H), 2.82 (brs, 2H), 2.45 (brs, 6H), 2.32 (s, 3H), 2.11 (qn, 2H), 2.04 (qn, 2H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 155.4, 151.8, 151.4, 151.4, 147.5, 142.4, 136.2, 135, 129.1, 129.1, 119.2, 115.5, 114.8, 82.3, 80.3, 68.3, 56.3, 52.0, 46.4, 46.4, 44.6, 43.1, 30.7, 28.5, 24.2, 23, 19.7, 15.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{43}ClFN_6O_5S$: 701.2683, found 701.2678.

Step B: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[tert-butoxycarbonyl-[2-(dimethylamino)ethyl]amino]prop-1-

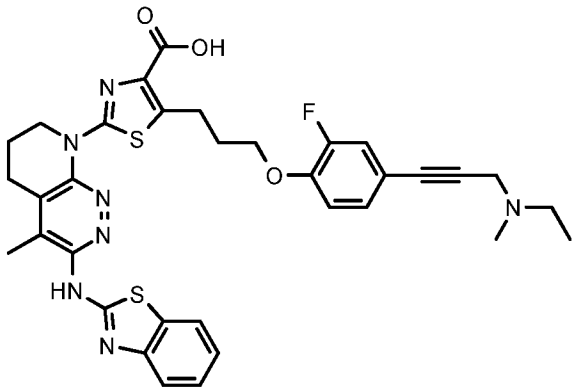
ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[644] Using **Buchwald General Procedure II** starting from the product from *Step A* and *1,3-benzothiazol-2-amine*, the desired product was obtained. LC-MS-ESI (m/z): $[M+H]^+$ calcd for $C_{41}H_{48}FN_8O_5S_2$: 815.3, found 815.4.

Step C: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[2-(dimethylamino)ethylamino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

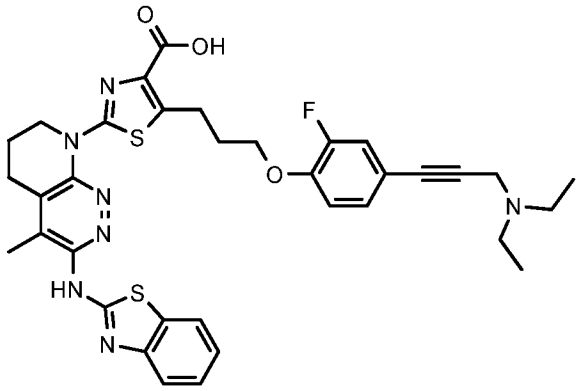
[645] Using **Deprotection and Hydrolysis General Procedure** followed by repurification via reverse phase preparative chromatography (C18, 25 mM NH_4HCO_3 in water : MeCN) starting from the product from *Step B*, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{35}H_{38}FN_8O_3S_2$: 701.2487, found 701.2483.

Preparation of P13: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-[ethyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



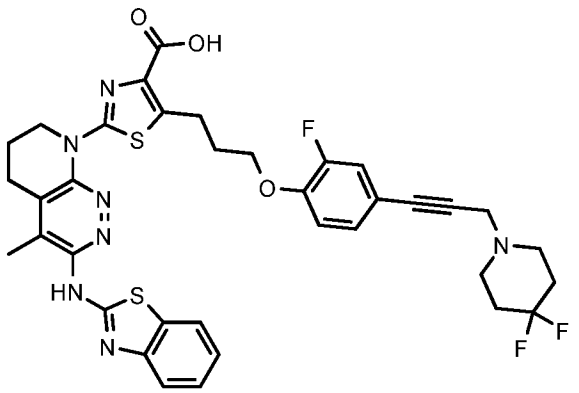
[646] Using **Silver catalyzed propargylic amine preparation General Procedure** starting from **Preparation 3c**, paraformaldehyde as the aldehyde and *N*-methylethanamine as the appropriate secondary amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{35}FN_7O_3S_2$: 672.2221, found 672.2206.

Preparation of P14: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-(diethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



[647] Using **Silver catalyzed propargylic amine preparation General Procedure** starting from **Preparation 3c**, paraformaldehyde as the aldehyde and diethyl amine as the appropriate secondary amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{35}H_{37}FN_7O_3S_2$: 686.2377, found 686.2386.

Preparation of P15: 2-{3-[(1,3-Benzothiazol-2-yl)amino]-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{4-[3-(4,4-difluoropiperidin-1-yl)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylic acid



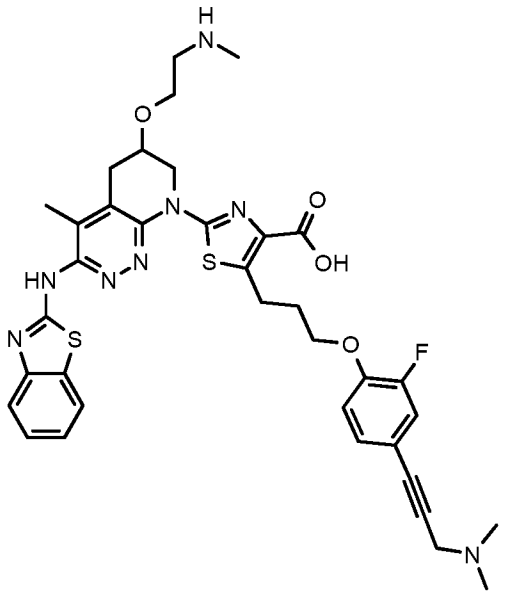
Step A: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(4,4-difluoro-1-piperidyl)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[648] Using **Propargylic amine preparation General Procedure** starting from 100 mg of **Preparation 3d** (0.155 mmol, 1eq.) as the appropriate propargylic alcohol and 4,4-difluoropiperidine (20 eq.), 120 mg of the desired product (72%) was obtained.

Step B: 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{4-[3-(4,4-difluoropiperidin-1-yl)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylic acid

[649] Using **Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd $C_{36}H_{35}F_3N_7O_3S_2$: 734.2189, found 734.2185.

Preparation of P16: 2-{3-[(1,3-Benzothiazol-2-yl)amino]-4-methyl-6-[2-(methylamino)ethoxy]-5*H*,6*H*,7*H*,8*H*-pyrido[2,3-*c*]pyridazin-8-yl}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylic acid



Step A: 4-methylmorpholin-3-one

[650] A solution of 2-(methylamino)ethanol (5.32 mL, 66.6 mmol, 1 eq) in ethanol (100 mL) and 35% aqueous sodium hydroxide (6.25 mL) was cooled to 15-20 °C and chloroacetyl chloride (13.3 mL, 166 mmol, 2.5 eq) and 35% aqueous sodium hydroxide (22 mL) were added simultaneously with vigorous stirring over 1 h. The mixture was stirred for 20 min, then neutralised with aqueous hydrochloric acid and extracted with dichloromethane (3 x 100 mL). The combined organic extracts were washed with water, dried (PTFE phase separator) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded the desired product as a colourless oil (4.4 g, 38.2 mmol, 58%). ^{1}H NMR (400 MHz, DMSO-d6) δ 4.00 (s, 2H), 3.84 – 3.78 (m, 2H), 3.36 – 3.29 (m, 2H), 2.86 (s, 3H).

Step B: 2-(but-2-yn-1-yl)-4-methylmorpholin-3-one

[651] To a solution of diisopropylamine (6.45 mL, 45.9 mmol, 1.2 eq) in tetrahydrofuran (130 mL), cooled to -78 °C, was added *n*-butyllithium (2.06M in hexanes; 20.4 mL, 42 mmol,

1.1 eq) dropwise. After 1 minute a solution of the product from Step A (4.4 g, 38.2 mmol, 1 eq) in tetrahydrofuran (30 mL) was added dropwise. After 15 minutes a solution of 1-bromo-2-butyne (4.02 mL, 45.9 mmol, 1.2 eq) in tetrahydrofuran (15 mL) was added dropwise and the mixture was stirred at -78 °C for 1 h then allowed to warm to ambient temperature. Saturated aqueous ammonium chloride was added and the mixture was extracted with ethyl acetate (x3), and the combined organic extracts were dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded the desired product as a yellow oil (5.15 g, 30.8 mmol, 81%). ^{1}H NMR (400 MHz, DMSO-d6) δ 4.09 (dd, J = 7.6, 3.5 Hz, 1H), 4.01 – 3.94 (m, 1H), 3.76 (ddd, J = 11.9, 10.0, 3.6 Hz, 1H), 3.52 – 3.41 (m, 1H), 3.26 – 3.18 (m, 1H), 2.86 (s, 3H), 2.67 – 2.58 (m, 1H), 2.57 – 2.44 (m, 1H), 1.73 (t, J = 2.6 Hz, 3H).

Step C: 2-[2-(methylamino)ethoxy]hex-4-ynoic acid

[652] To a solution of the product from Step B (3.25 g, 19.4 mmol, 1 eq) in methanol (110 mL) was added 1M aqueous lithium hydroxide (60.3 mL, 60.3 mmol, 3.1 eq) and the mixture was heated at reflux overnight. The reaction was concentrated *in vacuo* to afford the desired product as an orange gum (5.15 g, 27.8 mmol, 100%) that was used directly in the subsequent step without further characterisation.

Step D: 2-[2-({[(9H-fluoren-9-yl)methoxy]carbonyl}(methyl)amino)ethoxy]hex-4-ynoic acid

[653] To a solution of the product from Step C (5.15 g, 27.8 mmol, 1 eq) in 1,4-dioxane (45 mL) and water (160 mL) was added potassium carbonate (15.4 g, 111 mmol, 4 eq) at 0 °C, followed by 9*H*-fluoren-9-yl-methyl chloroformate (7.19 g, 27.8 mmol, 1 eq) and the mixture was allowed to warm to ambient temperature and stir for 2 h. The reaction was partitioned between water and ethyl acetate, and the aqueous phase was acidified with aqueous hydrochloric acid to pH 2-3 and extracted with ethyl acetate (3 x 300 mL). The combined organic extracts were washed with brine, dried (magnesium sulfate) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 120 g RediSep™ silica cartridge) eluting with a gradient of 0 – 20% methanol in dichloromethane afforded the desired product as a dark yellow gum (7.06 g, 17.3 mmol, 62%). LC/MS ($C_{24}H_{25}NO_5$) 408 $[M+H]^+$; RT 0.74 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.90 (t, J = 6.8 Hz, 2H), 7.65 (dd, J = 7.5, 1.1 Hz, 2H), 7.42 (td, J = 7.4, 3.0 Hz, 2H), 7.34 (td, J = 7.4, 1.3 Hz, 2H), 4.43 – 4.22 (m, 3H), 3.50 – 3.42 (m, 1H), 3.39 – 3.28 (m, 1H), 3.26 – 3.15 (m, 3H), 2.90 – 2.82 (m, 3H), 2.51 – 2.44 (m, 2H), 1.71 (dt, J = 13.8, 2.5 Hz, 3H).

Step E: (9H-fluoren-9-yl)methyl N-{2-[(1-hydroxyhex-4-yn-2-yl)oxy]ethyl}-N-methylcarbamate

[654] A solution of the product from Step D (7.06 g, 17.33 mmol, 1 eq) in tetrahydrofuran (120 mL) was cooled to -10 ºC, then triethylamine (2.65 mL, 19.1 mmol, 1.1 eq) and isobutyl chloroformate (2.7 mL, 20.8 mmol, 1.2 eq) in THF (40 mL) were added dropwise. The precipitate was removed by filtration and the solution was cooled to -10 °C. Sodium borohydride (2.62 g, 69.3 mmol, 4 eq) in water (40 mL) was added dropwise and the mixture was stirred for 1 h at -10 ºC. The pH of the solution was adjusted to pH 5 using 1N aqueous hydrochloric acid, and then adjusted to pH 10 using saturated aqueous sodium bicarbonate. The layers were separated and the organic phase was successively washed water (100 mL) and brine (50 mL), dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 80 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded the desired product as a colourless gum (4.64 g, 11.8 mmol, 68%). LC/MS ($C_{24}H_{27}NO_4$) 394 $[M+H]^+$; RT 0.77 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.90 (d, J = 7.5 Hz, 2H), 7.65 (dt, J = 7.4, 0.9 Hz, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.35 (td, J = 7.4, 1.2 Hz, 2H), 4.68 – 4.60 (m, 1H), 4.39 (d, J = 6.0 Hz, 1H), 4.34 (d, J = 6.7 Hz, 1H), 4.28 (t, J = 6.4 Hz, 1H), 3.60 – 3.51 (m, 1H), 3.46 – 3.36 (m, 2H), 3.34 – 3.28 (m, 2H), 3.19 (dd, J = 16.6, 5.5 Hz, 2H), 2.84 (d, J = 10.8 Hz, 3H), 2.38 – 2.15 (m, 2H), 1.71 (t, J = 2.5 Hz, 3H).

Step F: (9H-fluoren-9-yl)methyl N-[2-({1-[(tert-butyldiphenylsilyl)oxy]hex-4-yn-2-yl}oxy)ethyl]-N-methylcarbamate

[655] To a cooled solution of the product from Step E (4.64 g, 11.8 mmol, 1 eq) and imidazole (1.56 mL, 23.6 mmol, 2 eq) in dichloromethane (200 mL) was added *tert*-butyl(chloro)diphenylsilane (6.13 mL, 23.6 mmol, 2 eq) dropwise and the mixture was allowed to warm to ambient temperature and stir overnight. The reaction was quenched with 2M aqueous ammonium chloride and the mixture was extracted with dichloromethane (3 x 200 mL). The combined organic extracts were washed with brine, dried (PTFE phase separator) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 120 g RediSep™ silica cartridge) eluting with a gradient of 0 – 25% ethyl acetate in *iso*-heptane afforded the desired product as a colourless gum (5.86 g, 9.27 mmol, 79%). LC/MS ($C_{40}H_{45}NO_4Si$) 632 $[M+H]^+$; RT 1.38 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.87 (dd, J = 20.0, 7.5 Hz, 2H), 7.67 – 7.56 (m, 6H), 7.53 – 7.39 (m, 7H), 7.39 – 7.22 (m, 3H), 4.38 (t, J = 4.8 Hz, 1H), 4.31 (s, 1H), 4.24 (t, J = 5.7 Hz, 1H), 3.73 –

3.61 (m, 1H), 3.60 – 3.44 (m, 2H), 3.34 – 3.29 (m, 2H), 3.29 – 3.18 (m, 1H), 3.16 – 3.06 (m, 1H), 2.81 (d, J = 14.1 Hz, 3H), 2.43 – 2.26 (m, 2H), 1.69 (t, J = 2.4 Hz, 3H), 0.98 (s, 9H).

Step G: (9H-fluoren-9-yl)methyl N-[2-({1-[(tert-butyldiphenylsilyl)oxy]-3-(3,6-dichloro-5-methylpyridazin-4-yl)propan-2-yl}oxy)ethyl]-N-methylcarbamate

[656] A solution of the product from Step F (5.86 g, 9.27 mmol, 1 eq) and 3,6-dichloro-1,2,4,5-tetrazine (5.6 g, 37.1 mmol, 4 eq) in toluene (130 mL) was heated at 150 °C overnight in a sealed flask. The reaction was concentrated *in vacuo* and purification by automated flash column chromatography (CombiFlash Rf, 120 g RediSep™ silica cartridge) eluting with a gradient of 0 – 30% ethyl acetate in *iso*-heptane afforded the desired product as a pink foam (2.99 g, 3.97 mmol, 43%). **L**C/MS ($C_{42}H_{45}Cl_2N_3O_4Si$) 754 $[M+H]^+$; RT 1.37 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.90 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 7.4 Hz, 1H), 7.68 – 7.59 (m, 5H), 7.57 – 7.50 (m, 1H), 7.47 – 7.41 (m, 6H), 7.45 – 7.37 (m, 1H), 7.36 – 7.28 (m, 2H), 7.23 (t, J = 7.5 Hz, 1H), 4.30 (d, J = 5.7 Hz, 1H), 4.27 – 4.11 (m, 2H), 3.81 – 3.60 (m, 3H), 3.55 – 3.45 (m 1H), 3.20 – 2.98 (m, 4H), 2.89 – 2.77 (m, 1H), 2.58 (d, J = 23.0 Hz, 3H), 2.39 (d, J = 13.1 Hz, 3H), 1.01 (s, 9H).

Step H: 4-{3-[(tert-butyldiphenylsilyl)oxy]-2-[2-(methylamino)ethoxy]propyl}-3,6-dichloro-5-methylpyridazine

[657] A solution of the product from Step G (2.79 g, 3.7 mmol, 1 eq) and diethylamine (0.77 mL, 7.39 mmol, 2 eq) in acetonitrile (60 mL) was stirred at ambient temperature overnight. Water was added and the mixture was extracted with ethyl acetate (3 x 70 mL). The combined organic extracts were washed with brine (100 mL), dried (magnesium sulfate) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™ silica cartridge) eluting with a gradient of 0 – 16% methanol in dichloromethane afforded the desired product as an orange/ pink gum (1.9 g, 3.57 mmol, 96%). LC/MS ($C_{27}H_{35}Cl_2N_3O_2Si$) 532 $[M+H]^+$; RT 0.84 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.69 – 7.62 (m, 4H), 7.54 – 7.41 (m, 6H), 3.83 – 3.60 (m, 3H), 3.42 – 3.36 (m, 1H), 3.16 – 2.97 (m, 3H), 2.45 (s, 3H), 2.39 – 2.23 (m, 2H), 2.06 (s, 3H), 1.02 (s, 9H).

Step I: tert-butyl N-[2-({1-[(tert-butyldiphenylsilyl)oxy]-3-(3,6-dichloro-5-methylpyridazin-4-yl)propan-2-yl}oxy)ethyl]-N-methylcarbamate

[658] To a solution of the product from Step H (1.9 g, 3.57 mmol, 1 eq) in dichloromethane (100 mL) was added di-*tert*-butyl dicarbonate (1.53 mL, 7.14 mmol, 2 eq) followed by triethylamine (1.99 mL, 14.3 mmol, 4 eq) and the mixture was stirred at ambient temperature

for 4 h. The reaction was partitioned between dichloromethane and water, and the aqueous phase was acidified to pH 4 and extracted with dichloromethane (3 x 80 mL). The combined organic extracts were washed with brine, dried (PTFE phase separator) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 40 g RediSep™ silica cartridge) eluting with a gradient of 0 – 25% ethyl acetate in *iso*-heptane afforded the desired product as a colourless gum (1.83 g, 2.9 mmol, 81%). LC/MS ($C_{32}H_{43}Cl_2N_3O_4Si$) 532 $[M\text{-}Boc+H]^+$; RT 1.33 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.69 – 7.62 (m, 4H), 7.54 – 7.41 (m, 6H), 3.76 (qd, J = 10.7, 4.7 Hz, 2H), 3.66 (d, J = 5.5 Hz, 1H), 3.44 (q, J = 7.9, 6.3 Hz, 1H), 3.20 – 3.10 (m, 3H), 3.04 (dd, J = 14.0, 4.1 Hz, 2H), 2.58 (s, 3H), 2.44 (s, 3H), 1.31 (d, J = 22.6 Hz, 9H), 1.02 (s, 9H).

Step J: tert-butyl N-(2-{[1-(3,6-dichloro-5-methylpyridazin-4-yl)-3-hydroxypropan-2-yl]oxy}ethyl)-N-methylcarbamate

[659] A solution of the product from Step I (1.83 g, 2.9 mmol, 1 eq) in tetrahydrofuran (75 mL) was cooled to 0 °C before the addition of tetrabutylammonium fluoride (1M in tetrahydrofuran; 2.9 mL, 2.9 mmol, 1 eq) and stirring at 0 °C for 30 min, then at ambient temperature for 1 h. The reaction was partitioned between dichloromethane and water, and the aqueous phase was extracted with dichloromethane (x2). The combined organic extracts were washed with brine, dried (PTFE phase separator) and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 24 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded the desired product as a pale orange gum (0.73 g, 1.86 mmol, 64%). ^{1}H NMR (400 MHz, DMSO-d6) δ 4.93 (t, J = 5.5 Hz, 1H), 3.62 – 3.44 (m, 4H), 3.23 (dt, J = 9.6, 6.0 Hz, 1H), 3.11 (d, J = 23.9 Hz, 2H), 3.02 (dd, J = 6.5, 2.0 Hz, 2H), 2.60 (d, J = 8.1 Hz, 3H), 2.45 (s, 3H), 1.35 (d, J = 13.0 Hz, 9H).

Step K: methyl 2-{[(tert-butoxy)carbonyl][2-(2-{[(tert-butoxy)carbonyl](methyl)amino}ethoxy)-3-(3,6-dichloro-5-methylpyridazin-4-yl)propyl]amino}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylate

[660] To a solution of the product from Step J (125 mg, 0.32 mmol, 1 eq) in toluene (20 mL) was added the product from **Preparation 1c** (171 mg, 0.35 mmol, 1.1 eq), di-*tert*-butyl azodicarboxylate (146 mg, 0.63 mmol, 2 eq) and triphenylphosphine (166 mg, 0.63 mmol, 2 eq) and the mixture was stirred at 50 °C for 1 h. The reaction was partitioned between dichloromethane and water, and the aqueous phase was extracted with dichloromethane (x2), and the combined organic extracts were washed with brine, dried (magnesium sulfate)

and concentrated *in vacuo.* Purification by automated flash column chromatography (CombiFlash Rf, 12 g RediSep™ silica cartridge) eluting with a gradient of 0 – 100% ethyl acetate in *iso*-heptane afforded the desired product as a pale yellow gum (282 mg, 0.32 mmol, 102%). LC/MS ($C_{40}H_{53}Cl_2FN_6O_8S$) 867 $[M+H]^+$; RT 0.97 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.30 (dd, 1H), 7.23 – 7.17 (m, 1H), 7.12 (t, 1H), 4.29 (dd, J = 13.9, 5.7 Hz, 1H), 4.10 (t, J = 6.0 Hz, 2H), 3.96 – 3.87 (m, 1H), 3.74 (s, 3H), 3.61 – 3.48 (m, 1H), 3.42 (s, 3H), 3.32 (s, 2H), 3.25 (dt, J = 7.1, 3.9 Hz, 3H), 3.16 – 2.99 (m, 2H), 2.97 – 2.89 (m, 1H), 2.58 (d, J = 11.6 Hz, 2H), 2.45 (s, 3H), 2.23 (s, 6H), 2.10 (t, J = 6.9 Hz, 2H), 1.52 (s, 9H), 1.31 (d, J = 39.6 Hz, 9H).

Step L: methyl 2-{[2-(2-{[(tert-butoxy)carbonyl](methyl)amino}ethoxy)-3-(3,6-dichloro-5-methylpyridazin-4-yl)propyl]amino}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylate

[661] A solution of the product from Step K (275 mg, 0.32 mmol, 1 eq) in 1,1,1,3,3,3-hexafluoro-2-propanol (2.5 mL, 23.7 mmol, 74.7 eq) was heated at 100 °C for 60 min under microwave irradiation. The reaction was concentrated *in vacuo* and purification by automated flash column chromatography (CombiFlash Rf, 12 g RediSep™ silica cartridge) eluting with a gradient of 0 – 7% methanol in dichloromethane afforded the desired product as a white solid (154 mg, 0.2 mmol, 63%). LC/MS ($C_{35}H_{45}Cl_2FN_6O_6S$) 767 $[M+H]^+$; RT 0.70 (LCMS-V-B2). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.83 (br s, 1H), 7.30 (dd, J = 11.9, 2.0 Hz, 1H), 7.24 – 7.17 (m, 1H), 7.12 (t, J = 8.7 Hz, 1H), 4.08 (t, J = 6.1 Hz, 2H), 3.82 (dt, J = 9.0, 4.5 Hz, 1H), 3.70 (s, 3H), 3.60 – 3.49 (m, 1H), 3.46 – 3.39 (m, 4H), 3.33 (s, 2H), 3.29 – 3.18 (m, 1H), 3.14 (t, 2H), 3.10 – 3.02 (m, 2H), 2.98 (dd, J = 13.9, 3.8 Hz, 1H), 2.64 – 2.53 (m, 2H), 2.44 (s, 3H), 2.23 (s, 6H), 2.07 – 1.95 (m, 2H), 1.32 (d, J = 30.8 Hz, 9H).

Step M: methyl 2-[6-(2-{[(tert-butoxy)carbonyl](methyl)amino}ethoxy)-3-chloro-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl]-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylate

[662] To a solution of the product from Step L (154 mg, 0.2 mmol, 1 eq) in 1,4-dioxane (14 mL) was added cesium carbonate (131 mg, 0.4 mmol, 2 eq), *N,N*-diisopropylethylamine (0.07 mL, 0.4 mmol, 2 eq) and bis(di-*tert*-butyl(4-dimethylaminophenyl)phosphine) dichloropalladium(II) (14.2 mg, 0.02 mmol, 0.1 eq) and the mixture was heated at 80 °C for 45 min. The reaction was partitioned between dichloromethane and water, and the aqueous phase was extracted with dichloromethane (x2). The combined organic extracts were washed with brine, dried (magnesium sulfate) and concentrated *in vacuo*. Purification by automated flash column chromatography (CombiFlash Rf, 12 g RediSep™ silica cartridge)

eluting with a gradient of 0 – 8% methanol in dichloromethane afforded the desired product as a cream solid (136 mg, 0.19 mmol, 93%). LC/MS ($C_{35}H_{44}ClFN_6O_6S$) 731 $[M+H]^+$; RT 0.75 (LCMS-V-B2). ^1H NMR (400 MHz, DMSO-d6) δ 7.31 (dt, J = 12.0, 1.9 Hz, 1H), 7.25 – 7.19 (m, 1H), 7.14 (t, 1H), 4.86 (dd, 1H), 4.25 (s, 1H), 4.13 (t, J = 6.2 Hz, 2H), 3.93 (d, J = 13.5 Hz, 1H), 3.78 (s, 3H), 3.56 (t, J = 5.6 Hz, 2H), 3.42 (s, 3H), 3.32 (s, 2H), 3.30 – 3.23 (m, 2H), 3.21 – 3.09 (m, 2H), 3.08 – 3.00 (m, 1H), 2.58 – 2.52 (m, 1H), 2.34 (s, 3H), 2.23 (s, 6H), 2.12 (p, J = 6.7 Hz, 2H), 1.27 (d, J = 28.5 Hz, 9H).

Step N: methyl 2-{3-[(1,3-benzothiazol-2-yl)amino]-6-(2-{[(tert-butoxy)carbonyl](methyl)amino}ethoxy)-4-methyl-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylate

[663] To a solution of the product from Step M (136 mg, 0.19 mmol, 1 eq) in cyclohexanol (4.5 mL) was added 2-aminobenzothiazole (55.7 mg, 0.37 mmol, 2 eq) and *N,N*-diisopropylethylamine (0.1 mL, 0.56 mmol, 3 eq) and the mixture was sparged with nitrogen (10 min). Xantphos (21.5 mg, 0.04 mmol, 0.2 eq) and tris(dibenzylideneacetone)dipalladium(0) (17 mg, 0.02 mmol, 0.1 eq) were added and the mixture was heated at 140 °C for 1 h under microwave irradiation. The reaction was partitioned between dichloromethane and water, and the aqueous phase was extracted with dichloromethane (3 x 40 mL). The combined organic extracts were washed with brine, dried (PTFE phase separator) and concentrated *in vacuo.* Purification by reverse phase automated flash chromatography (CombiFlash Rf, C18 15.5g Gold RediSep column) eluting with a gradient of 5 – 95% acetonitrile in water afforded the desired product as a yellow solid (70.8 mg, 0.08 mmol, 45%). LC/MS ($C_{42}H_{49}FN_8O_6S_2$) 845 $[M+H]^+$; RT 0.86 (LCMS-V-B2). ^1H NMR (400 MHz, DMSO-d6) δ 11.52 (br s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.37 (ddd, J = 8.2, 7.3, 1.3 Hz, 1H), 7.31 (dd, J = 11.9, 1.9 Hz, 1H), 7.24 – 7.12 (m, 3H), 4.80 (dd, 1H), 4.22 (s, 1H), 4.15 (t, J = 6.2 Hz, 2H), 3.94 (d, J = 13.4 Hz, 1H), 3.78 (s, 3H), 3.56 (t, J = 5.7 Hz, 2H), 3.44 – 3.37 (m, 1H), 3.31 (s, 2H), 3.28 (d, 1H), 3.24 – 3.14 (m, 2H), 3.12 – 2.97 (m, 2H), 2.58 (d, J = 12.3 Hz, 3H), 2.33 (s, 3H), 2.19 (s, 6H), 2.14 (q, J = 7.0 Hz, 2H), 1.27 (d, 9H).

Step O: methyl 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-6-[2-(methylamino)ethoxy]-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylate

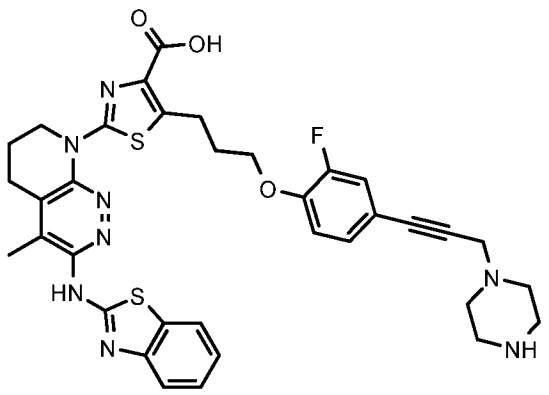
[664] To a solution of the product from Step N (70.8 mg, 0.08 mmol, 1 eq) in dichloromethane (5 mL) was added trifluoroacetic acid (1 mL) slowly and the mixture was

stirred at ambient temperature for 1 h. The reaction was partitioned between dichloromethane and saturated aqueous sodium bicarbonate and the aqueous phase was extracted with dichloromethane (3 x 30 mL). The combined organic extracts were washed with brine, dried (PTFE phase separator) and concentrated *in vacuo* to afford the desired product as a bright yellow solid (59.8 mg, 0.08 mmol, 96%). LC/MS ($C_{37}H_{41}FN_8O_4S_2$) 745 $[M+H]^+$; RT 1.07 (LCMS-V-B1). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.88 (dd, J = 7.8, 1.2 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.37 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 7.32 (dd, J = 11.9, 1.9 Hz, 1H), 7.24 – 7.12 (m, 3H), 4.79 – 4.69 (m, 1H), 4.26 – 4.19 (m, 1H), 4.15 (t, J = 6.2 Hz, 2H), 4.03 (dd, J = 13.5, 2.4 Hz, 1H), 3.78 (s, 3H), 3.60 (t, J = 5.5 Hz, 2H), 3.39 (s, 2H), 3.32 – 3.27 (m, 2H), 3.15 (d, J = 14.6 Hz, 1H), 3.08 – 2.99 (m, 1H), 2.70 (t, J = 5.5 Hz, 2H), 2.38 (s, 3H), 2.29 (s, 3H), 2.22 (s, 6H), 2.17 – 2.08 (m, 2H).

Step P: 2-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-6-[2-(methylamino)ethoxy]-5H,6H,7H,8H-pyrido[2,3-c]pyridazin-8-yl}-5-(3-{4-[3-(dimethylamino)prop-1-yn-1-yl]-2-fluorophenoxy}propyl)-1,3-thiazole-4-carboxylic acid

[665] To a solution of the product from Step O (59.8 mg, 0.08 mmol, 1 eq) in 1,4-dioxane (2 mL) was added 1M aqueous lithium hydroxide (0.24 mL, 0.24 mmol, 3 eq) and the mixture was heated at 50 °C for 2 h. The solid was collected by filtration and dried under vacuum to afford the desired product as a bright yellow solid (43 mg, 0.06 mmol, 73%), as a lithium salt. HRMS-ESI (m/z) [M+H]+ calcd for $C_{36}H_{40}FN_8O_4S_2$: 731.2598, found 731.2623.

Preparation of P17: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-piperazin-1-ylprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylic acid



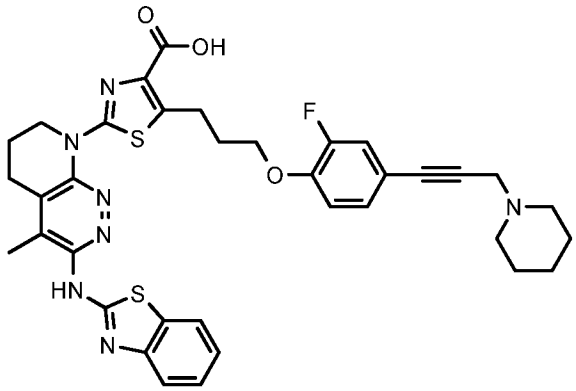
Step A: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(4-tert-butoxycarbonylpiperazin-1-yl)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[666] Using **Silver catalyzed propargylic amine preparation General Procedure** starting from **Preparation 3c**, paraformaldehyde as the aldehyde and *tert*-butyl piperazine-1-carboxylate as the appropriate secondary amine, the desired product was obtained.

Step B: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-(3-piperazin-1-ylprop-1-ynyl)phenoxy]propyl]thiazole-4-carboxylic acid

[667] The mixture of the product from *Step A* (207 mg, 0.25 mmol) and HFxPyr (2.5 mmol, 10 eq.) in acetonitrile (4.3 mL) was stirred at 60 °C for 2.5 h. The product was purified via flash chromatography on 24 g silica gel column using DCM and MeOH (NH_3) as eluents to give 143 mg (79%) of the desired product. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{35}H_{36}FN_8O_3S_2$: 699.2330, found 699.2322.

Preparation of P18: 2-[3-(1,3-Benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(1-piperidyl)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



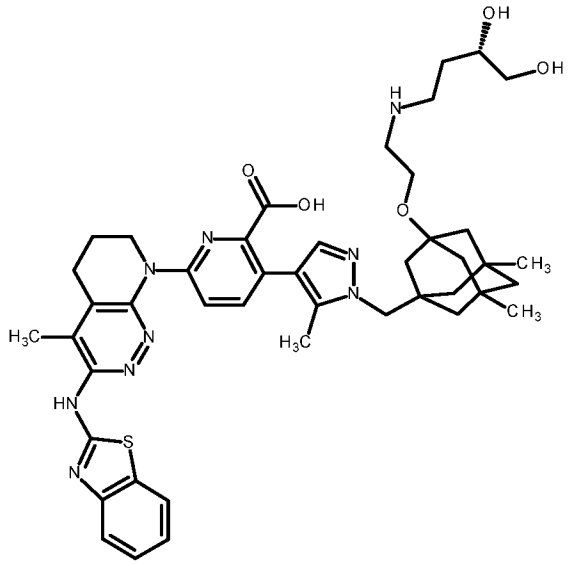
Step A: methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(1-piperidyl)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[668] Using **Propargylic amine preparation General Procedure** starting from 100 mg of **Preparation 3d** (0.155 mmol, 1eq.) as the appropriate propargylic alcohol and piperidine (264.2 mg, 20 eq.), 55 mg of the desired product (50%) was obtained.

Step B: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(1-piperidyl)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

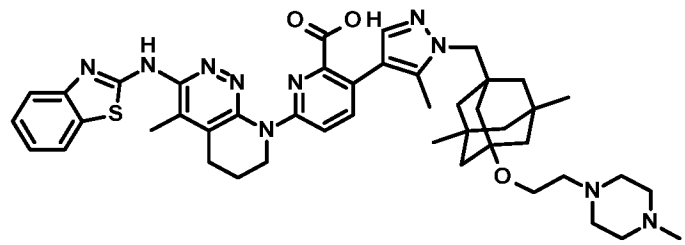
[669] Using **Hydrolysis General Procedure** starting from the product of *Step A* as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{36}H_{37}FN_7O_3S_2$: 698.2377, found 698.2373.

Preparation of P19: 6-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydropyrido[2,3-*c*]pyridazin-8(5*H*)-yl}-3-(1-{[3-(2-{[(3*S*)-3,4-dihydroxybutyl]amino}ethoxy)-5,7-dimethyladamantan-1-yl]methyl}-5-methyl-1*H*-pyrazol-4-yl)pyridine-2-carboxylic acid



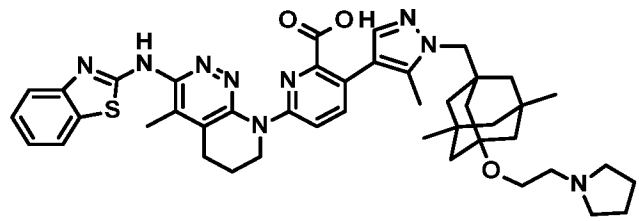
[670] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 2-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine as the appropriate amine, a compound with a dihydroxy protected amine was obtained. Hydrolysis with a 10% HCl solution (rt, 1 h) and purification by preparative HPLC (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) afforded the desired product. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{55}N_9O_5$: 822.4125, found: 822.4120.

Preparation of P20: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(4-methylpiperazin-1-yl)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



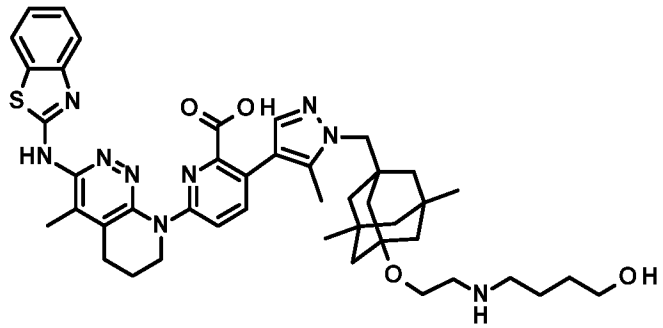
[671] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 1-methylpiperazine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+2H]2+ calcd for $C_{45}H_{58}N_{10}O_3S$: 409.2207, found: 409.2208.

Preparation of P21: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



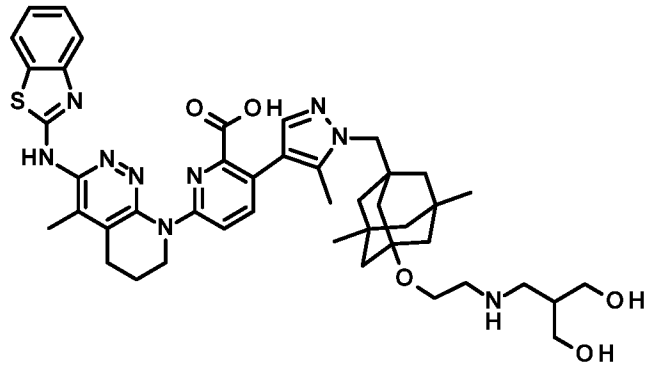
[672] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and pyrrolidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{54}N_9O_3S$: 788,4070, found: 788.4068.

Preparation of P22: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-(4-hydroxybutylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



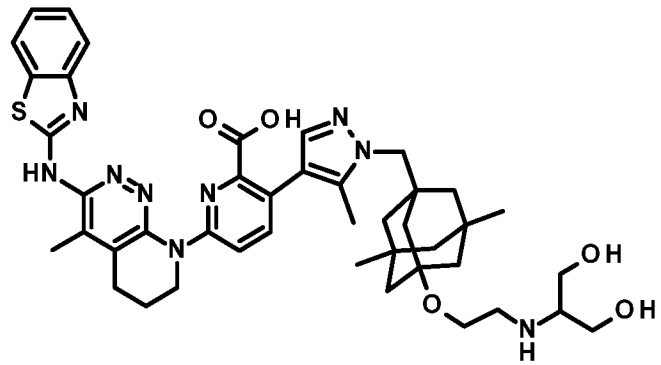
[673] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 4-aminobutan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{56}N_9O_4S$: 806.4176, found: 806.4174.

Preparation of P23: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[[3-hydroxy-2-(hydroxymethyl)propyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



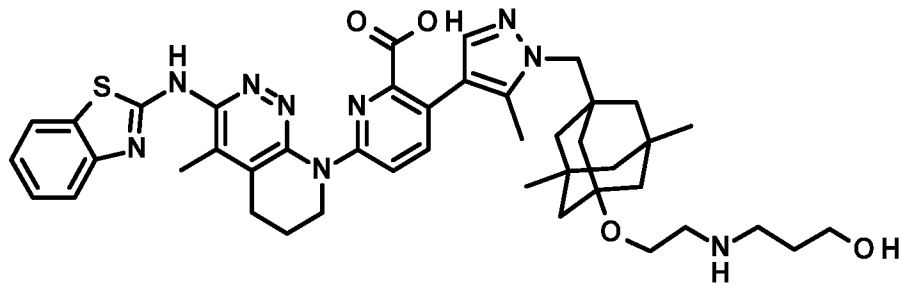
[674] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and (2,2-dimethyl-1,3-dioxan-5-yl)methanamine as the appropriate amine a compound with a dihydroxy protected amine was obtained. Hydrolysis with a 10% HCl solution (rt, 1 h) and purification by preparative HPLC (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) afforded the desired product. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{56}N_9O_5S$: 822,4125, found: 822.4099.

Preparation of P24: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[[2-hydroxy-1-(hydroxymethyl)ethyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



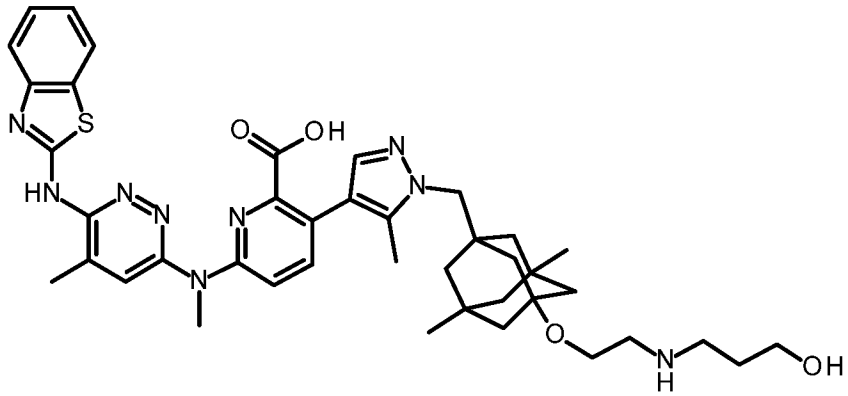
[675] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 2-aminopropane-1,3-diol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{43}H_{54}N_9O_5S$: 808.3969, found: 808.3965.

Preparation of P25: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-(3-hydroxypropylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



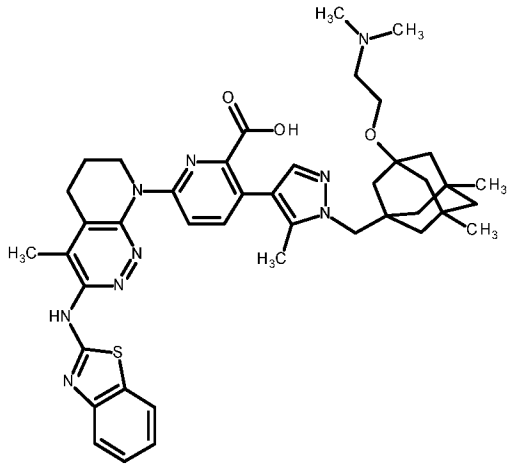
[676] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 3-aminopropan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{43}H_{54}N_9O_4S$: 792.4019, found: 792.4012.

Preparation of P26: 6-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-3-[1-[[3-[2-(3-hydroxypropylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



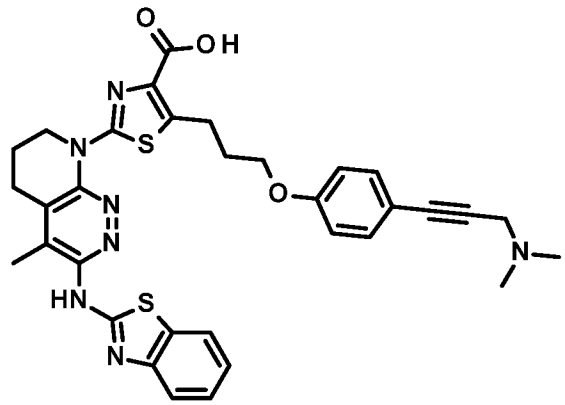
[677] Using the **Amine Substitution and Hydrolysis General procedure II** starting from **Preparation 14_01** and 3-aminopropane-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{41}H_{52}N_9O_4S$: 766.3863, found: 766.3860.

Preparation of P27: 6-{3-[(1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydropyrido[2,3-*c*]pyridazin-8(5*H*)-yl}-3-[1-({3-[2-(dimethylamino)ethoxy]-5,7-dimethyladamantan-1-yl}methyl)-5-methyl-1*H*-pyrazol-4-yl]pyridine-2-carboxylic acid



[678] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and dimethylamine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{42}H_{52}N_9O_3S$: 762.3914, found: 762.3912.

Preparation of P28: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



Step A*: *4-[3-(dimethylamino)prop-1-ynyl]phenol

[679] Using **Sonogashira General Procedure** starting from 10.0 g of 4-iodophenol (45.45 mmol) and 4.91 g (1.3 eq) of *N,N*-dimethylprop-2-yn-1-amine, 3.29 g (41%) of the desired product was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.83 (brs, 1H), 7.25 (d, 2H), 6.74 (d,2H), 3.44 (s, 2H), 2.26 (s, 6H); LC/MS ($C_{11}H_{14}NO$) 176$[M+H]^+$.

Step B: methyl 2-(tert-butoxycarbonylamino)-5-[3-[tert-butyl(diphenyl)silyl]oxypropyl]thiazole-4-carboxylate

[680] To the product of **Preparation 1a**, *Step C* (77.0 g, 243.7 mmol), imidazole (33.14 g, 2 eq) and DMAP (1.49 g, 0.05 eq) in DMF (973 mL) was added dropwise *tert*-butyl(chloro)diphenylsilane (93.5 mL, 1.5 eq) and the reaction mixture was stirred at rt for 16

h. After removal of the volatiles, purification by column chromatography (silica gel, using heptane and EtOAc as eluents) afforded 13.56 g (99%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.63 (s, 1H), 7.60 (d, 4H), 7.45 (t, 2H), 7.42 (t, 4H), 3.74 (s, 3H), 3.67 (t, 2H), 3.20 (t, 2H), 1.87 (qn, 2H), 1.47 (s, 9H), 0.99 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.8, 156.0, 142.6, 135.6, 135.5, 133.5, 130.3, 128.3, 81.8, 62.9, 51.9, 34.0, 28.3, 27.1, 23.2, 19.2; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{29}H_{39}N_2O_5SSi$: 555.2349, found: 555.2336.

Step C*: *methyl 2-[tert-butoxycarbonyl-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propyl]amino]-5-[3-[tert-butyl(diphenyl)silyl]oxypropyl]thiazole-4-carboxylate

[681] Using **Alkylation General Procedure** starting from 34.95 g (63 mmol) of the product from *Step B* and 25.0 g (1.2 eq) of 3,6-dichloro-4-(3-iodopropyl)-5-methyl-pyridazine as the appropriate iodine compound, 51.0 g (quantitative yield) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.63-7.37 (m, 10H), 4.09 (t, 2H), 3.75 (s, 3H), 3.67 (t, 2H), 3.20 (t, 2H), 2.82 (m, 2H), 2.40 (s, 3H), 1.87 (m, 2H), 1.87 (m, 2H), 1.50 (s, 9H), 0.97 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 62.9, 52.0, 46.1, 33.9, 28.1, 27.5, 27.1, 25.9, 23.8, 16.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{37}H_{47}Cl_2N_4O_5SSi$: 757.2413, found: 757.2395.

Step D: methyl 5-[3-[tert-butyl(diphenyl)silyl]oxypropyl]-2-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]thiazole-4-carboxylate

[682] Using **Deprotection with HFIP General Procedure** starting from 51.70 g of the product from *Step C* (68 mmol), 36.32 g (81%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.71 (t, 1H), 7.63-7.37 (m, 10H), 3.69 (s, 3H), 3.67 (t, 2H), 3.30 (m, 2H), 3.10 (t, 2H), 2.85 (m, 2H), 2.83 (s, 3H), 1.79 (m, 2H), 1.78 (m, 2H), 0.98 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 62.9, 51.7, 44.1, 34.2, 28.0, 27.1, 27.0, 23.4, 16.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{32}H_{39}Cl_2N_4O_3SSi$: 657.1889, found: 657.1875.

Step E*: *methyl 5-[3-[tert-butyl(diphenyl)silyl]oxypropyl]-2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)thiazole-4-carboxylate

[683] The mixture of 36.0 g (54.7 mmol) of the product from *Step D* and 35.7 g (2 eq) of Cs_2CO_3 in 1,4-dioxane (383 mL) was stirred at 90 °C for 18 h. After dilution with water, the precipitated solid was filtered off, washed with diethylether, and dried to give 34.0 g (99%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.61 (d, 4H), 7.43 (t, 2H), 7.42 (t, 4H), 4.26 (t, 2H), 3.77 (s, 3H), 3.70 (t, 2H), 3.23 (t, 2H), 2.90 (t, 2H), 2.33 (s, 3H), 2.04 (qn,

2H), 1.90 (qn, 2H), 1.00 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 155.3, 151.8, 151.4, 143.2, 136.2, 135.5, 134.7, 133.6, 130.3, 129.0, 128.3, 63.1, 51.9, 46.3, 34.1, 27.1, 24.2, 23.1, 19.8, 19.2, 15.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{32}H_{38}ClN_4O_3SSi$: 621.2122, found: 621.2097.

Step F*: *methyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-(3-hydroxypropyl)thiazole-4-carboxylate

[684] The mixture of 23.36 g (37.6 mmol) of the product from *Step E* and 45 mL (1.2 eq.) of 1 M TBAF solution in THF (5 mL/mmol) was stirred at rt for 2 h. After the removal of the volatiles, purificationby column chromatography (silica gel, using EtOAc and MeOH/NH_3 as eluents) afforded 12.88 g (89%) of the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.54 (br., 1H), 4.25 (m, 2H), 3.80 (s, 3H), 3.45 (t, 2H), 3.11 (m, 2H), 2.88 (t, 2H), 2.31 (s, 3H), 2.04 (m, 2H), 1.77 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 155.2, 151.2, 143.8, 136.1, 134.5, 129.0, 60.5, 52.0, 46.3, 34.6, 24.2, 23.2, 19.7, 15.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{16}H_{20}ClN_4O_3S$: 383.0945, found: 383.0937.

Step G*: *methyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

[685] Using **Mitsunobu General Procedure I** starting from 0.65 g (1.2 eq) of the product from *Step F* and 250 mg (1.43 mmol) of 4-[3-(dimethylamino)prop-1-ynyl]phenol in THF (9 mL/mmol), 0.28 g (37%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.34 (d, 2H), 6.91 (d, 2H), 4.26 (t, 2H), 4.03 (t, 2H), 3.78 (s, 3H), 3.40 (s, 2H), 3.25 (t, 2H), 2.88 (t, 2H), 2.31 (s, 3H), 2.22 (s, 6H), 2.08 (qn, 2H), 2.03 (qn, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 158.9, 155.3, 151.7, 151.3, 142.7, 136.2, 134.9, 133.3, 129.0, 115.2, 115.0, 85.2, 84.1, 67.1, 52.0, 48.3, 46.3, 44.3, 30.8, 24.1, 23.1, 19.7, 15.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{27}H_{31}ClN_5O_3S$: 540.1836, found: 540.1834.

Step H*: *methyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylate

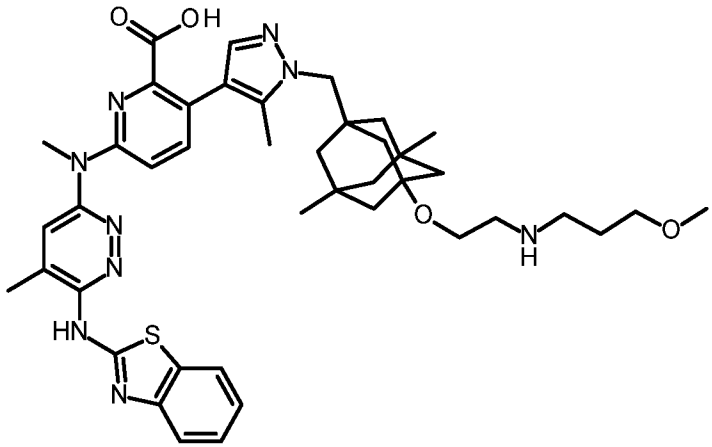
[686] Using **Buchwald General Procedure I** starting from 0.27 g of the product from *Step G* (0.5 mmol), 0.29 g (89%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.83 (dm, 1H), 7.50 (dm, 1H), 7.36 (m, 1H), 7.35 (m, 2H), 7.18 (m, 1H), 6.94 (m, 2H), 4.28 (m, 2H), 4.09 (t, 2H), 3.80 (s, 3H), 3.39 (s, 2H), 3.29 (t, 2H), 2.88 (t, 2H), 2.35 (s,

3H), 2.23 (s, 6H), 2.13 (m, 2H), 2.07 (m, 2H); HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{36}N_7O_3S_2$: 654.2321, found: 654.2322.

Step I*: *2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

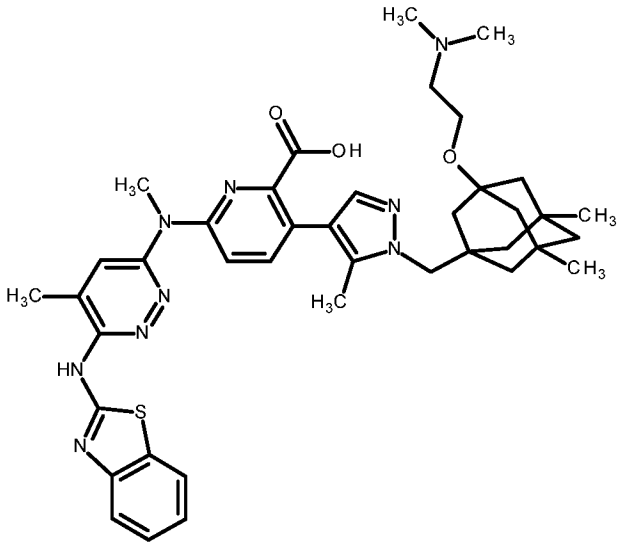
[687] To the product from *Step H* (280 mg, 0.43 mmol) in a 1:1 mixture of THF and water (10 mL/mmol) was added 90 mg (5 eq) of $LiOHxH_2O$, and the reaction mixture was stirred at 50 °C for 18 h. After the removal of the volatiles, purificationby reverse phase preparative chromatography (C18, 0.1% TFA in water and MeCN as eluents) afforded 132 mg (48%) of the desired compound. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{33}H_{34}N_7O_3S_2$: 640.2165, found: 640.2160.

Preparation of P29: 6-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-3-[1-[[3-[2-(3-methoxypropylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



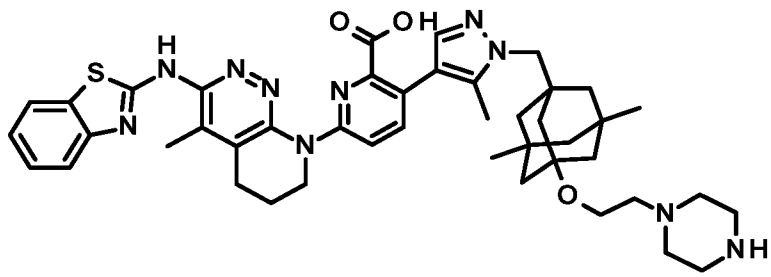
[688] Using the **Amine Substitution and Hydrolysis General procedure II** starting from **Preparation 14_01** and 3-methoxypropan-1-amine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{42}H_{54}N_9O_4S$: 780.4019, found: 780.4019.

Preparation of P30:6-[{6-[(1,3-benzothiazol-2-yl)amino]-5-methylpyridazin-3-yl}(methyl)amino]-3-[1-({3-[2-(dimethylamino)ethoxy]-5,7-dimethyladamantan-1-yl}methyl)-5-methyl-1*H*-pyrazol-4-yl]pyridine-2-carboxylic acid



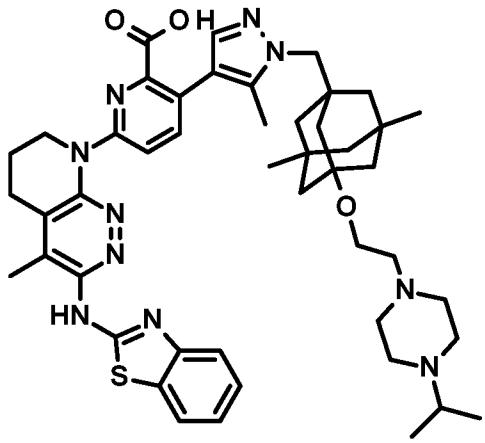
[689] Using the **Amine Substitution and Hydrolysis General procedure II** starting from **Preparation 14_01** and dimethylamine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{40}H_{50}N_9O_3S$: 736.3757, found: 736.3751.

Preparation of P31: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-piperazin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



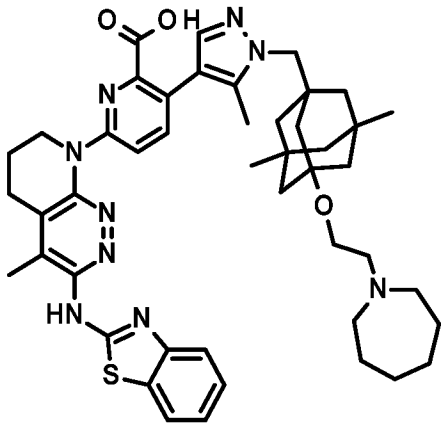
[690] Using the **Amine substitution and Hydrolysis General procedure I** starting from Preparation 12 and piperazine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{55}N_{10}O_3S$: 803.4179, found: 803.4177.

Preparation of P32: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-(4-isopropylpiperazin-1-yl)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



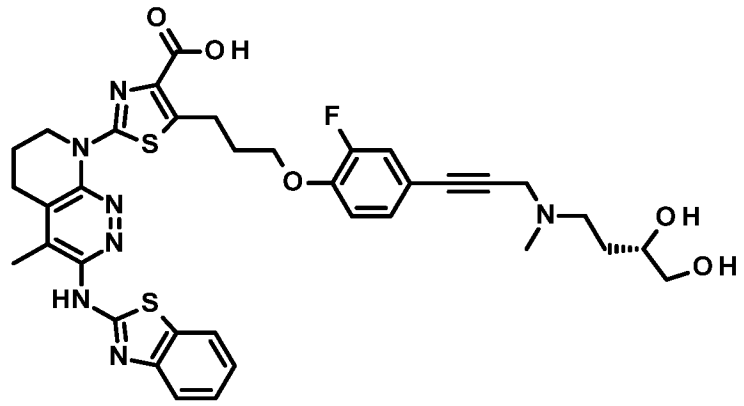
[691] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 1-isopropylpiperazine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{47}H_{61}N_{10}O_3S$: 845.4649, found: 845.4646.

Preparation of P33: 3-[1-[[3-[2-(azepan-1-yl)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]pyridine-2-carboxylic acid



[692] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and azepane as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{46}H_{58}N_9O_3S$: 816.4383, found: 816.4379.

Preparation of P34: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[4-[3-[[(3*S*)-3,4-dihydroxybutyl]-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid



Step A: 2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl 4-methylbenzenesulfonate

[693] To 1.0 g (6.8 mmol) of 2-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanol and 3.8 mL (4 eq) of triethylamine in 34 mL of DCM was added 4.5 g (2 eq) of *p*-tolylsulfonyl 4-methylbenzenesulfonate at 0 °C. The reaction mixture was stirred until no further conversion was observed, concentrated and treated with diisopropyl ether. Then, the precipitated hydrochloric salt was filtered off and the mother liquour was concentrated and purified via flash chromatography (silica gel, using heptane and EtOAc as eluents) to give 1.6 g (81%) of desired product. ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.79 (dm, 2H), 7.49 (dm, 2H), 4.08 (m, 2H), 4.00 (m, 1H), 3.91/3.44 (dd+dd, 2H), 2.42 (s, 3H), 1.83/1.77 (m+m, 2H), 1.24/1.20 (s+s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 132.7, 132.7, 130.7, 128.1, 108.6, 72.3, 68.7, 68.4, 32.9, 27.2/25.9, 21.6; HRMS-ESI (m/z): [M+H]+ calcd for C14H21O5S: 301.1110, found: 301.1107.

Step B: N-[2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]prop-2-yn-1-amine

[694] The mixture of the product from Step A (7.6 g, 25.3 mmol), prop-2-yn-1-amine (16 mL, 10 eq) and DIPEA (13.22 mL, 3 eq) in 127 mL of MeCN was stirred at 50 °C for 16 h. After concentration, taken up in DCM and extraction with cc. $NaHCO_3$ solution and brine, the combined organic layers were dried and concentrated to give 5.0 g (107%) of the desired product, which was used without any further purification. ^{1}H NMR (500 MHz, dmso-d6) δ ppm 4.07 (m, 1H), 3.98/3.43 (dd+t, 2H), 3.28 (m, 2H), 3.05 (t, 1H), 2.62/2.55 (m+m, 2H), 2.23 (brs, 1H), 1.63/1.59 (m+m, 2H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 108.2, 83.4, 74.6, 74.1, 69.2, 45.1, 37.8, 33.6, 27.3, 26.2; HRMS (EI) (m/z): [M]+ calcd for $C_{10}H_{17}NO_2$: 183.1259, found: 183.1260.

Step C: N-[2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]-N-methyl-prop-2-yn-1-amine

[695] To the product from Step B (500 mg, 2.73 mmol) in *N,N*-dimethylformamide (14 mL) was added portionwise sodium hydride (120 mg, 1.1 eq) at 0°C. After stirring at 0 °C for 0.5 h, the mixture was treated with iodomethane (0.17 mL, 1 eq) and stirred at rt for 18 h. After quenching with a saturated solution of NH_4Cl and water, the mixture was extracted with

Et_2O. The combined organic phases were dried and concentrated to give the desired product (362 mg, 67%). GC/MS ($C_{11}H_{19}NO_2$) 197 [M^+].

Step D*: *ethyl 2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-5-[3-[4-[3-[2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[696] Using **Sonogashira General Procedure** starting from 0.548 g (0.89 mmol) of the product of **Preparation 15** and 350 mg (2 eq) of the product from Step C as the appropriate acetylene, 510 mg (82%) of the desired product was obtained. LC/MS ($C_{34}H_{42}ClFN_5O_5S$) 686 [M+H]$^+$.

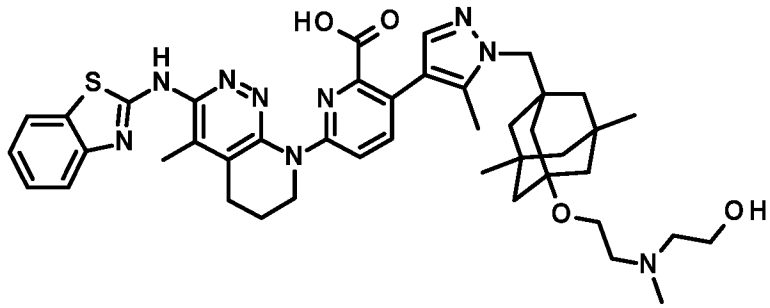
Step E*: *ethyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[697] Using **Buchwald General Procedure I** starting from 510 mg (0.52 mmol) of the product from *Step D* and 234 mg (3 eq) of *1,3-benzothiazol-2-amine*, 200 mg (48%) of the desired product was obtained. ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.88 (dm, 1H), 7.49 (brd, 1H), 7.37 (m, 1H), 7.3 (dd, 1H), 7.20 (dm, 1H), 7.19 (m, 1H), 7.16 (t, 1H), 4.26 (m, 2H), 4.25 (q, 2H), 4.14 (t, 2H), 4.04 (m, 1H), 3.98/3.45 (dd+dd, 2H), 3.46 (s, 2H), 3.28 (m, 2H), 2.87 (t, 2H), 2.45/2.39 (m+m, 2H), 2.34 (s, 3H), 2.21 (s, 3H), 2.13 (m, 2H), 2.04 (m, 2H), 1.63 (m, 2H), 1.29 (t, 3H), 1.29 (s, 3H), 1.24 (s, 3H); HRMS (ESI) (m/z): [M+H]+ calcd for $C_{41}H_{47}FN_7O_5S_2$: 800.3064, found: 800.3064.

Step F: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[(3S)-3,4-dihydroxybutyl]-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

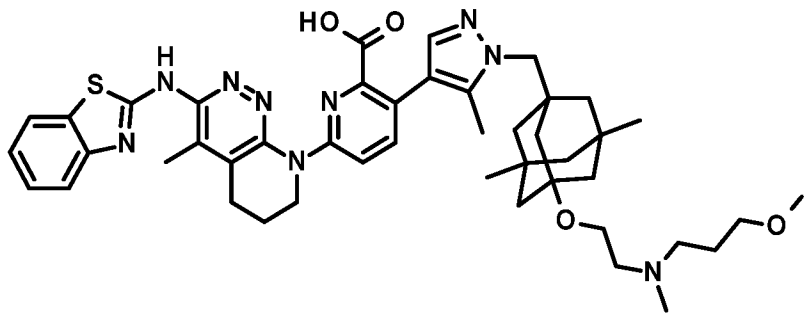
[698] The mixture of 200 mg (0.25 mmol) of product from *Step E* and 53 mg of $LiOHxH_2O$ (5 eq) in 5 mL of THF / water (1:1) was stirred at 60 °C for 18 h. The reaction mixture was treated with 0.125 mL (6 eq) of concentrated hydrogen chloride at 0 °C (pH = 2-3) and stirred at rt, then at 60 °C for 0.5 h. After the reaction mixture was concentrated to remove THF and lyophilization, the solid was dissolved in 6 N NH_3 solution in MeOH and purified by reverse phase chromatography (using 5 mM NH_4HCO_3 and MeCN as eluents) to give 47 mg (25%) of the desired product. HRMS (ESI) (m/z): [M+H]+ calcd for $C_{36}H_{39}FN_7O_5S_2$: 732.2438, found: 732.2441.

Preparation of P35: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[2-hydroxyethyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



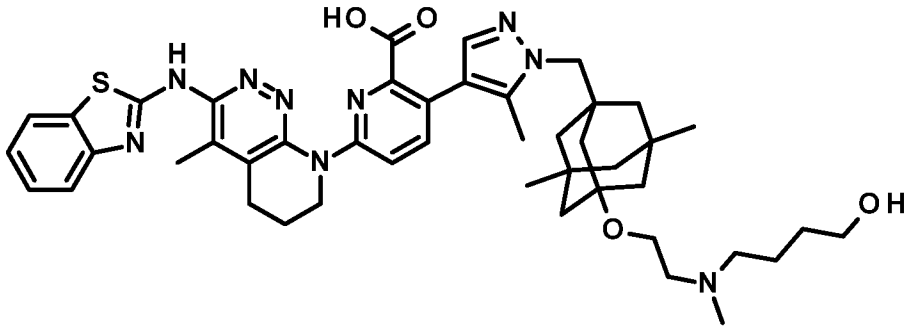
[699] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 2-(methylamino)ethanol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{43}H_{54}N_9O_4S$: 792.4019, found: 792.4019.

Preparation of P36: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[3-methoxypropyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



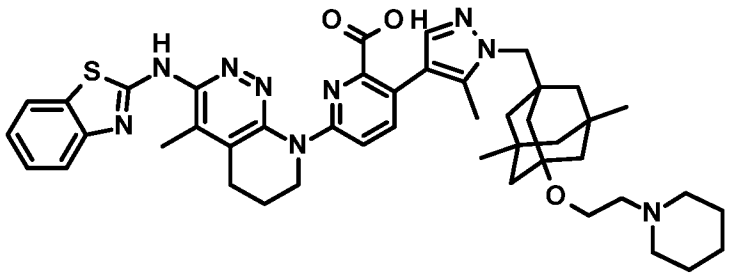
[700] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 3-methoxy-N-methyl-propan-1-amine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_4S$: 820.4332, found: 820.4328.

Preparation of P37: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[4-hydroxybutyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



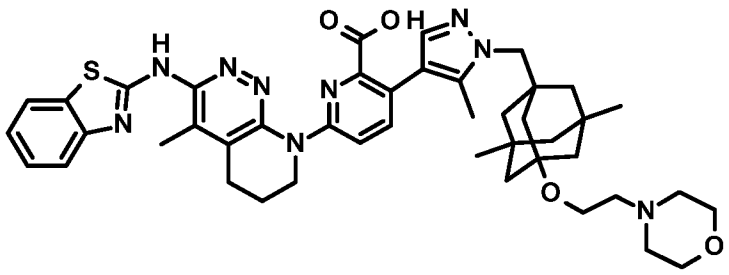
[701] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and 4-(methylamino)butan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_4S$: 820.4332, found: 820.4339.

Preparation of P38: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(1-piperidyl)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



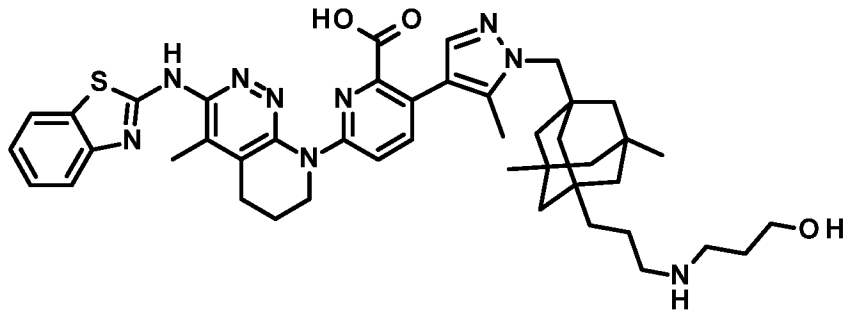
[702] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and piperidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{56}N_9O_3S$: 802.4227, found: 802.4223.

Preparation of P39: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-morpholinoethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



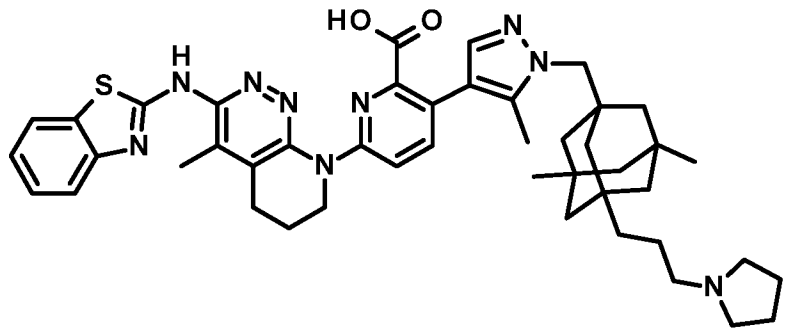
[703] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 12** and morpholine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{44}H_{54}N_9O_4S$: 804.4019, found: 804.4012.

Preparation of P40: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[3-(3-hydroxypropylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



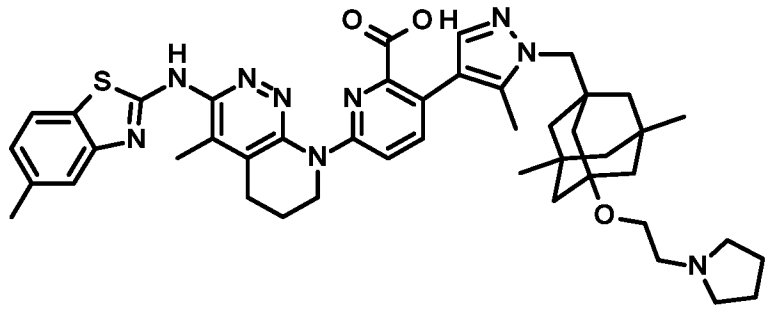
[704] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 13** and 3-aminopropan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{44}H_{56}N_9O_3S$: 790.4227, found: 790.4220.

Preparation of P41: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(3-pyrrolidin-1-ylpropyl)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



[705] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 13** and pyrrolidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{45}H_{56}N_9O_2S$: 786.4278, found: 786.4273.

Preparation of P42: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(5-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]pyridine-2-carboxylic acid



Step A: methyl 3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(5-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate

[706] Using **Buchwald General Procedure I** at 130 °C for 1.5 h, starting from 140 mg (0.22 mmol) of the product from **Preparation 12**, Step C and 54.3 mg (1.5 eq) of the 5-methyl-1,3-benzothiazol-2-amine, 126 mg (75%) of the desired product was obtained. ^{1}H NMR (500 MHz, dmso-d6) δ ppm 12.08/10.89 (brs/brs, 1H), 7.95 (d, 1H), 7.69 (d, 1H), 7.67 (br, 1H), 7.38 (s, 1H), 7.30 (br, 1H), 7.00 (d, 1H), 4.46 (brs, 1H), 4.00 (t, 2H), 3.88 (s, 2H), 3.70 (s, 3H), 3.41 (t, 2H), 3.35 (t, 2H), 2.85 (t, 2H), 2.39 (s, 3H), 2.32 (s, 3H), 2.16 (s, 3H), 1.98 (qn, 2H), 1.39 (s, 2H), 1.30/1.25 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.08/1.02 (d+d, 2H), 0.87 (s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 139.8, 137.5, 123.6, 121.6, 119.0, 62.1, 61.5, 59.0, 52.7, 50.1, 47.0, 46.0, 45.4, 43.3, 30.2, 24.3, 21.7, 21.6, 12.6, 10.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{42}H_{51}N_8O_4S$: 763.3760, found: 763.3754.

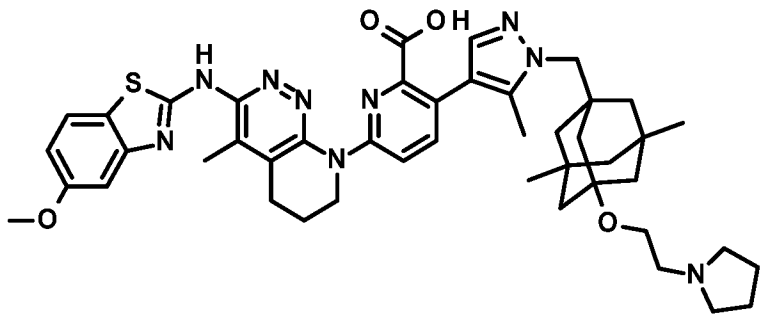
Step B: methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(5-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate

[707] To the product from Step A (119 mg, 0.16 mmol) and triethylamine (0.066 mL, 3 eq) in DCM (2 mL) was added *p*-tolylsulfonyl 4-methylbenzenesulfonate (76 mg, 1.5 eq) and the reaction mixture was stirred for 1 h. Purification by column chromatography (silica gel, DCM and EtOAc as eluents) afforded the desired product (93 mg, 65%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 12.17/10.83 (brs/brs, 1H), 7.95 (d, 1H), 7.77 (d, 2H), 7.7 (d, 1H), 7.69 (br, 1H), 7.46 (d, 2H), 7.42 (br, 1H), 7.39 (s, 1H), 7.00 (d, 1H), 4.07 (t, 2H), 4 (t, 2H), 3.96 (s, 3H), 3.85 (s, 2H), 3.49 (t, 2H), 2.85 (t, 2H), 2.40 (s, 3H), 2.39 (s, 3H), 2.32 (s, 3H), 2.15 (s, 3H), 1.99 (qn, 2H), 1.29 (s, 2H), 1.17/1.1 (d+d, 4H), 1.12/1.1 (d+d, 4H), 1.02/0.97 (d+d, 2H), 0.84 (s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 139.8, 137.6, 130.6, 128.1, 123.6, 119.0, 71.5, 58.8, 58.4, 52.7, 49.9, 46.6, 45.9, 45.4, 43.0, 30.1, 24.3, 21.6, 21.6, 21.6, 12.6, 10.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{49}H_{57}N_8O_6S_2$: 917.3842, found: 917.3840.

Step C: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(5-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid

[708] Using the **Amine substitution and Hydrolysis General procedure I** starting from the product from Step B and pyrrolidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{56}N_9O_3S$: 802.4227, found: 802.4220.

Preparation of P43: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-methoxy-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]pyridine-2-carboxylic acid



Step A: methyl 3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-methoxy-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate

[709] Using **Buchwald General Procedure I** at 130 °C for 2.5 h, starting from 140 mg (0.22 mmol) of the product from **Preparation 12**, *Step C* and 60 mg (1.5 eq) of the 5-methyl-1,3-benzothiazol-2-amine, 129 mg (75%) of the desired product was obtained. ^{1}H NMR (500 MHz, dmso-d6) δ ppm 7.95 (d, 1H), 7.69 (d, 1H), 7.67 (br., 1H), 7.38 (s, 1H), 7.02 (br., 1H), 6.80 (dd, 1H), 4.46 (br., 1H), 4.00 (t, 2H), 3.88 (s, 2H), 3.80 (s, 3H), 3.70 (s, 3H), 3.41 (t, 2H), 3.35 (t, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 2.16 (s, 3H), 1.98 (m, 2H), 1.39 (s, 2H), 1.30/1.25 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.08/1 (d+d, 2H), 0.87 (s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 139.8, 137.5, 122.6, 119.0, 110.5, 62.1, 61.5, 58.9, 55.8, 52.6, 50.1, 47.0, 46.0, 45.4, 43.3, 30.2, 24.3, 21.7, 12.6, 10.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{42}H_{51}N_8O_5S$: 779.3703, found: 779.3687.

Step B: methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-methoxy-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate

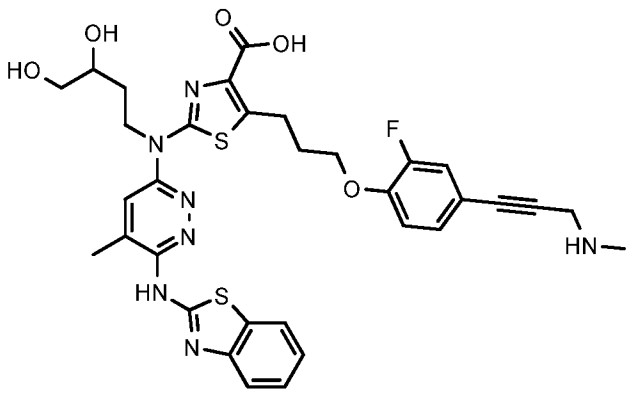
[710] To the product from Step A (122 mg, 0.16 mmol) and triethylamine (0.066 mL, 3 eq) in DCM (2 mL) was added *p*-tolylsulfonyl 4-methylbenzenesulfonate (77 mg, 1.5 eq) and the

reaction mixture was stirred for 1 h. Purificationby column chromatography (silica gel, DCM and EtOAc as eluents) afforded the desired product (79 mg, 54%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm 12.17/10.83 (brs/brs, 1H), 7.95 (d, 1H), 7.77 (d, 2H), 7.72 (d, 1H), 7.67 (brd, 1H), 7.46 (d, 2H), 7.39 (s, 1H), 7.02 (br, 1H), 6.80 (d, 1H), 4.07 (t, 2H), 4.00 (t, 2H), 3.86 (s, 2H), 3.80 (s, 3H), 3.69 (s, 3H), 3.49 (t, 2H), 2.86 (t, 2H), 2.41 (s, 3H), 2.33 (s, 3H), 2.15 (s, 3H), 1.99 (qn, 2H), 1.29 (s, 2H), 1.17/1.1 (d+d, 4H), 1.12/1.10 (d+d, 4H), 1.02/0.97 (d+d, 2H), 0.84 (s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 139.9, 137.6, 130.6, 128.1, 119.0, 110.6, 71.5, 58.8, 58.4, 55.9, 52.6, 49.9, 46.6, 45.9, 45.8, 43.0, 30.1, 24.3, 21.6, 21.6, 12.7, 10.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{49}H_{57}N_8O_7S_2$: 933.3792, found: 933.3794.

Step C: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-methoxy-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid

[711] Using the **Amine substitution and Hydrolysis General procedure I** starting from the product from Step B and pyrrolidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+H]+ calcd for $C_{45}H_{57}N_9O_4S$: 818.4176, found: 818.4172.

Preparation of P44: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3,4-dihydroxybutyl)amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

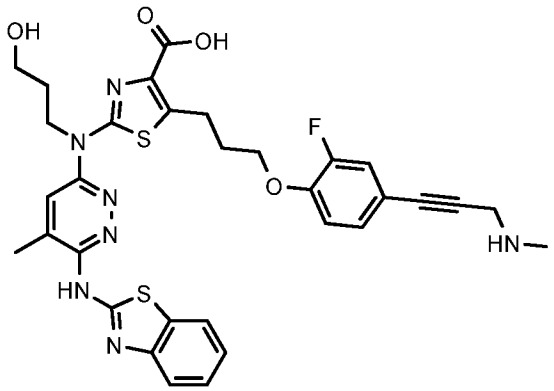
[712] Using **Buchwald General Procedure III** starting from 350 mg of **Preparation 3h_01** (0.57 mmol, 1 eq.) and 235 mg of **Preparation 4a_01** (0.57 mmol, 1 eq.) as the appropriate halide, 490 mg (87%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.84 (d, 1H), 7.68 (s, 1H), 7.47 (d, 1H), 7.44 (td, 1H), 7.32 (brd., 1H), 7.25 (td, 1H), 7.22

(d, 1H), 7.16 (t, 1H), 5.86 (s, 2H), 4.49/4.33 (m+m, 2H), 4.20 (br., 2H), 4.17 (m, 1H), 4.15 (t, 2H), 4.04/3.63 (dd+dd, 2H), 3.77 (s, 3H), 3.72 (t, 2H), 3.27 (t, 2H), 2.84 (br., 3H), 2.45 (s, 3H), 2.13 (m, 2H), 1.75 (m, 2H), 1.40 (s, 9H), 1.37/1.24 (s+s, 6H), 0.92 (t, 2H), -0.11 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 129.1, 127.2, 123.5, 123.2, 119.3, 117.5, 115.5, 112.0, 108.6, 73.7, 72.8, 68.9, 68.4, 66.7, 51.9, 44.4, 38.5, 33.8, 30.9, 28.5, 27.3/26.0, 23.3, 23.1, 17.9, 17.8, -1.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{48}H_{63}FN_7O_8S_2Si$: 976.3927, found 976.3916.

Step B: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3,4-dihydroxybutyl)amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[713] Using **Deprotection and Hydrolysis General Procedure** starting from the product from Step A as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{33}H_{35}FN_7O_5S_2$: 692.2120, found 692.2114.

Preparation of P45: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3-hydroxypropyl)amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



Step A: methyl 5-[3-[4-[3-[tert-butoxycarbonyl(methyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-[tert-butyl(dimethyl)silyl]oxypropyl-[5-methyl-6-[(Z)-[3-(2-trimethylsilylethoxymethyl)-1,3-benzothiazol-2-ylidene]amino]pyridazin-3-yl]amino]thiazole-4-carboxylate

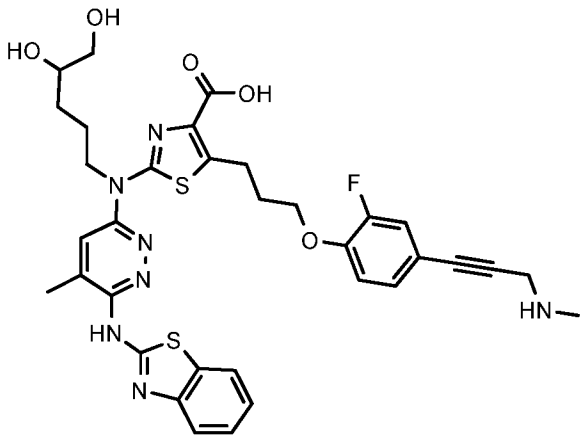
[714] Using **Buchwald General Procedure III** starting from 300 mg of **Preparation 3n_01** (0.46 mmol, 1 eq.) and 187 mg of **Preparation 4a_01** (0.46 mmol, 1 eq.) as the appropriate halide, 395 mg (83%) of the desired product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.82 (dd, 1H), 7.60 (s, 1H), 7.44 (m, 1H), 7.44 (dd, 1H), 7.31 (dd, 1H), 7.24 (m, 1H), 7.20 (m, 1H), 7.15 (t, 1H), 5.84 (s, 2H), 4.39 (t, 2H), 4.20 (s, 2H), 4.14 (t, 2H), 3.76 (s, 3H), 3.70 (t, 2H), 3.70 (t, 2H), 3.25 (t, 2H), 2.84 (s, 3H), 2.42 (s, 3H), 2.11 (m, 2H), 1.91 (m, 2H),

1.40 (s, 9H), 0.91 (t, 2H), 0.85 (s, 9H), 0.01 (s, 6H), -0.12 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 162.2, 147.5, 137.6, 129.1, 127.2, 123.4, 123.2, 119.3, 117.5, 115.4, 112.0, 79.7, 72.8, 68.4, 66.7, 60.5, 51.9, 44.6, 38.1, 33.8, 30.9, 30.4, 28.6, 26.3, 23.1, 17.9, 17.8, -0.9, -5.0; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{50}H_{71}FN_7O_7S_2Si_2$: 1020.4373, found 1020.4365.

Step B: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3-hydroxypropyl)amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

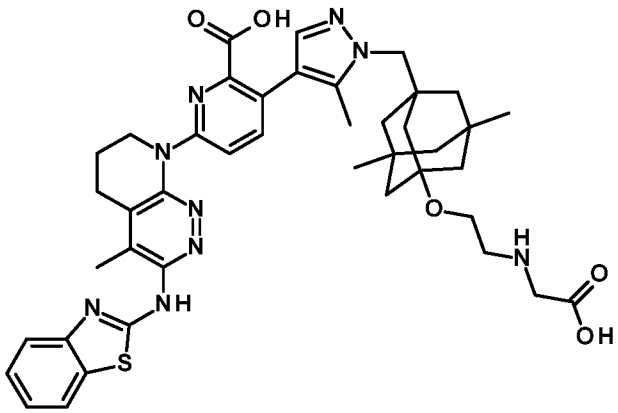
[715] Using **Deprotection and Hydrolysis General Procedure** starting from the product from *Step A* as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{32}H_{33}FN_7O_4S_2$: 662.2014, found 662.2016.

Preparation of P46: 2-[[6-(1,3-Benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(4,5-dihydroxypentyl) amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



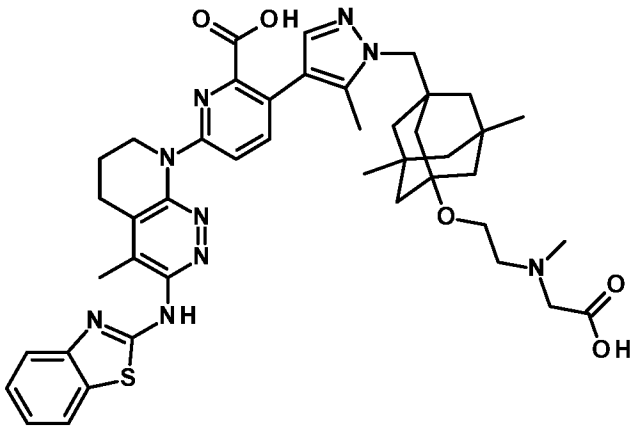
[716] Using **Deprotection and Hydrolysis General Procedure** starting from the product from **Preparation 5a_01**, *Step A* as the appropriate methyl ester, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{34}H_{37}FN_7O_5S_2$: 706.2276, found 706.2274.

Preparation of P47: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-(carboxymethylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



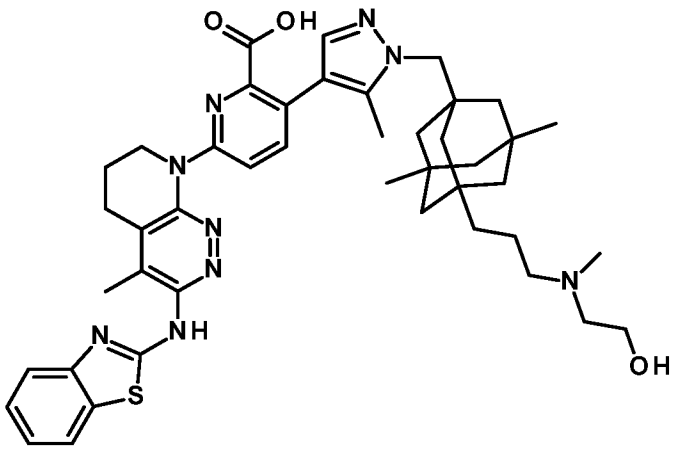
[717] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 16** and methyl 2-aminoacetate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained.**HRMS-ESI** (m/z): [M+H]+ calcd for $C_{42}H_{50}N_9O_5S$: 792.3656, found: 792.3651.

Preparation of P48: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[carboxymethyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



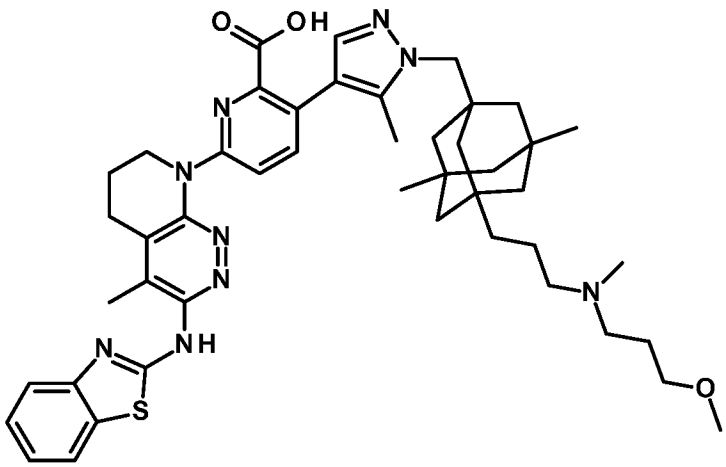
[718] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 16** and methyl 2-(methylamino)acetate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{43}H_{52}N_9O_5S$: 806.3812, found: 806.3807.

Preparation of P49: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[2-hydroxyethyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



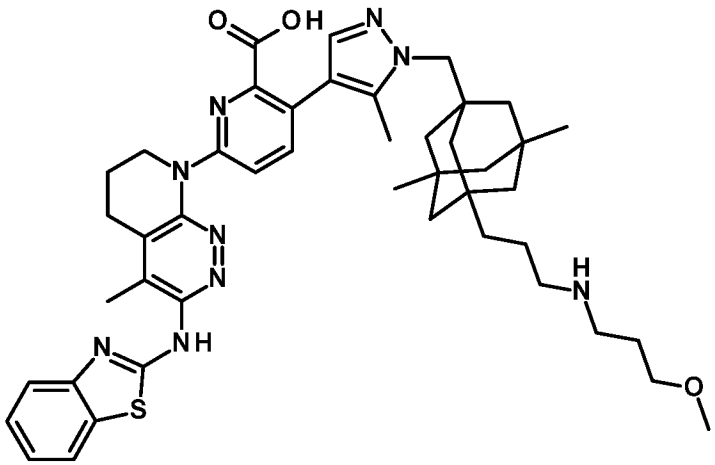
[719] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 13** and 2-(methylamino)ethanol as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{56}N_9O_3S$: 790.4227, found: 790.4227.

Preparation of P50: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[3-methoxypropyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



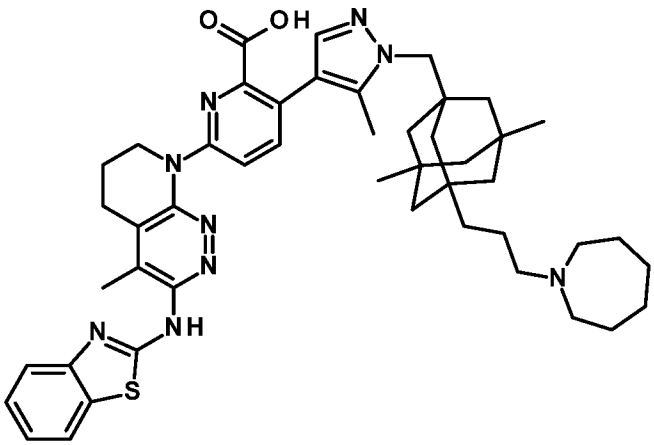
[720] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 13** and 3-methoxy-*N*-methyl-propan-1-amine as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{46}H_{60}N_9O_3S$: 818.4540, found: 818.4537.

Preparation of P51: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-(3-methoxypropylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



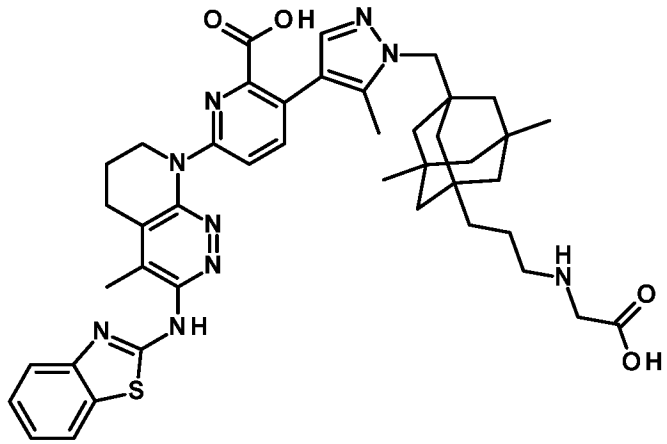
[721] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 13** and 3-methoxypropan-1-amine as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_3S$: 804.4383, found: 804.4380.

Preparation of P52: 3-[1-[[3-[3-(azepan-1-yl)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid



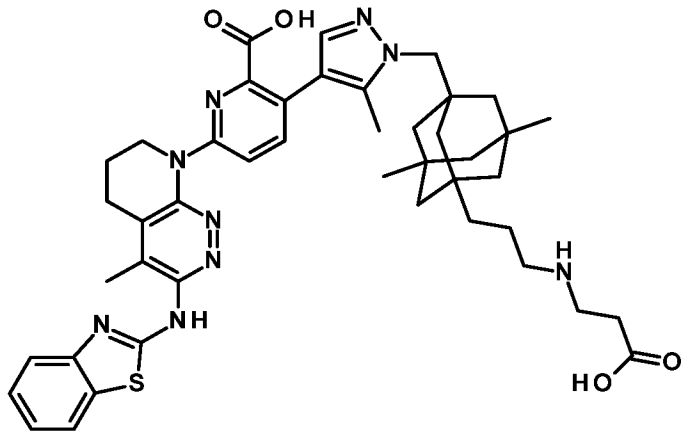
[722] Using the **Amine substitution and Hydrolysis General procedure I** starting from **Preparation 13** and azepane as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{47}H_{60}N_9O_2S$: 814.4591, found: 814.4588.

Preparation of P53: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-(carboxymethylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



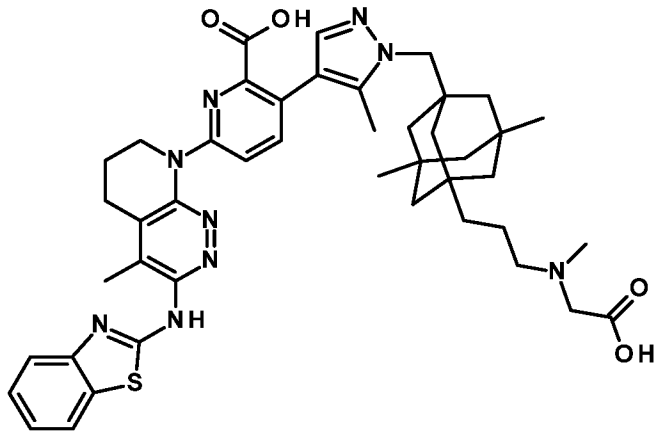
[723] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 13** and methyl 2-aminoacetate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{43}H_{52}N_9O_4S$: 790.3863, found: 790.3855.

Preparation of P54: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-(2-carboxyethylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



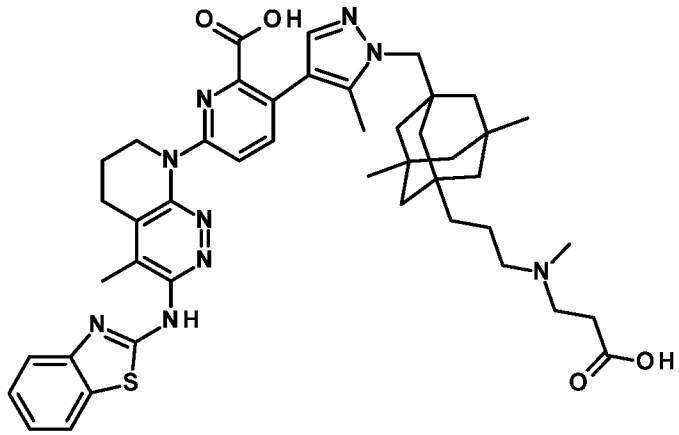
[724] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 13** and methyl 3-aminopropanoate as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{54}N_9O_4S$: 804.4019, found: 804.4015.

Preparation of P55: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[carboxymethyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



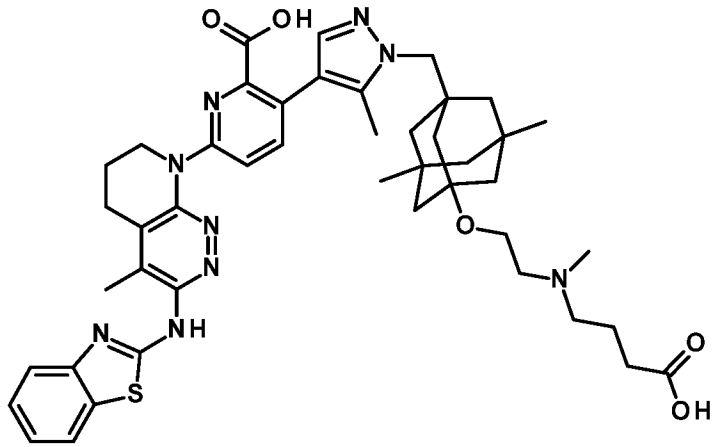
[725] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 13** and methyl 2-(methylamino)acetate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{54}N_9O_4S$: 804.4019, found: 804.4014.

Preparation of P56: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[2-carboxyethyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



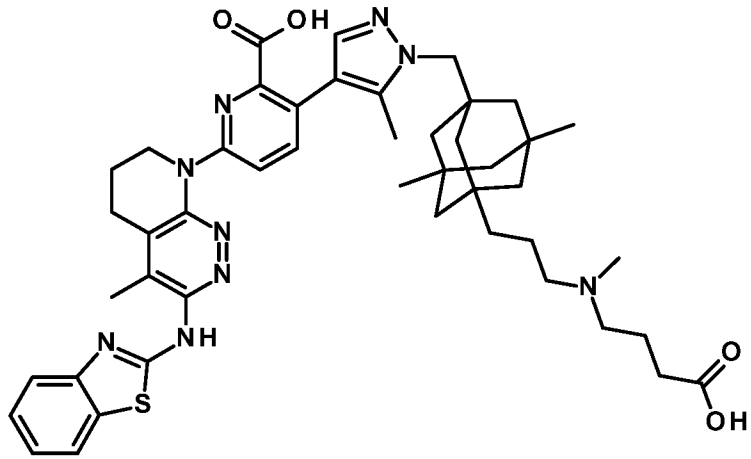
[726] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 13** and ethyl 3-(methylamino)propanoate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{56}N_9O_4S$: 818.4176, found: 818.4167.

Preparation of P57: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[3-carboxypropyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



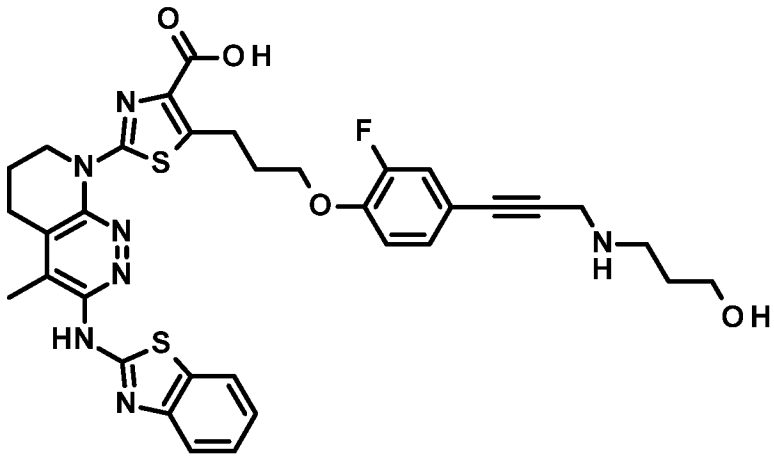
[727] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 16** and methyl 4-(methylamino)butanoate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{56}N_9O_5S$: 834.4125, found: 834.4115.

Preparation of P58: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[3-carboxypropyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



[728] Using the **Amine Substitution and Hydrolysis General procedure III** starting from **Preparation 13** and methyl 4-(methylamino)butanoate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{46}H_{58}N_9O_4S$: 832.4332, found: 832.4324.

Preparation of P59: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(3-hydroxypropylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid



***Step A*: 3-[tert-butyl(dimethyl)silyl]oxy-N-prop-2-ynyl-propan-1-amine**

[729] The mixture of 0.70 mL (3.0 mmol) of 3-bromopropoxy-*tert*-butyl-dimethyl-silane, 1.9 mL (10 eq) of propargylic amine and 1.6 mL (3 eq) of DIPEA in acetonitrile (15 mL) was stirred at 50 °C until no further conversion was observed. The reaction mixture was concentrated, diluted with DCM, and extracted with saturated $NaHCO_3$ and brine. The combined organic layers were dried and concentrated to give the desired product in quantitative yield. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 3.62 (t, 2H), 3.27 (d, 2H), 3.02 (t, 1H), 2.59 (t, 2H), 2.19 (brs, 1H), 1.57 (m, 2H), 0.86 (s, 9H), 0.02 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 73.9, 61.5, 45.2, 37.9, 32.7, 26.3, -4.8; **HRMS (EI)** (m/z): [M-CH3]+ calcd for $C_{11}H_{22}NOSi$: 212.1471, found: 212.1467.

***Step B*: ethyl 5-[3-[4-[3-[3-[tert-butyl(dimethyl)silyl]oxypropylamino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)thiazole-4-carboxylate**

[730] Using **Sonogashira General Procedure** starting from 1.0 g (1.64 mmol) of the product of Preparation 15 and 737 mg (2 eq.) of the product from Step A as the appropriate acetylene, 1.16 g (96%) of the desired product was obtained. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 45.2 (t, 2H), 7.24 (dd, 1H), 7.17 (dd, 1H), 7.14 (t, 1H), 4.27 (br., 2H), 4.25 (q, 2H), 4.12 (t, 2H), 3.65 (t, 2H), 3.6 (s, 2H), 3.25 (t, 2H), 2.89 (t, 2H), 2.32 (s, 3H), 2.11 (m, 2H), 2.04 (m, 2H), 1.63 (m, 2H), 1.28 (t, 3H), 0.84 (s, 9H), 0.02 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 128.8, 119.1, 115.4, 68.3, 61.3, 60.7, 46.3, 45.2, 38.4, 32.4, 30.8, 26.3, 24.2, 23.1, 19.7, 15.7, 14.6, -4.8; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{35}H_{48}ClFN_5O_4SSi$: 716.2869, found: 716.2868.

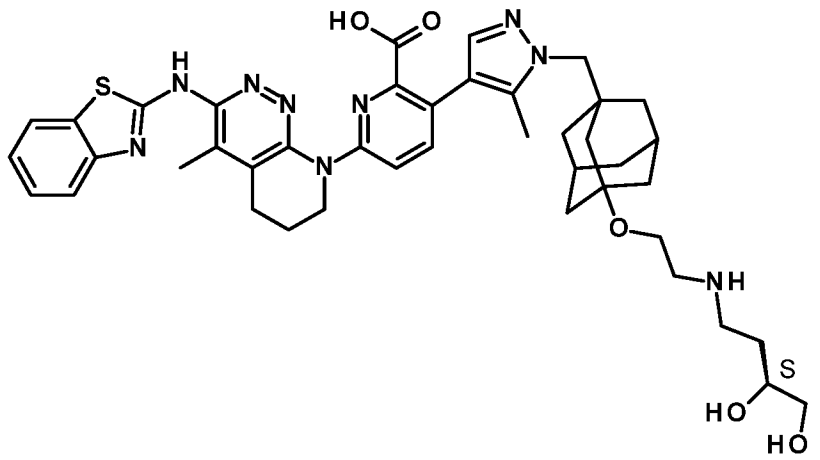
***Step C*: ethyl 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[3-[tert-butyl(dimethyl)silyl]oxypropylamino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate**

[731] Using **Buchwald General Procedure I** starting from 1.16 g (1.57 mmol) of the product from *Step B* and 730 mg (2 eq) of *1,3-benzothiazol-2-amine*, 598 mg (45%) of the desired product was obtained. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.87 (d, 1H), 7.49 (d, 1H), 7.37 (td, 1H), 7.25 (dd, 1H), 7.19 (t, 1H), 7.17 (t, 1H), 7.17 (m, 1H), 4.26 (br., 2H), 4.25 (q, 2H), 4.14 (t, 2H), 3.63 (t, 2H), 3.57 (s, 2H), 3.27 (t, 2H), 2.87 (t, 2H), 2.69 (t, 2H), 2.34 (s, 3H), 2.13 (m, 2H), 2.04 (m, 2H), 1.61 (m, 2H), 1.28 (t, 3H), 0.84 (s, 9H), 0.02 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 128.9, 126.5, 122.5, 122.3, 119.1, 116.3, 115.5, 68.4, 61.3, 60.6, 46.3, 45.2, 38.4, 32.4, 31.1, 26.3, 23.9, 23.2, 20.3, 14.6, 12.9, -4.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{42}H_{53}FN_7O_4S_2Si$: 830.3354, found: 830.3347.

***Step D*: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(3-hydroxypropylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid**

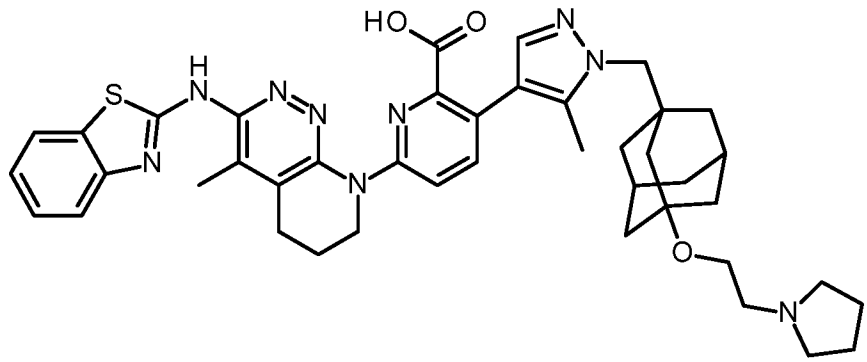
[732] The mixture of 590 mg (0.71 mmol) of the product from *Step C* and 298 mg of $LiOHxH_2O$ (10 eq) in 7 mL of THF / water (1:1) was stirred at 60 °C until no further conversion was observed. The reaction mixture was treated with 0.71 mL (12 eq) of concentrated hydrogen chloride at 0°C (pH = 2-3) and stirred until no further conversion was observed. After the reaction mixture was concentrated to remove THF and lyophilization, the solid was dissolved in a 6N NH_3 solution in MeOH and purified by reverse phase chromatography (using 25 mM NH_4HCO_3 and MeCN as eluents) to give 100 mg (21%) of the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{34}H_{35}FN_7O_4S_2$: 688.2176, found: 688.2179.

Preparation of P60: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[[(3S)-3,4-dihydroxybutyl]amino]ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



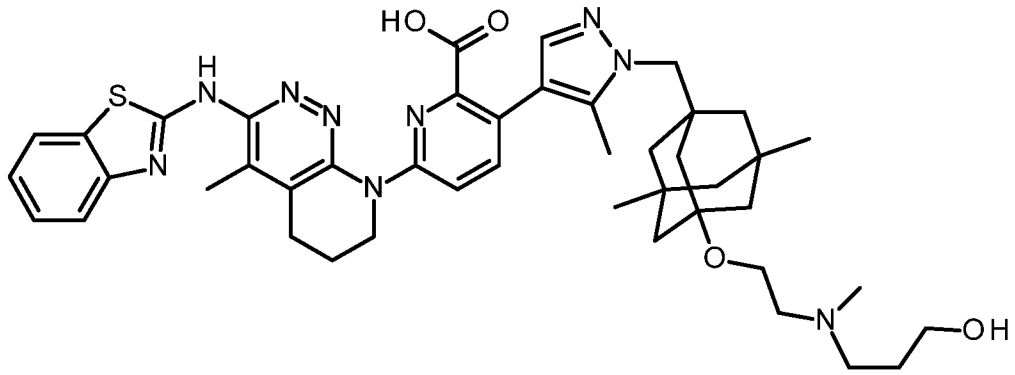
[733] To the product from the **Preparation 18** (0.066 mmol) in acetonitrile (30 ml/mmol) was added 2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine, hydrogen chloride (1:1) (3 eq) and the reaction mixture was stirred at 60 °C for 48 h. After the addition of KOH solution (5 eq), the reaction mixture was stirred at 60 °C for 1 h. After the addition of HCl solution (10 eq), the reaction mixture was stirred at 60 °C for 1 h. The product was purified by preparative HPLC chromatography (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{42}H_{52}N_9O_5S$: 794.3812, found: 794.3807.

Preparation of P61: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[5-methyl-1-[[3-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylic acid



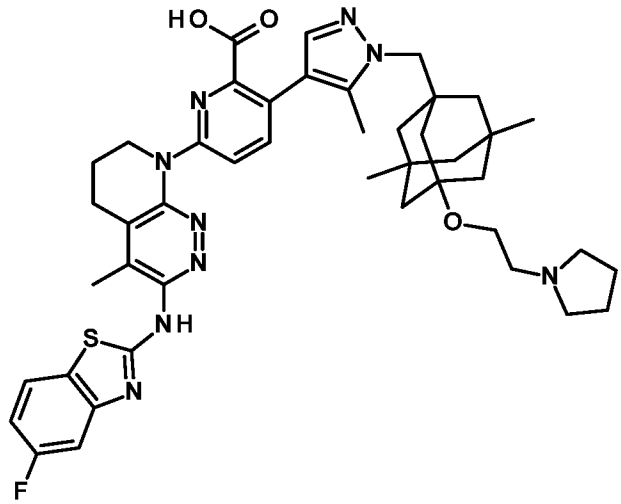
[734] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 18** and pyrrolidine as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{42}H_{50}N_9O_3S$: 760.3757, found: 760.3753.

Preparation of P62: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[3-hydroxypropyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



[735] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 16** and 3-(methylamino)propan-1-ol as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+2H]2+ calcd for $C_{44}H_{56}N_9O_4S$: 403.7127, found: 403.7126.

Preparation of P63: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid



***Step A:* (4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate**

[736] To 260 mg (0.35 mmol) of **Preparation 16, *Step C*** in 2 mL of dichloromethane were added 0.5 mL (10 eq) of *N,N*-diethylethanamine and 457 mg (4 eq) of p-tolylsulfonyl 4-methylbenzenesulfonate, then the mixture was stirred for 0.5 h. The product was purified by column chromatography (silica gel, using DCM and EtOAc as eluents) to give 259 mg (85%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.85 (d, 1H), 7.76 (d, 2H), 7.71 (d, 1H), 7.45 (d, 2H), 7.40 (s, 1H), 7.16 (d, 2H), 6.89 (d, 2H), 5.09 (s, 2H), 4.05 (t, 2H), 3.96

(t, 2H), 3.81 (s, 2H), 3.74 (s, 3H), 3.46 (t, 2H), 2.87 (t, 2H), 2.40 (s, 3H), 2.29 (s, 3H), 2.08 (s, 3H), 1.98 (qn, 2H), 1.29 (s, 2H), 1.13/1.11 (d+d, 4H), 1.11/1.06 (d+d, 4H), 0.98/0.90 (d+d, 2H), 0.81 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.1, 137.7, 130.6, 130.2, 128.2, 120.5, 114.3, 71.4, 66.8, 58.9, 58.4, 55.6, 49.8, 46.5, 46.0, 45.8, 42.9, 30.0, 24.6, 21.6, 21.0, 15.5, 10.8; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{48}H_{56}ClN_6O_7S$: 895.3620, found: 895.3619.

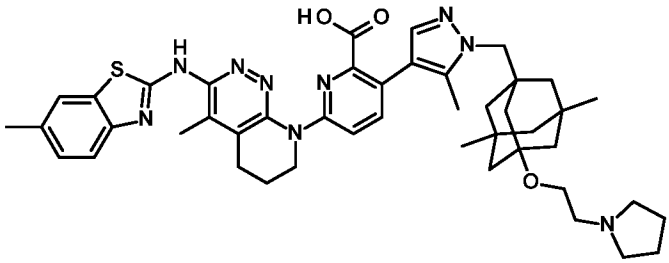
Step B: ***(4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate***

[737] To 259 mg (0.29 mmol) of the product from *Step A* in 3 mL acetonitrile was added pyrrolidine (3 eq), and the reaction mixture was stirred at 55 °C for 18 h. The product was purified by column chromatography (silica gel, using DCM and MeOH as eluents) to give 221 mg (98%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.85 (d, 1H), 7.70 (d, 1H), 7.40 (s, 1H), 7.18 (m, 2H), 6.91 (m, 2H), 5.10 (s, 2H), 3.96 (m, 2H), 3.86 (s, 2H), 3.75 (s, 3H), 3.60-2.90 (brs, 6H), 3.59 (brt, 2H), 2.87 (t, 2H), 2.29 (s, 3H), 2.11 (s, 3H), 2.10-1.70 (brs, 4H), 1.98 (m, 2H), 1.48-0.94 (m, 12H), 0.86 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.1, 137.7, 130.2, 120.5, 114.3, 66.8, 58.9, 56.9, 55.6, 46.0, 30.0, 24.6, 21.0, 15.5, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{57}ClN_7O_4$: 794.4161, found: 794.4160.

Step C: ***3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid***

[738] The mixture of 0.22 g (0.28 mmol) of the product from *Step B*, 93.5 mg (2 eq) of 5-fluoro-1,3-benzothiazol-2-amine, 25 mg (0.1 eq) of $Pd_2(dba)_3$, 32 mg (0.2 eq) of XantPhos, and 0.14 mL (3 eq) of DIPEA in 2 mL of butan-2-ol was kept at 100 °C in a microwave reactor for 1 h. The product was purified by column chromatography (using DCM / MeOH as eluents) to give the coupled product, which was treated with 3 eq of KOH in 2 mL of acetonitrile at 50 °C for 18 h. The hydrolysed product was purified by preparative HPLC chromatography (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{53}FN_9O_3S$: 806.3976, found: 806.3971.

Preparation of P64: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(6-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid



Step A: ***(4-methoxyphenyl)methyl 3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(6-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate***

[739] The mixture of 250 mg (0.34 mmol) of **Preparation 16, *Step C***, 112 mg (2 eq) of 6-methyl-1,3-benzothiazol-2-amine, 31 mg (0.1 eq) of $Pd_2(dba)_3$, 39 mg (0.2 eq) of XantPhos, and 0.17 mL (3 eq) of DIPEA in 2.5 mL of cyclohexanol was kept at 130 °C for 2 h. The product was purified by column chromatography (using DCM / MeOH as eluents) to give 206 mg (71%) of the desired product. **^{1}H NMR** (300 MHz, dmso-d6) δ ppm 7.93 (d, 1H), 7.69 (d, 1H), 7.62 (brs, 1H), 7.45 (brs, 1H), 7.39 (s, 1H), 7.19 (m, 2H), 7.16 (brd, 1H), 6.91 (m, 2H), 5.10 (s, 2H), 4.45 (brs, 1H), 3.99 (m, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.40 (t, 2H), 3.34 (t, 2H), 2.85 (t, 2H), 2.37 (s, 3H), 2.31 (s, 3H), 2.11 (s, 3H), 1.98 (m, 2H), 1.43-0.9 (m, 12H), 0.84 (s, 6H); **^{13}C NMR** (300 MHz, dmso-d6) δ ppm 140.0, 137.6, 130.2, 127.5, 121.7, 118.9, 114.3, 66.7, 62.1, 61.5, 59.0, 55.6, 45.4, 30.1, 24.2, 21.7, 21.4, 12.6, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{49}H_{57}N_8O_5S$: 869.4173, found: 869.4167.

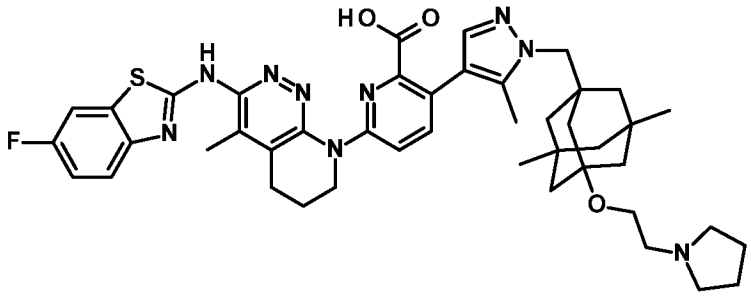
Step B: ***(4-methoxyphenyl)methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(6-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate***

[740] To 203 mg (0.23 mmol) of the product from *Step A* in 2 mL of dichloromethane was added 0.16 mL (5 eq) of *N,N*-diethylethanamine and 150 mg (2 eq) of p-tolylsulfonyl 4-methylbenzenesulfonate, then the mixture was stirred for 18 h. The product was purified by column chromatography (silica gel, using DCM and EtOAc as eluents) to give 84 mg (38%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 10.74 (br., 1H), 7.94 (d, 1H), 7.76 (dm, 2H), 7.69 (d, 1H), 7.61 (br., 1H), 7.45 (dm, 2H), 7.44 (br., 1H), 7.40 (s, 1H), 7.18 (dm, 2H), 7.17 (brd., 1H), 6.90 (dm, 2H), 5.09 (s, 2H), 4.05 (t, 2H), 3.99 (t, 2H), 3.82 (s, 2H), 3.74 (s, 3H), 3.47 (t, 2H), 2.84 (t, 2H), 2.40 (s, 3H), 2.37 (brs., 3H), 2.31 (s, 3H), 2.10 (s, 3H), 1.98 (m, 2H), 1.35-0.87 (m, 12H), 0.81 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.0, 137.7, 130.6, 130.1, 128.1, 127.5, 121.8, 118.9, 114.3, 71.5, 66.7, 58.9, 58.4, 55.6, 45.4, 30.0, 24.3, 21.6, 21.6, 21.4, 12.5, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{56}H_{63}N_8O_7S_2$: 1023.4261, found: 1023.4265.

Step C: ***3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(6-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid***

[741] To 84 mg (0.082 mmol) of the product from *Step B* in 1 mL acetonitrile was added pyrrolidine (3 eq) and the reaction mixture was stirred at 55 °C for 18 h. After treatment with 5 eq of KOH, the mixture was stirred at 55 °C for 1 h and the product was purified by preparative HPLC chromatography (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{56}N_9O_3S$: 802.4227, found: 802.4227.

Preparation of P65: 3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid



Step A: ***(4-methoxyphenyl)methyl 6-[3-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate***

[742] The mixture of 250 mg (0.34 mmol) of **Preparation 16, *Step C***, 114 mg (2 eq) of 6-fluoro-1,3-benzothiazol-2-amine, 31 mg (0.1 eq) of $Pd_2(dba)_3$, 39 mg (0.2 eq) of XantPhos, and 0.17 mL (3 eq) of DIPEA in 2.5 mL of cyclohexanol was kept at 130 °C for 2 h. The product was purified by column chromatography (using DCM / MeOH as eluents) to give 158 mg (55%) of the desired product.**^{1}H NMR** (500 MHz, dmso-d6) δ ppm 10.87 (brs, 1H), 7.94 (d, 1H), 7.77 (brd, 1H), 7.69 (d, 1H), 7.57 (brs, 1H), 7.39 (s, 1H), 7.20 (m, 1H), 7.19 (m, 2H), 6.91 (m, 2H), 5.10 (s, 2H), 4.45 (brs, 1H), 3.99 (m, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.40 (t, 2H), 3.34 (t, 2H), 2.85 (t, 2H), 2.31 (s, 3H), 2.11 (s, 3H), 1.98 (m, 2H), 1.43-0.91 (m, 12H), 0.84 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.0, 137.7, 130.2, 118.9, 114.3, 114.0, 108.4, 66.7, 62.1, 61.5, 59.0, 55.6, 45.4, 30.1, 24.3, 21.6, 12.5, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{48}H_{54}FN_8O_5S$: 873.3922, found: 873.3917.

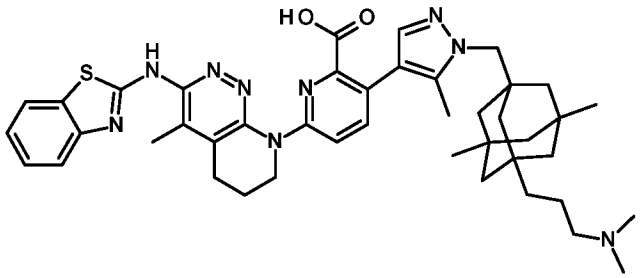
Step B: ***(4-methoxyphenyl)methyl 3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylate***

[743] To 158 mg (0.23 mmol) of the product from *Step A* in 2 mL of dichloromethane was added 0.125 mL (5 eq) of *N,N*-diethylethanamine and 117 mg (2 eq) of p-tolylsulfonyl 4-methylbenzenesulfonate, then the mixture was stirred for 18 h. The product was purified by column chromatography (silica gel, using DCM and EtOAc as eluents) to give 71 mg (41%) of the desired product. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 10.88 (brs, 1H), 7.94 (d, 1H), 7.77 (br., 1H), 7.76 (dm, 2H), 7.69 (d, 1H), 7.59 (br., 1H), 7.45 (dm, 2H), 7.40 (s, 1H), 7.21 (t, 1H), 7.17 (dm, 2H), 6.90 (dm, 2H), 5.09 (s, 2H), 4.05 (t, 2H), 4.00 (m, 2H), 3.82 (s, 2H), 3.74 (s, 3H), 3.47 (t, 2H), 2.85 (t, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.10 (s, 3H), 1.98 (m, 2H), 1.35-0.87 (m, 12H), 0.81 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.0, 137.7, 130.6, 130.1, 128.1, 118.9, 114.3, 114.0, 108.4, 71.5, 66.7, 58.9, 58.4, 55.6, 45.4, 30.0, 24.3, 21.6, 21.6, 12.5, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{55}H_{60}FN_8O_7S_2$: 1027.4010, found: 1027.4003.

Step C: ***3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid***

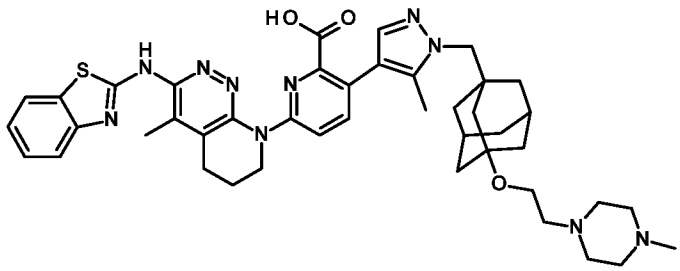
[744] To 71 mg (0.069 mmol) of the product from *Step B* in 1 mL acetonitrile was added pyrrolidine (3 eq) and the reaction mixture was stirred at 55 °C for 18 h. After treatment with 5 eq of KOH, the mixture was stirred at 55 °C for 1 h and the product was purified by preparative HPLC chromatography (using acetonitrile and 5 mM aqueous NH_4HCO_3 solution as eluents) to give the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{53}FN_9O_3S$: 806.3976, found: 806.3969.

Preparation of P66: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-(dimethylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



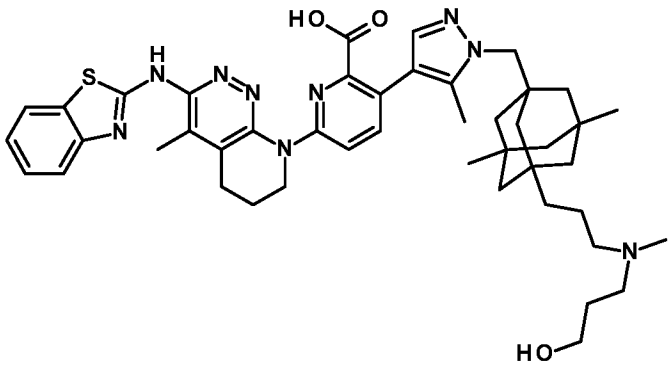
[745] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 13** and *N*-methylmethanamine as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{43}H_{54}N_9O_2S$: 760.4121, found: 760.4114.

Preparation of P67: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[5-methyl-1-[[3-[2-(4-methylpiperazin-1-yl)ethoxy]-1-adamantyl]methyl]pyrazol-4-yl]pyridine-2-carboxylic acid



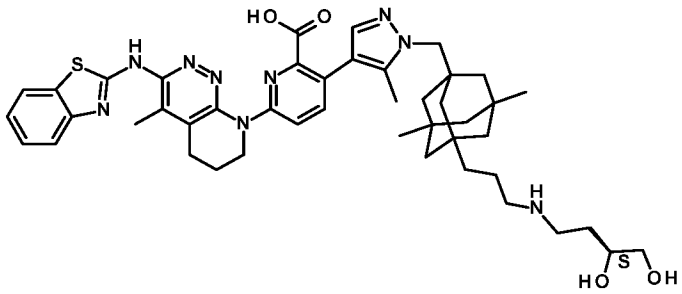
[746] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 18** and 1-methylpiperazine as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{43}H_{53}N_{10}O_3S$: 789.4022, found: 789.4014.

Preparation of P68: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[3-hydroxypropyl(methyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



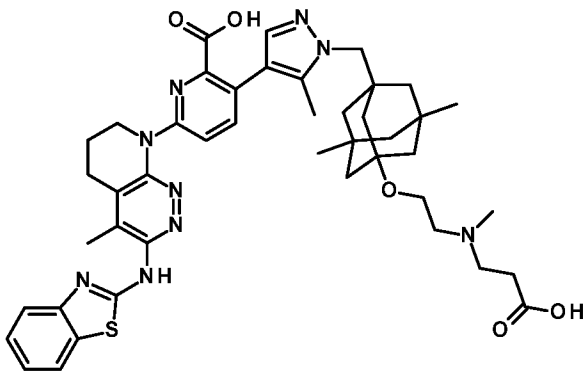
[747] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 13** and 3-(methylamino)propan-1-ol as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_3S$: 804.4383, found: 804.4375.

Preparation of P69: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-[[(3S)-3,4-dihydroxybutyl]amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



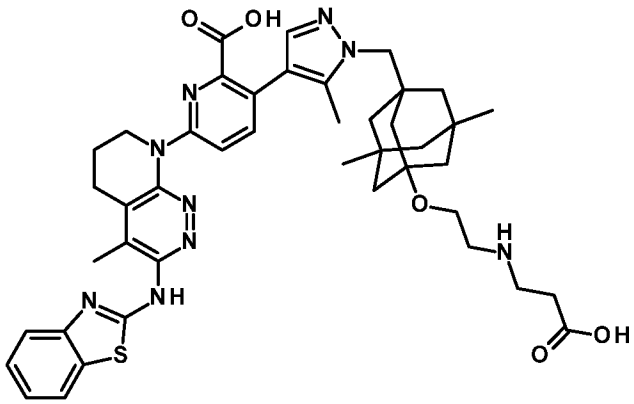
[748] To the product from the **Preparation 13** (0.074 mmol) in 2 mL of acetonitrile was added the 2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine, hydrogen chloride (1:1) (4 eq) and the reaction mixture was stirred at 60 °C for 18 h. After the addition of KOH solution (5 eq), the reaction mixture was stirred at 60 °C for 1 h. After the addition of HCl solution (10 eq), the reaction mixture was stirred at 60 °C for 0.5 h. The product was purified by preparative HPLC chromatography (using acetonitrile and 5mM aqueous NH_4HCO_3 solution as eluents) to give the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_4S$: 820.4332, found: 820.4323.

Preparation of P70: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[2-carboxyethyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



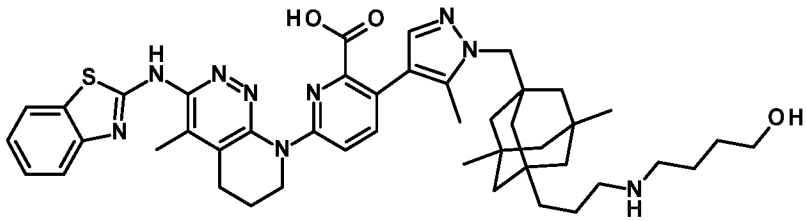
[749] Using the **Amine Substitution and Hydrolysis General procedure III**, starting from **Preparation 16** and ethyl 3-(methylamino)propanoate, hydrogen chloride (1:1) as the appropriate amine, the desired product was obtained.**HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{54}N_9O_5S$: 820.3968, found: 820.3962.

Preparation of P71: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-(2-carboxyethylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



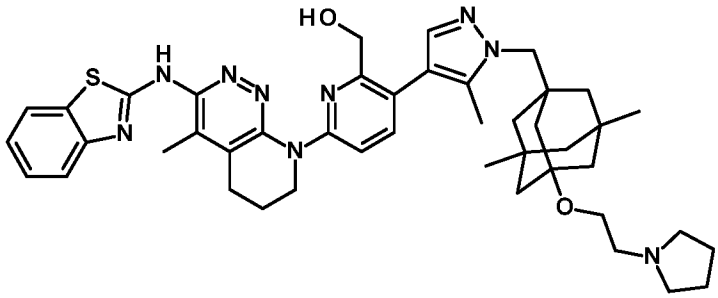
[750] Using the **Amine Substitution and Hydrolysis General procedure III**, starting from **Preparation 16** and methyl 3-aminopropanoate as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{43}H_{52}N_9O_5S$: 806.3812, found: 806.3793.

Preparation of P72: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[3-(4-hydroxybutylamino)propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



[751] Using the **Amine substitution and Hydrolysis General procedure I**, starting from **Preparation 13** and 4-aminobutan-1-ol as the appropriate amine, the desired product was obtained. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{45}H_{58}N_9O_3S$: 804.4383, found: 804.4383.

Preparation of P73: [6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-2-pyridyl]methanol



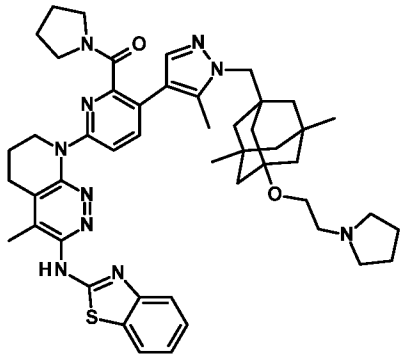
Step A: ***(4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate***

[752] Using the **Amine substitution and Hydrolysis General procedure I** without the hydrolysis step, starting from **Preparation 16** and pyrrolidine as the appropriate amine, 190 mg of the desired product was obtained. **^{1}H NMR** (500 MHz, dmso-d6) δ ppm 7.95 (d, 1H), 7.81 (d, 1H), 7.68 (d, 1H), 7.50 (brd., 1H), 7.39 (s, 1H), 7.35 (t, 1H), 7.19 (dm, 2H), 7.16 (t, 1H), 6.91 (dm, 2H), 5.10 (s, 2H), 3.99 (t, 2H), 3.85 (s, 2H), 3.74 (s, 3H), 3.41 (t, 2H), 2.85 (t, 2H), 2.46 (t, 2H), 2.41 (br., 4H), 2.32 (s, 3H), 2.11 (s, 3H), 1.98 (m, 2H), 1.62 (m, 4H), 1.40 (s, 2H), 1.28/1.22 (d+d, 4H), 1.19/1.13 (d+d, 4H), 1.03/0.94 (d+d, 2H), 0.84 (s, 6H); **^{13}C NMR** (500 MHz, dmso-d6) δ ppm 140.0, 137.7, 130.2, 126.4, 122.4, 122.1, 118.9, 114.3, 66.7, 59.5, 59.0, 56.6, 55.6, 54.5, 50.0, 46.9, 46.0, 45.4, 43.2, 30.1, 24.3, 23.6, 21.7, 12.6, 10.9; **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{52}H_{62}N_9O_4S$: 908.4645, found: 908.4633.

Step B: ***[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-2-pyridyl]methanol***

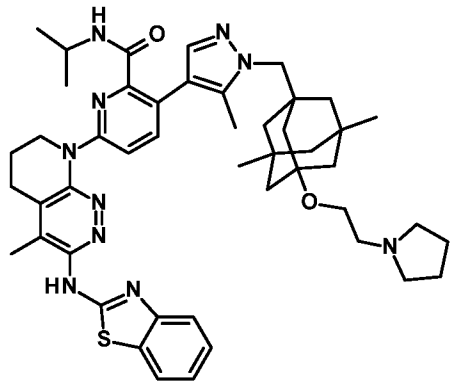
[753] To 190 mg (0.21 mmol) of the product from *Step A* in 4.2 mL of tetrahydrofuran was added 24 mg (3 eq) of $LiAlH_4$, and the mixture was stirred for 40 min. After quenching with 0.1% TFA in MeOH and filtration, the product was purified via preparative HPLC (MeCN and 0.1% TFA solution as eluents) to give 110 mg (67%) of the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{56}N_9O_2S$: 774.4277, found: 774.4269.

Preparation of P74: [6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-2-pyridyl]-pyrrolidin-1-yl-methanone



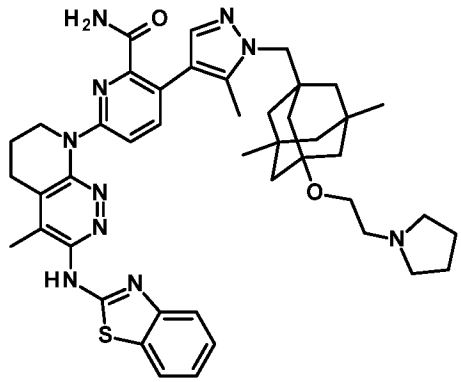
[754] To 50 mg (0.063 mmol) of **P21**, 9.37 mg (2.1 eq) of pyrrolidine, and 0.032 mL (3 eq) of DIPEA in 0.5 mL of DMF were added 36 mg (1.5 eq) of HATU at 0 °C, then the mixture was stirred for 18 h at room temperature. After pouring the reaction mixture into water, the precipitated solid was filtered out, washed with water, and dried. The product was purified by column chromatography (amino column, using DCM and MeOH as eluents) to give 29 mg (65%) of the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{48}H_{61}N_{10}O_2S$: 841.4699, found: 841.4698.

<u>Preparation of P75</u>: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-*N*-isopropyl-pyridine-2-carboxamide



[755] To 50 mg (0.063 mmol) of **P21**, 9.37 mg (2 eq) of propan-2-amine, and 0.032 mL (3 eq) of DIPEA in 0.5 mL of DMF were added 36 mg (1.5 eq) of HATU at 0 °C, then the mixture was stirred for 18 h at room temperature. After pouring the reaction mixture into water, the precipitated solid was filtered out, washed with water, and dried. The product was purified by column chromatography (amino column, using DCM and MeOH as eluents) to give 34 mg (76%) of the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{47}H_{61}N_{10}O_2S$: 829.4699, found: 829.4694.

Preparation of P76: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxamide



[756] To 50 mg (0.063 mmol) of **P21** and 18 mg (1.3 eq) of tert-butoxycarbonyl tert-butyl carbonate in 0.5 mL of dioxane was added 0.006 mL of pyridine, then the mixture was stirred for 10 min. After treating the mixture with 6.5 mg (1.3 eq) of NH_4HCO_3, the reaction was stirred for 5 days. The product was purified by column chromatography (amino column, using DCM and MeOH as eluents) to give 17 mg (47%) of the desired product. **HRMS-ESI** (m/z): [M+H]+ calcd for $C_{44}H_{55}N_{10}O_2S$: 787.4230, found: 787.4226.

Example 2. Synthesis and Characterization of Payload Precursors

[757] "PMB-protected payload" is also referred to as a precursor of the considered payload for the purpose of the preparation of a Linker/Payload.

Preparation A for Precursors: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(*p*-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

Step A: (4-methoxyphenyl)methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-(3,6-dichloro-5-methyl-pyridazin-4-yl)propylamino]pyridine-2-carboxylate

[758] The mixture of the product from **Preparation 11** (9.78 g, 18.1 mmol), the product from **Preparation 7** (13.6 g, 1.1 eq), $Pd(AtaPhos)_2Cl_2$ (801 mg, 0.1 eq), and Cs_2CO_3 (17.7 g, 3 eq) in 1,4-dioxane (109 mL) and H_2O (18 mL) was stirred at 80 °C for 8 h. After quenching the cooled reaction with brine, the mixture was extracted with EtOAc and the combined organic layers were dried and concentrated to give the desired product (21.9 g, 119%), which was used in the next step without further purification. ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.68-7.35 (m, 10H), 7.31 (d, 1H), 7.27 (s, 1H), 7.11 (dm, 2H), 6.98 (t, 1H), 6.83 (dm,

2H), 6.62 (d, 1H), 4.99 (s, 2H), 3.80 (s, 2H), 3.70 (s, 3H), 3.65 (t, 2H), 3.44 (t, 2H), 3.34 (q, 2H), 2.84 (m, 2H), 2.34 (s, 3H), 2.01 (s, 3H), 1.77 (m, 2H), 1.38-0.89 (m, 12H), 0.97 (s, 9H), 0.82 (s, 6H); ^{13}C NMR (500 MHz, dmso-d6) δ ppm 140.4, 137.6, 130.1, 114.2, 110.3, 66.3, 64.4, 61.7, 59.0, 55.5, 40.9, 30.1, 28.1, 27.3, 27.1, 16.4, 10.8; HRMS-ESI (m/z): [M+H]+ calcd for $C_{57}H_{69}Cl_2N_6O_5Si$: 1015.4475 found: 1015.4474.

Step B: (4-methoxyphenyl)methyl 3-[1-[[3-[2-[tert-butyl(diphenyl)silyl]oxyethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)pyridine-2-carboxylate

[759] The mixture of the product from Step A (21.9 g, 21.6 mmol), Cs_2CO_3 (14 g, 2 eq), DIPEA (7.5 mL, 2 eq) and $Pd(Ataphos)_2Cl_2$ (954 mg, 0.1 eq) in 1,4-dioxane (108 mL) was stirred at 110 °C for 18 h. After quenching with water and extracting with EtOAc, the combined organic phases were dried, concentrated, and purified by column chromatography (silica gel, DCM and EtOAc as eluents) to give the desired product (8.4 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.84 (d, 1H), 7.67 (d, 1H), 7.65 (d, 4H), 7.44 (t, 2H), 7.41 (s, 1H), 7.40 (t, 4H), 7.15 (d, 2H), 6.87 (d, 2H), 5.07 (s, 2H), 3.96 (t, 2H), 3.83 (s, 2H), 3.71 (s, 3H), 3.66 (t, 2H), 3.45 (t, 2H), 2.86 (t, 2H), 2.29 (s, 3H), 2.08 (s, 3H), 1.97 (qn, 2H), 1.38 (s, 2H), 1.25/1.18 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.01/0.93 (d+d, 2H), 0.97 (s, 9H), 0.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.8, 159.7, 156.3, 153.6, 150.8, 147.7, 140.1, 137.6, 137.3, 136.0, 135.6, 133.8, 130.2, 130.2, 129.1, 128.2, 127.7, 123.0, 120.4, 115.6, 114.3, 74.2, 66.8, 64.4, 61.7, 59.3, 55.6, 49.9, 46.8, 46.0, 46.0, 43.3, 39.7, 33.6, 30.1, 27.1, 24.6, 21.0, 19.3, 15.5, 10.8; HRMS-ESI (m/z): [M+H]+ calcd for $C_{57}H_{68}ClN_6O_5Si$: 979.4709 found: 979.4710.

Step C: (4-methoxyphenyl)methyl 6-(3-chloro-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl)-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[760] To the product from Step B (8.4 g, 8.6 mmol) in THF (86 mL) was added a 1 M solution of TBAF in THF (9.4 mL, 1.1 eq) at 0 °C and the reaction mixture was stirred at room temperature for 1.5 h. After quenching with a saturated solution of NH_4Cl and extracted with EtOAc, the combined organic phases were washed with brine, dried, concentrated, and purified by column chromatography (silica gel, DCM and MeOH as eluents) to give the desired product (4.7 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.85 (d, 1H), 7.70 (d, 1H), 7.39 (s, 1H), 7.18 (d, 2H), 6.90 (d, 2H), 5.10 (s, 2H), 4.45 (t, 1H), 3.96 (t, 2H), 3.84 (s, 2H), 3.74 (s, 3H), 3.40 (q, 2H), 3.33 (t, 2H), 2.86 (t, 2H), 2.29 (s, 3H), 2.09 (s, 3H), 1.98 (qn, 2H), 1.39 (s, 2H), 1.27/1.21 (d+d, 4H), 1.18/1.12 (d+d, 4H), 1.03/0.94 (d+d, 2H), 0.84 (s,

6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.8, 159.7, 156.3, 153.6, 150.8, 147.8, 140.2, 137.6, 137.3, 136.0, 130.2, 129.1, 127.7, 123.0, 120.4, 115.6, 114.3, 74.0, 66.8, 62.2, 61.5, 59.0, 55.6, 50.0, 46.9, 46.0, 46.0, 43.3, 39.7, 33.5, 30.1, 24.6, 21.0, 15.5, 10.9; HRMS-ESI (m/z): [M+H]+ calcd for $C_{41}H_{50}ClN_6O_5$: 741.3531 found: 741.3530.

Step D: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-(2-hydroxyethoxy)-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[761] The mixture of the product from Step C (4.7 g, 6.3 mmol), 1,3-benzothiazol-2-amine (1.9 g, 2 eq), Pd_2dba_3 (580 mg, 0.1 eq), XantPhos (730 mg, 0.2 eq), and DIPEA (3.3 mL, 3 eq) in cyclohexanol (38 mL) was stirred at 130 °C for 2 h. Purification by column chromatography (silica gel, heptane, EtOAc and MeCN as eluents) afforded the desired product (3.83 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.95 (d, 1H), 7.81 (brd, 1H), 7.69 (d, 1H), 7.49 (brs, 1H), 7.39 (s, 1H), 7.35 (m, 1H), 7.19 (m, 2H), 7.16 (m, 1H), 6.91 (m, 2H), 5.10 (s, 2H), 4.46 (t, 1H), 3.99 (m, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.40 (m, 2H), 3.34 (t, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 2.11 (s, 3H), 1.99 (m, 2H), 1.45-0.9 (m, 12H), 0.84 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{48}H_{55}N_8O_5S$: 855.4016 found: 855.4011.

Step E: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-[2-(p-tolylsulfonyloxy)ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[762] To the product from Step D (3.83 g, 4.48 mmol) and triethylamine (1.87 mL, 3 eq) in DCM (45 mL) was added *p*-tolylsulfonyl 4-methylbenzenesulfonate (2.19 g, 1.5 eq) and the reaction mixture was stirred for 2 h. Purification by column chromatography (silica gel, heptane and EtOAc as eluents) afforded 2.5 g (55%) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.95 (d, 1H), 7.81 (brs, 1H), 7.76 (m, 2H), 7.45 (brs, 1H), 7.45 (m, 2H), 7.40 (s, 1H), 7.35 (m, 1H), 7.18 (m, 2H), 7.17 (m, 1H), 6.97 (d, 1H), 6.90 (m, 2H), 5.10 (s, 2H), 4.05 (m, 2H), 4.00 (m, 2H), 3.82 (s, 2H), 3.74 (s, 3H), 3.47 (m, 2H), 2.85 (m, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.10 (s, 3H), 1.98 (m, 2H), 1.87-1.34 (m, 12H), 0.81 (s, 6H); HRMS-ESI (m/z): [M+H]+ calcd for $C_{55}H_{61}N_8O_7S_2$: 1009.4104 found: 1009.4102.

Amine substitution procedure III

[763] To the product from **Preparation A for Precursors** in a 1:1 mixture of acetonitrile and *N*-methyl-2-pyrrolidone (10 ml/mmol) was added the appropriate amine (3-10 eq) and the reaction mixture was stirred at 50 °C for 2-24 h. After the purification of the product by preparative reversed phase chromatography, the desired product was obtained.

Precursor of P37: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[4-hydroxybutyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[764] Using **Amine substitution procedure III** and 4-(methylamino)butan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{53}H_{66}N_9O_5S$: 940.4907 found 940.4906.

Precursor of P36: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[3-methoxypropyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[765] Using **Amine substitution procedure III** and 3-methoxy-*N*-methyl-propan-1-amine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{53}H_{66}N_9O_5S$: 940.4907 found 940.4904.

Precursor of P35: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[2-hydroxyethyl(methyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[766] Using **Amine substitution procedure III** and 2-(methylamino)ethanol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{51}H_{62}N_9O_5S$: 912.4594 found 912.4592.

Precursor of P27: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-(dimethylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[767] Using **Amine substitution procedure III** and dimethylamine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{50}H_{60}N_9O_4S$: 882.4489 found 882.4490.

Precursor of P21: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3,5-dimethyl-7-(2-pyrrolidin-1-ylethoxy)-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[768] Using **Amine substitution procedure III** and pyrrolidine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): [M+2H]2+ calcd for $C_{52}H_{62}N_9O_4S$: 454.7362 found 454.7365.

Precursor of P25: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-(3-hydroxypropylamino)ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[769] Using **Amine substitution procedure III** and 3-aminopropan-1-ol as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{51}H_{62}N_9O_5S$: 912.4591, found 912.4581.

Precursor of P19: (4-methoxyphenyl)methyl 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5*H*-pyrido[2,3-*c*]pyridazin-8-yl]-3-[1-[[3-[2-[2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethylamino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate

[770] Using **Amine substitution procedure III** and 2-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]ethanamine as the appropriate amine, the desired product was obtained. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{55}H_{68}N_9O_6S$: 982.5013, found 982.5000.

Example 3. Synthesis and Characterization of Linkers, Linker-Payloads, and Precursors thereof

[771] Exemplary linkers, linker-payloads, and precursors thereof were synthesized using exemplary methods described in this example.

Abbreviations:

CuI	cupper (I) iodide
DCC	dicyclohexyl carbodiimide
DCM	dichloromethane
DEA	N-ethylethanamine
DIPEA:	N,N-Diisopropylethylamine
DMF:	dimethylformamide
DMSO:	dimethyl sulfoxide
EDC:	*N*-Ethyl,*N'*-dimethylamino-propylcarbodiimide
EEDQ	ethyl 2-ethoxy-2H-quinoline-1-carboxylate
Fmoc :	Fluorenylmethyloxycarbonyl
Fmoc-Cit-OH	(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-5-ureido-pentanoic acid
HBTU:	(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HOAt:	1-Hydroxy-7-azabenzotriazole
$MgSO_4$	magnesium sulfate
MMAE:	(2S)-N-[(1S)-1-[[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[[(1R,2S)-2-hydroxy-1-methyl-2-phenyl-ethyl]amino]-1-methoxy-2-methyl-3-oxo-propyl]pyrrolidin-1-yl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxo-butyl]-methyl-carbamoyl]-2-methyl-propyl]-3-methyl-2-(methylamino)butanamide (MMAE)
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
NMP	N-methylpyrrolidone
$Pd(PPh_3)_2Cl_2$	dichloro-tri(triphenylphosphine)palladium
PBr_3	tribromophosphane
Pt/C 10%	platinum over carbon 10%
RT	room temperature
$SOCl_2$	thionyl chloride
THF	tetrahydrofuran
TBAF	tetrabutylammonium, fluoride
TBAI	tetrabutylammonium, iodide
TFA	trifluoroacetic acid
TSTU:	[dimethylamino-(2,5-dioxopyrrolidin-1-yl)oxy-methylene]-dimethyl-ammonium; tetrafluoroborate

Chemical naming

[772] IUPAC-preferred names were generated using the chemical naming functionality provided by Biovia® Draw 2018 (Version 18.1 .NET).

Materials, Methods & General Procedures

[773] All reagents obtained from commercial sources were used without further purification. Anhydrous solvents were obtained from commercial sources and used without further drying. Flash chromatography was performed on CombiFlash Rf (Teledyne ISCO) with pre-packed silica-gel cartridges (Macherey-Nagel Chromabond Flash). Thin layer chromatography was conducted with 5 x 10 cm plates coated with Merck Type 60 F254 silica-gel. Microwave heating was performed in CEM Discover® instrument.

[774] ^{1}H-NMR measurements were performed on 400 MHz Bruker Avance or 500 MHz Avance Neo spectrometer, using DMSO-d_6 or $CDCl_3$ as solvent. ^{1}H NMR data is in the form of chemical shift values, given in part per million (ppm), using the residual peak of the solvent (2.50 ppm for DMSO-d_6 and 7.26 ppm for $CDCl_3$) as internal standard. Splitting

patterns are designated as: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br s (broad singlet), br t (broad triplet) dd (doublet of doublets), td (triplet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets). IR measurements were performed on a Bruker Tensor 27 equipped with ATR Golden Gate device (SPECAC). HRMS measurements were performed on a LTQ OrbiTrap Velos Pro mass spectrometer (ThermoFisher Scientific). Samples were dissolved in $CH_3CN/H2O$ (2/1:v/v) at a concentration range from 0.01 to 0.05 mg/mL approximately and introduced in the source by an injection of 2 µL in a flow of 0.1 mL/min. ESI ionization parameters were as follow: 3.5 kV and 350°C transfer ion capillary. All the spectra were acquired in positive ion mode with a resolving power of 30,000 or 60,000 using a lock mass.

[775] HRMS measurements were performed on an LTQ OrbiTrap Velos Pro mass spectrometer (ThermoFisher Scientific GmbH, Bremen, Germany). Samples were dissolved in CH_3CN/H_2O (2/1:v/v) at a concentration range from 0.01 to 0.05 mg/mL approximately and introduced in the source by an injection of 2 µL in a flow of 0.1 mL/min. ESI ionization parameters were as follows: 3.5 kV and 350°C transfer ion capillary. All the spectra were acquired in positive ion mode with a resolving power of 30 000 or 60 000 using a lock mass.

UPLC®-MS:

[776] UPLC®-MS data were acquired using an instrument with the following parameters (Table 10):

Table 10. UPLC®-MS Parameters

Instrument(s)	Waters Aquity A-class with diode array UV detector “PDA” and “ZQ detector 2” mass device and MassLinks software.
ZQ detector 2	MS scan from 0.15 to 6 min and from 100 to 2372 Da
PDA detector	from 190 to 400 nm
Columns	Aquity UPLC ®BEH column C18, 1.7 µm, 130 Å, 2.1x50 mm Column used at 40°C with a flowrate of 0.6mL/min
Solvent A	water + 0.02% TFA
Solvent B	acetonitrile + 0.02% TFA
Gradient	from 2% B to 100% B in 5 min, then 0.3 min washing with 100% B and 0.5 min equilibration at 2% B for the next injection (total gradient of 6 min).

Preparative-HPLC:

[777] Preparative-HPLC (“Prep-HPLC”) data were acquired using an instrument with the following parameters (Table 11):

Table 11. Prep-HPLC Parameters

Instrument(s)	Columns Waters X-Bridge 5 or 10 µm with sizes (flowrate) of: 19x50 mm (12 ml/min), 19x100 mm (12 ml/min), 30x100 mm (30-50 ml/min), 30x250 mm (30-50 ml/min), 50x250 mm (80-150 ml/min); Interchim Puriflash 4100 with a maximum of 100 bars and a maximum flowrate of 250 ml/min, or Interchim Puriflash 4250 with a maximum of 250 bars and a maximum flowrate of 250 ml/min; Quaternary solvent pump with the possibility to use 4 solvents at the same time in a gradient
UV	2 wavelengths for the collection between 200 and 400 nm
Columns	Waters XBridge 10µm
Collection	8 ml or 32 ml tubes

[778] Three Prep-HPLC methods were used:

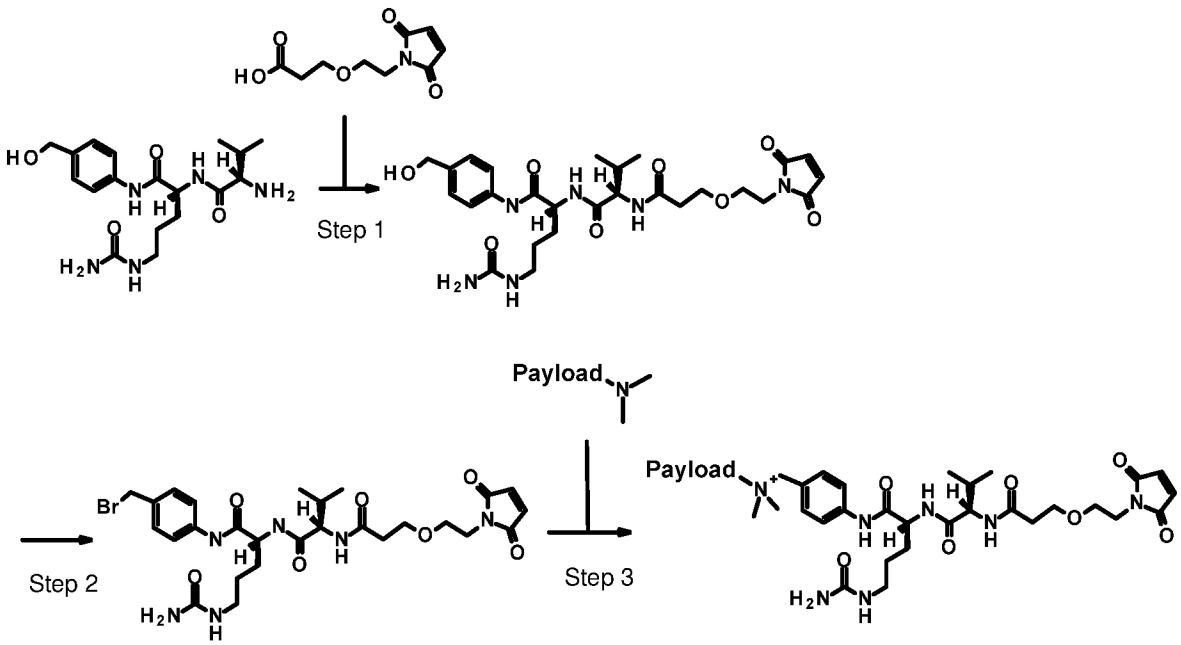
a. TFA method: solvent: A = water + 0.05 % TFA, B = acetonitrile + 0.05 % TFA, gradient from 5 to 100% B in 15 to 30 CV

b. NH_4HCO_3 method: solvent: A = water + 0.02 M NH_4HCO_3, B = acetonitrile/water 80/20 + 0.02 M NH_4HCO_3, gradient from 5 to 100 % B in 15 to 30 CV

c. Neutral method: solvent: A = water, B = acetonitrile, gradient from 5 to 100% B in 15 to 30 CV

[779] All the fractions containing the pure compound were combined and directly freeze-dried to afford the compound as an amorphous powder.

Method A



Step 1: (2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide

[780] To a solution of 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoic acid (855 mg, 4.01 mmol) in THF (42 mL) were added *N,N'*-dicyclohexylmethanediimine (1.05 g, 5.08 mmol) and 1-hydroxypyrrolidine-2,5-dione (510 mg, 4.43 mmol). The reaction mixture was stirred at room temperature for 20 h. The precipitate was removed by filtration and the filtrate was added to a solution of (2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (1.27 g, 3.35 mmol) in DMF (42 mL). The reaction mixture was stirred at room temperature for 20 h, diluted with diethyl ether (250 mL). The solid was recovered by filtration to afford (2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (1.81 g). ^{1}H NMR (400 MHz, dmso-d6): δ 9.87 (s, 1H), 8.05 (d, 1H), 7.82 (d, 1H), 7.53 (d, 2H), 7.21 (d, 2H), 7.00 (s, 2H), 5.95 (t, 1H), 5.39 (s, 2H), 5.07 (t, 1H), 4.41 (d, 2H), 4.34-4.40 (m, 1H), 4.18-4.22 (m, 1H), 3.42-3.65 (m, 4H), 2.88-3.02 (m, 2H), 2.73 (s, 2H), 2.28-2.45 (m, 2H), 1.91-1.99 (m, 1H), 1.53-1.75 (m, 2H), 1.30-1.147 (m, 2H), 0.85 (d, 3H), 0.81 (d, 3H). ^{13}C NMR (125 MHz, dmso-d6): δ 171.05, 170.83, 170.32, 170.09, 158.82, 137.49, 137.37, 134.50, 126.88, 118.81, 66.66, 66.53, 62.57, 57.49, 53.06, 36.74, 35.76, 30.51, 29.31, 26.79, 25.20, 19.16, 18.07. MS (ESI) m/z $[M + H]^+$ = 575.2.

Step 2: (2S)-N-[4-(bromomethyl)phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy] propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide

[781] To a solution of (2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (37.2 mg, 65 µmol) in THF (1 mL) was added dropwise phosphorus tribromide (45 µL, 97 mmol) at 0 °C under argon. The reaction was stirred at 0 °C for 1 h and at room temperature for 2 h. The progress of the reaction was followed by UPLC-MS: an aliquot was treated by a large excess of morpholine in acetonitrile, following the formation of the corresponding morpholine adduct. The reaction was diluted with THF (3 mL), quenched by the addition of 2 drops of a saturated solution of $NaHCO_3$, stirred for 5 min at room temperature, dried over magnesium sulfate and filtered. The residue, containing the crude (2S)-N-[4-(bromomethyl)phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide (45 mg) was used immediately in the next step. MS (ESI) m/z $[M + H]^+$ = 662.62 (morpholine adduct).

Step 3: General procedure for linker introduction

[782] To a suspension of the payload (19.6 µmol) in DMF (30 mL/mmol) was added a solution of the product of Step 2 (1.2 eq.) in THF (50 mL/mmol) and DIPEA (3 eq.). The reaction was stirred at room temperature for 2 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to give the desired compound.

Preparation of L9A-P27: 2-[[(5R,7S)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate

[783] Using **Method A** and **P27** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$=1318.6557 (δ=0.2 ppm)

Preparation of L9A-P30: 2-[[(5RS,7SR)-3-[[4-[6-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetic acid

[784] Using **Method A** and **P30** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$=1292.6386 (δ=-0.9 ppm).

Preparation of L9A-P33: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]azepan-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[785] Using **Method A** and **P33** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1372.7019 (δ = -0.3 ppm).

Preparation of L9A-P32: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4-isopropyl-piperazin-4-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[786] Using **Method A** and **P32** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$=1401.7287 (δ = -0.1 ppm).

Preparation of L9A-P38: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]piperidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[787] Using **Method A** and **P38** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$=1358.6803 (δ = -4.7 ppm).

Preparation of L9A-P39: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]morpholin-4-ium-4-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylate;2,2,2-trifluoroacetic acid

[788] Using **Method A** and **P39** as the appropriate payload, the desired product was obtained.

HRMS (ESI) $[M+H]^+$ found = 1360.6634 (δ = -1.9 ppm).

Preparation of L9A-P41: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[3-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[789] Using **Method A** and **P41** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1342.6844 (δ = -5.5 ppm).

Preparation of L9A-P42: 3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(5-methyl-1,3-benzothiazol-2-yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[790] Using **Method A** and **P42** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1358.6807 (δ = -4.4ppm).

Method B

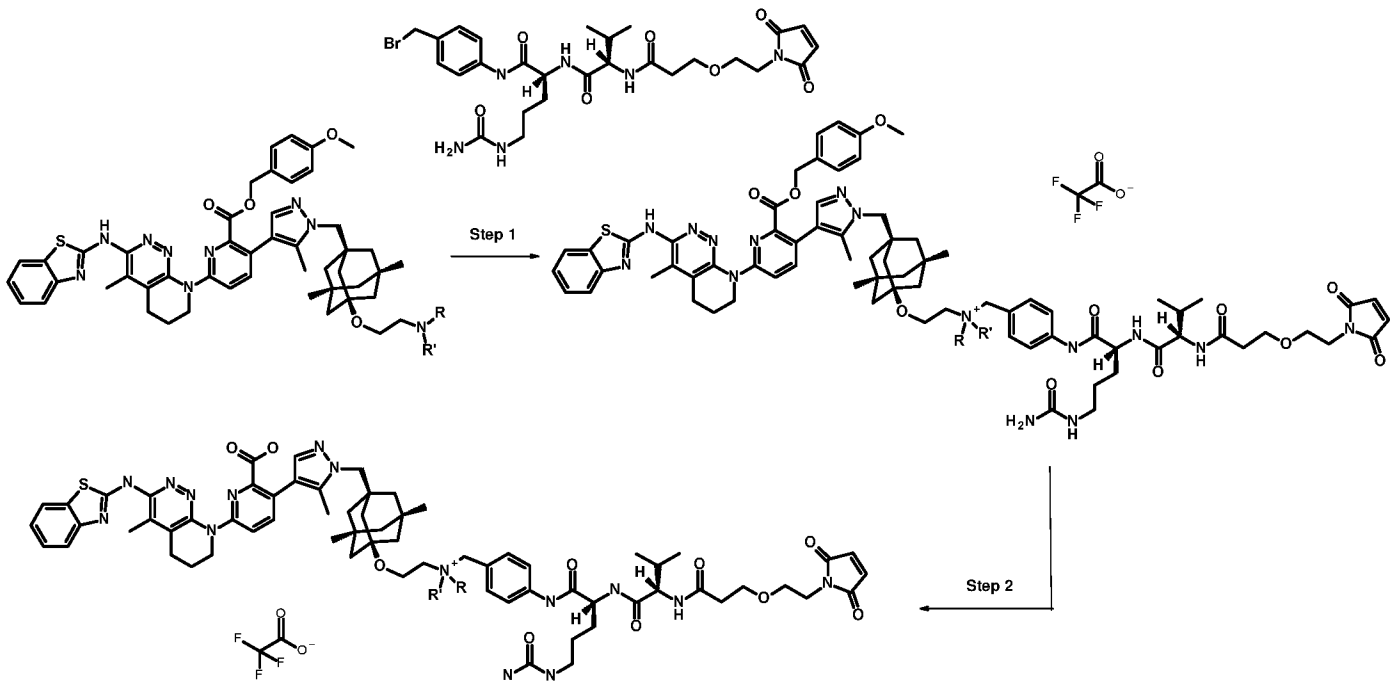
Step1:

[791] To a suspension of the *para* methoxy benzyl (PMB)-protected payload (11.3 µmol) in DMF (0.4 mL) was added a solution of (2S)-N-[4-(bromomethyl)phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide (12.4 mg, 13.6 µmol) in THF (0.2 mL) and DIPEA (9.8 µL, 56.7 µmol). The reaction was stirred at room temperature for 4 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the expected compound which was directly used in **Step 2**.

Step2:

[792] To a suspension of the product from **Step 1** in DCM (3.2 mL) was added TFA (320 µL, 4.18 mmol). The reaction was stirred at room temperature for 1 h. The solvent was evaporated and the residue dissolved in DMF (500 µL) This crude solution was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the desired product.

Preparation of L9A-P35: 2-[[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(2-hydroxyethyl)-methyl-ammonium;2,2,2-trifluoroacetic acid



[793] Using **Method B** and **the precursor of P35** as the appropriate PMB-protected payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1318.6531 (δ = -1.7 ppm).

Preparation of L9A-P36: 2-[[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(3-methoxypropyl)-methyl-ammonium;2,2,2-trifluoroacetic acid

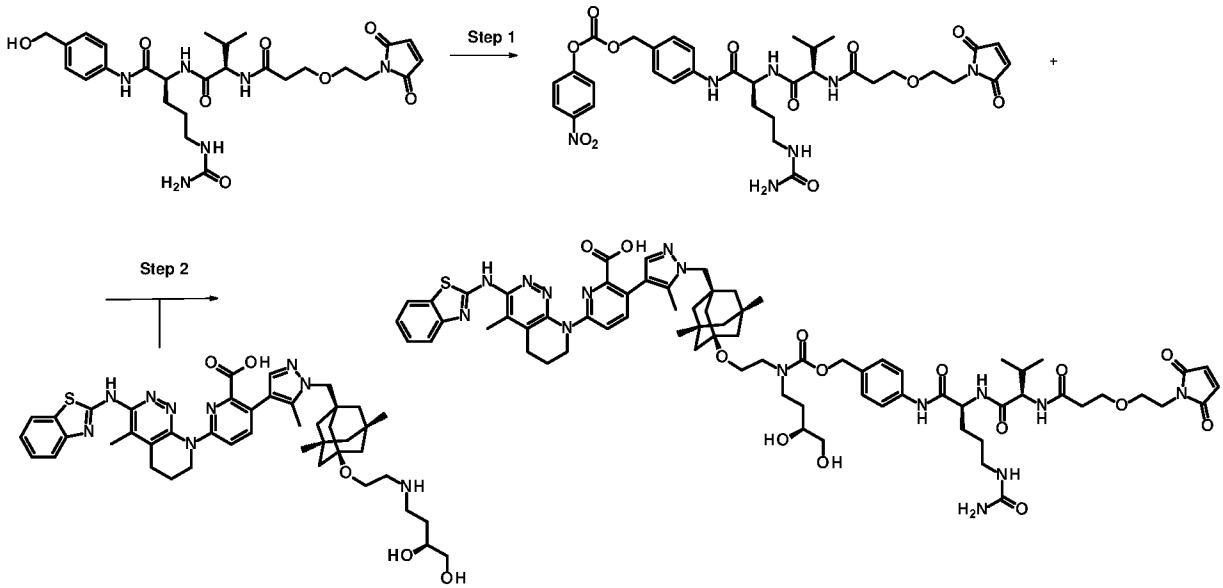
[794] Using **Method B** and **the precursor of P36** as the appropriate PMB-protected payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1376.6930 (δ = -3.1 ppm).

Preparation of L9A-P37: 2-[[(5SR,7RS)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(4-hydroxybutyl)-methyl-ammonium;2,2,2-trifluoroacetic acid

[795] Using **Method B** and **the precursor of P37** as the appropriate PMB-protected payload, the desired product was obtained. HRMS (ESI) $[M\text{-}CF_3CO_2]^+$ found = 1376.6918 (δ= -3.9 ppm).

Method C

Preparation of L9C-P19: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[[(3S)-3,4-dihydroxybutyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid



Step 1: [4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate

[796] To a solution of (2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (from **Method A**, **Step 1**) (580 mg; 1.0 mmol) in dry DMF were added DIPEA (0.5 mL; 3.025 mmol; 3 eq.) and bis(4-nitrophenyl)carbonate (615 mg; 2.02 mmol; 2 eq.). The reaction mixture was stirred at room temperature for 68 h. The reaction mixture was diluted with diethyl ether (15 mL) and the solid was filtered to afford the title compound (589 mg; 79%).^{1}**H** NMR (dmso-d_6): 0.82 (*d*, 3H, *J* = 6.8 Hz), 0.85 (*d*, 3H, *J* = 6.8 Hz), 1.47-1.33 (*m*, 2H), 1.74-1.54 (*m*, 2H), 1.92 -2.00 (*m*, 1H), 2.32-2.45 (*m*, 2H), 2.90-3.06 (*m*, 2H), 3.49-3.46 (*m*, 2H), 3.60-3.52 (*m*, 4H), 4.21 (*dd*, 1H, *J* = 8.7 and 6.8 Hz), 4.39 (*m*, 1H), 5.24 (*s*, 2H), 5.39 (*s*, 2H), 5.96 (*t*, 1H, *J* = 5.6 Hz), 7.00 (*s*, 2H), 7.41 (*d*, 2H, *J* = 8.8 Hz), 7.57 (*dd*, 2H, *J* = 6.8 and 2.4Hz), 7.65 (*d*, 2H, *J* = 8.4 Hz), 7.83 (*d*, 1H, *J* = 8.8 Hz), 8.10 (*d*, 1H, *J* = 7.6 Hz), 8.31 (*dd*, 2H, *J* = 6.8 and 2.4 Hz), 10.03 (*s*, 1H). LCMS Positive mode 740.14 detected ($M+H^+$).

Step 2: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[[(3S)-3,4-dihydroxybutyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid.

[797] To a suspension of **P19** (15 mg, 0.016 mmol) in DMF (0.5 mL) were added DIPEA (14 µL, 0.0801 mmol) and the carbonate of **Step 1** (14.2 mg, 0.0192 mmol) and the mixture was stirred at room temperature for 18 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the title compound (6.9 mg, yield 30%). ^{1}H NMR (500 MHz, dmso-d6) δ ppm (m, 2 H), (m, 4 H), (m, 10 H), (m, 2 H), 9.98 (s), 8.08 (d), 7.9 (d, 1 H), 7.82 (d), 7.8 (large, 1 H), 7.79 (largeNC, 1 H), 7.6 (m, 2 H), 7.49 (largeNC, 1 H), 7.43 (br s, 1 H), 7.37 (t, 1 H), 7.28 (d, 2 H), 7.19 (t, 1 H), 7 (s, 2 H), 5.97 (br s), 5.42 (large), 4.99 (s, 2 H), 4.38 (m, 1 H), 4.22 (t, 1 H), 4.03 (t, 2 H), 3.86 (m, 2 H), 3.57/3.46/3.28/3.21 (m, 6 H), 3.53 (m, 2 H), 3.42 (m, 2 H), 3.38 (m, 1 H), 3.01/2.94 (2m, 2 H), 2.89 (t, 2 H), 2.43/2.32 (2m, 2 H), 2.37 (s, 3 H), 2.2 (s, 3 H), 2.03 (m, 2 H), 1.95 (m, 1 H), 1.7/1.38 (2m, 2 H), 0.84 (m, 6 H), 0.84 (m, 6 H).^{13}C NMR (500 MHz, dmso-d6) δ ppm 137.6, 135.5, 128.7, 126.8, 122.7, 122.1, 119.1, 118.4, 69.7, 66.9, 66.2, 58.9, 58.4, 58.3, 53.7, 50.5/47.1/43.5, 48.3/46, 46, 39, 36.9, 36.6, 32.8, 30.9, 30.5, 30, 27.7, 24.4, 21.3, 19.8, 13.5, 10.8. HRMS (ESI) $[M+H]^+$ found = 1422.6688 (δ = 1.6 ppm).

Preparation of L9C-P22: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(4-hydroxybutyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[798] Using **Method C** and **P22** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found =1406.6728 (δ=1.0 ppm).

Preparation of L9C-P23: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-[3-hydroxy-2-(hydroxymethyl)propyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[799] Using **Method C** and **P23** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1422.6670 (δ = 0.5 ppm).

Preparation of L9C-P24: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-[2-hydroxy-1-(hydroxymethyl)ethyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[800] Using **Method C** and **P24** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1408.6518 (δ = 0.8 ppm).

Preparation of L9C-P25: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-hydroxypropyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[801] Using **Method C** and **P25** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found =1366.6396 (δ=-0.4 ppm).

Preparation of L9C-P26: 6-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-3-[1-[[(5SR,7RS)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-hydroxypropyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[802] Using **Method C** and **P26** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found =1366.6396 (δ=-0.4 ppm).

Preparation of L9C-P29: 6-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-3-[1-[[(5RS,7SR)-3-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-methoxypropyl)amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[803] Using **Method C** and **P29** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found =1380.6575 (δ = 1.2 ppm).

Preparation of L9C-P31: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]piperazin-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[804] Using **Method C** and **P31** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1403.6694 (δ = 1.7 ppm).

Preparation of L9C-P40: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-hydroxypropyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

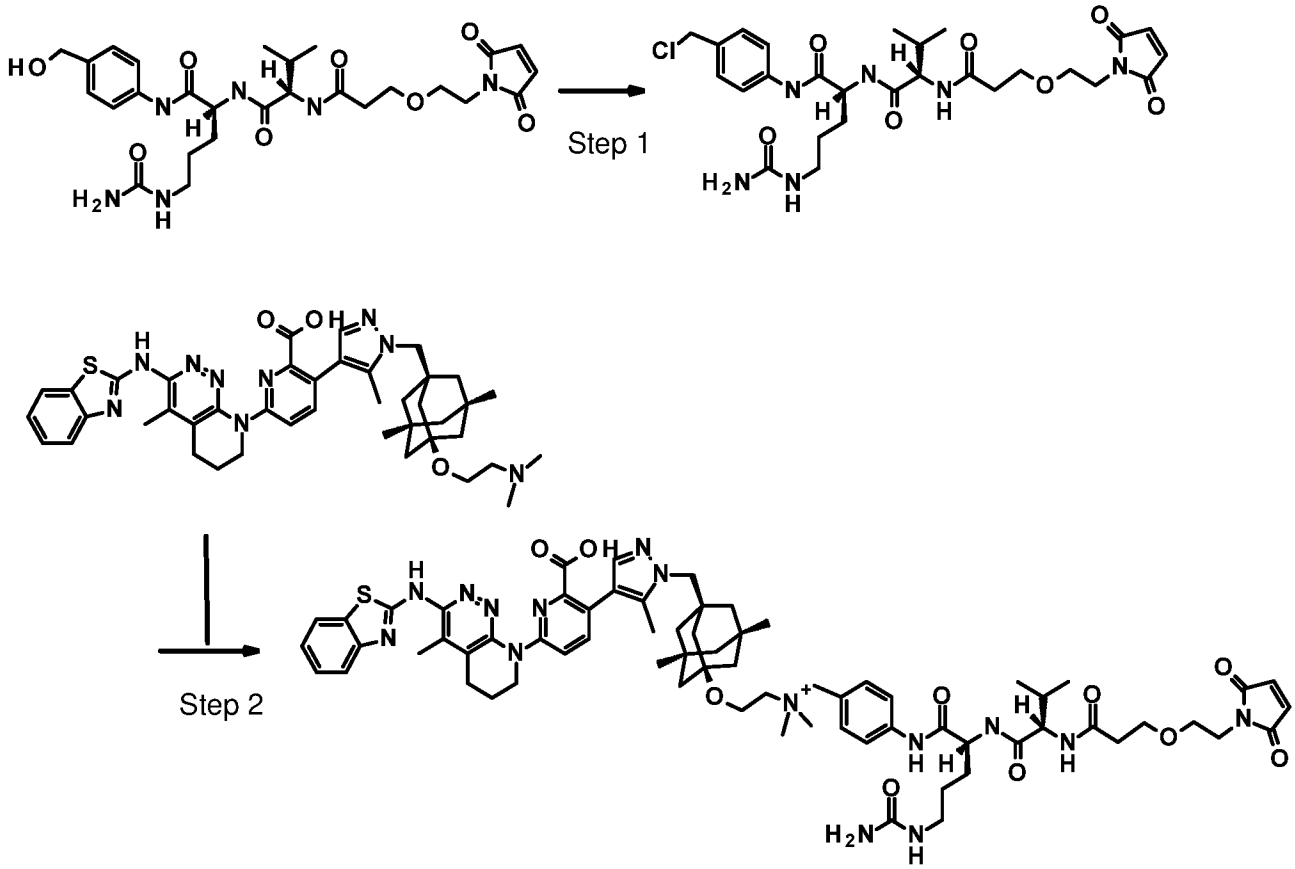
[805] Using **Method C** and **P40** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1390.6775 (δ = 0.7 ppm).

Preparation of L9A-P43: 3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-methoxy-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[806] Using **Method A** and **P43** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M-CF_3CO_2]^+$ found = 1374.6754 (δ = -4.5 ppm).

Method D

Preparation of L9A-P20: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4-methyl-piperazin-4-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid



Step 1: (2S)-N-[4-(chloromethyl)phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide

[807] A solution of $SOCl_2$ (102 µL, 1.39 mmol) in THF (8 ml) was prepared as Solution A. A solution of (2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (from **Method A, Step 1**) (100 mg, 0.174 mmol) in THF (4 ml) was prepared as Solution B. Then 500 µl of Solution A was added every 10 min to Solution B. The reaction was followed by UPLC-MS after addition of morpholine in the sample. After completion of the reaction, the mixture was evaporated under reduced pressure at room temperature and directly used in the next step (105 mg, 0.177 mmol). ^{1}H NMR (400 MHz, dmso-d6) δ ppm 10.00 (s, 1H), 8.10 (d, 1H), 7.85 (d, 1H), 7.60 (d, 2H), 7.35 (d, 2H), 7.00 (s, 2H), 6.05 (m, 1H), 5.25 (m, 2H), 4.70 (s, 2H), 4.40 (m, 1H), 4.20 (m, 1H), 3.65-3.40 (m, 6H), 3.00 (2m, 2H), 2.4/2.3 (2m, 2H), 2.00 (m, 1H), 1.7/1.6 (2m, 2H), 1.40 (2m, 2H), 0.80 (2d, 6H). IR: (v cm^{-1}) 3288, 1703, 1643. HR-ESI+: $[M+H]^+$ = found 593.2499 (δ = 2.4 ppm).

Step 2: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-

pentanoyl]amino]phenyl]methyl]-4-methyl-piperazin-4-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[808] To a solution of **P20** (15 mg, 14.4 µmol) in DMF (0.5 mL) was added a solution of the product from **Step 1** (14.6 mg, 17.2 µmol) and DIPEA (8 µL, 43.1 µmol). The reaction was stirred at 80 °C for 18 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the column and using the TFA method to afford the title compound (19.0 mg, yield 96%). HRMS (ESI) $[M]^+$ found = 1373.6974 (δ = -0.1 ppm).

Preparation of L9A-P21: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid;2,2,2-trifluoroacetic acid

[809] Using **Method D** and **P21** and as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M]^+$ found = 1344.6688 (δ = -1.7 ppm).

Preparation of L9A-P2: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl-dimethyl-ammonio]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate;2,2,2-trifluoroacetic acid

[810] Using **Method A** and **P2** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$=1188.4561 (δ=0.6 ppm).

Preparation of L9A-P1: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetic acid

[811] Using **Method A** and **P1** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$=1232.4802 (δ= -1.1 ppm).

Preparation of L9A-P10: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-

yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4-methyl-piperazin-4-ium-1-yl]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate;2,2,2-trifluoroacetic acid

[812] Using **Method A** and **P10** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1269.5176 (δ = 3.4 ppm).

Preparation of L9A-P9: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate;2,2,2-trifluoroacetic acid

[813] Using **Method A** and **P9** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1240.4887 (δ =1.6 ppm).

Preparation of L9A-P15: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4,4-difluoro-piperidin-1-ium-1-yl]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate;2,2,2-trifluoroacetic acid

[814] Using **Method A** and **P15** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1290.4831 (δ = -0.3 ppm).

Preparation of L9A-P18: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]piperidin-1-ium-1-yl]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate; 2,2,2-trifluoroacetic acid

[815] Using **Method A** and **P18** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1254.4990 (δ = -1.8 ppm).

Preparation of L9A-P28: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium; 2,2,2-trifluoroacetic acid

[816] Using **Method A** and **P28** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M-CF_3CO_2]^+$=1196.4827 (δ=1.9 ppm).

Preparation of L9C-P16: 2-[3-(1,3-benzothiazol-2-ylamino)-6-[2-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]ethoxy]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[817] Using **Method C** and **P16** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1331.5131 (δ = -0.4 ppm).

Preparation of L9C-P12: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[2-(dimethylamino)ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[818] Using **Method C** and **P12** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1301.5034 (δ = -0.3 ppm).

Preparation of L9C-P44: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3,4-dihydroxybutyl)amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[819] Using **Method C** and **P44** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1262.4527 (δ =-0.1 ppm).

Preparation of L9C-P45: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(3-hydroxypropyl)amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[820] Using **Method C** and **P45** as the appropriate payload, the desired product was obtained after a purification step based on the NH_4HCO_3 method (Prep-HPLC, general procedures). HRMS (ESI) $[M+H]^+$ found = 1262.4527 (δ = 0.4 ppm).

Preparation of L9C-P46: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-(4,5-dihydroxypentyl)amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-

yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[821] Using **Method C** and **P46** as the appropriate payload, the desired product was obtained after a purification step based on the NH_4HCO_3 method (Prep-HPLC, general procedures). HRMS (ESI) $[M+H]^+$ = 1324.4903 (δ=-1.7 ppm).

Preparation of L9C-P17: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]piperazin-1-yl]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[822] Using **Method C** and **P17** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1299.4880 (δ =0.5 ppm).

Preparation of L9A-P11: [3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]-1-methyl-prop-2-ynyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetic acid

[823] Using **Method A** and **P11** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1202.4722 (δ = 0.5 ppm).

Preparation of L9A-P8: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4-methyl-piperazin-4-ium-1-yl]but-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid;2,2,2-trifluoroacetic acid

[824] Using **Method D** and **P8** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M+H]^+$ found = 1 284.5343 (δ = -1.9 ppm).

Preparation of L9A-P14: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl-diethyl-ammonio]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[825] Using **Method D** and **P14** as the appropriate payload, the desired product was obtained after a purification step based on the NH_4HCO_3 method (Prep-HPLC, general procedures). HRMS (ESI) $[M+H]^+$ found = 1242.5021 (δ = -0.2ppm).

Preparation of L9A-P13: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl-ethyl-methyl-ammonio]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

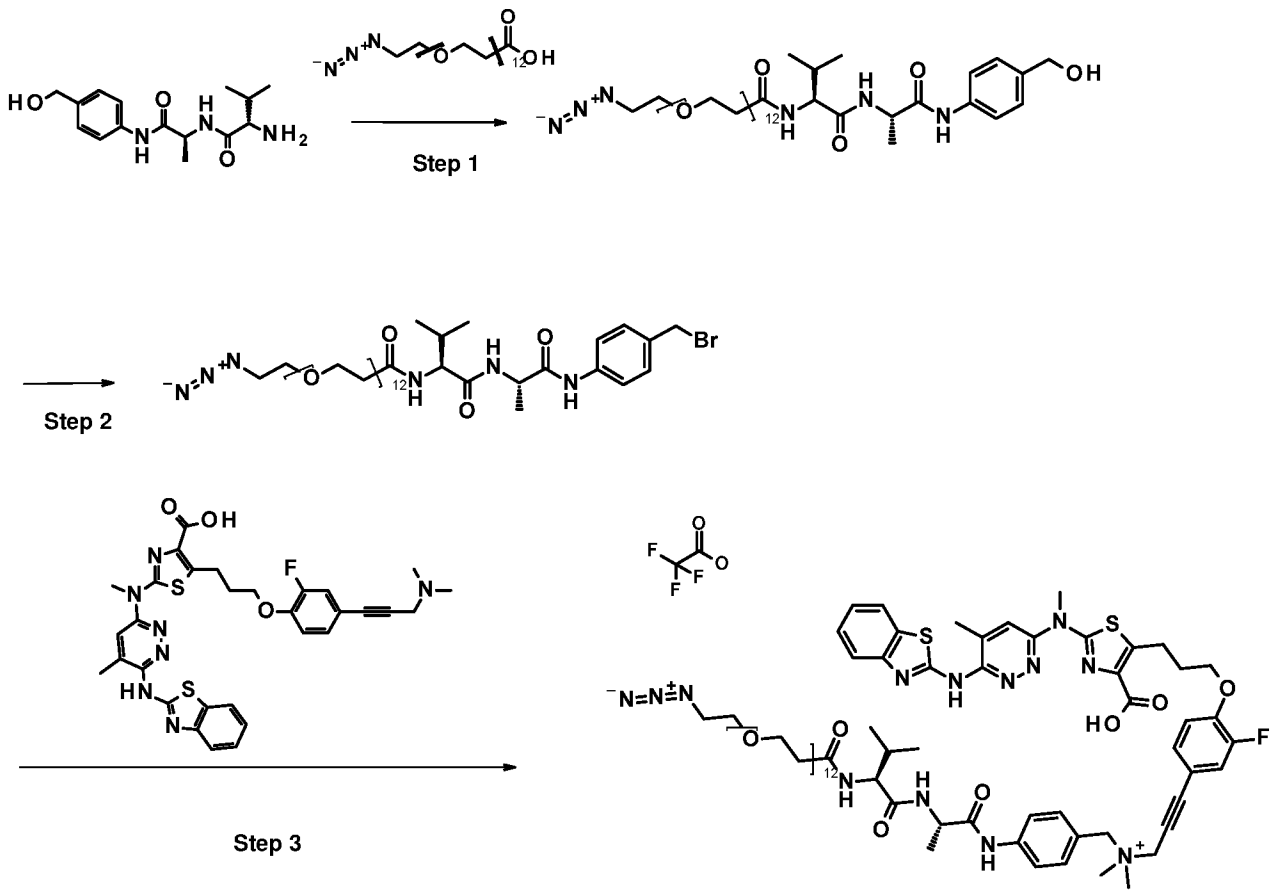
[826] Using **Method D** and **P13** as the appropriate payload, the desired product was obtained after a purification step based on the NH_4HCO_3 method (Prep-HPLC, general procedures). HRMS (ESI) $[M+H]^+$ found = 1228.4855 (δ = -1.0 ppm).

Preparation of L9A-P34: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[(3S)-3,4-dihydroxybutyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium;2,2,2-trifluoroacetic acid

[827] Using **Method D** and **P34** as the appropriate payload, the desired product was obtained. HRMS (ESI) $[M-CF_3CO_2]^+$ found = 1288.5086 (δ = 0.6 ppm).

Method F

Preparation of L13A-P2: [4-[[(2S)-2-[[(2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl-[3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl]-dimethyl-ammonium;2,2,2-trifluoroacetate



Step 1: (2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-N-[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide

[828] To a suspension of (2S)-2-amino-N-[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide (900 mg, 3.07 µmol) in DMF (10 mL) were successively added a solution of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid (2.00 g, 3.07 mmol) in DMF (10 mL), EDC (650 mg, 3.38 mmol) as a powder and DIPEA (1.00 mL, 6.14 mmol). The reaction was stirred at room temperature for 16 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the NH_4HCO_3 method to afford the desired product (1.64 g, 1.78 mmol). IR: (v cm^{-1}) 3600-3200, 3287, 2106, 1668, 1630, 1100. ^1H NMR (400 MHz, dmso-d6) δ ppm 9.82 (m, 1H), 8.14 (d, 1H), 7.87 (d, 1H), 7.54 (d, 2H), 7.23 (d, 2H), 5.08 (t, 1H), 4.43 (d, 2H), 4.39 (m, 1H), 4.20 (m, 1H), 3.65-3.44 (m, 48H), 3.39 (t, 2H), 2.5-2.3 (m, 2H), 1.97 (m, 1H), 1.31 (d, 3H), 0.87/0.84 (2d, 6H). HRMS (ESI) $[M+H]^+$ found: 919.5234 (δ = 3.4 ppm).

Step2: (2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-N-[(1S)-2-[4-(bromomethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide

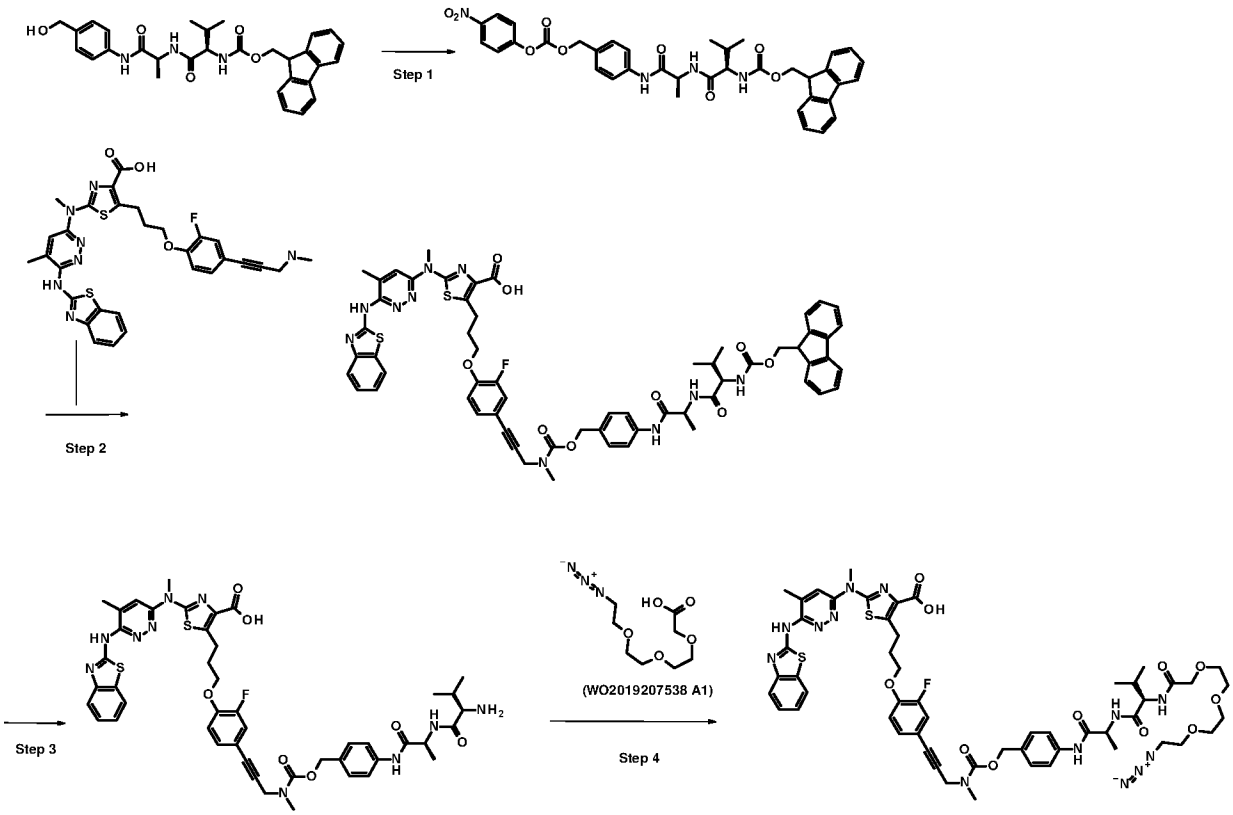
[829] To a solution of the product from **Step 1** (72 mg, 7.83 µmol) in THF (5 mL) was added at 0°C a 1M solution of PBr_3 in THF (157 µL, 157 µmol) and the reaction mixture was stirred for 1 h at 0°C and for 1 h at room temperature. The reaction mixture was diluted with AcOEt (5 mL), treated with an aqueous saturated solution of $NaHCO_3$ (0.5 mL), dried over $MgSO_4$, and used without further treatment in the next step. IR: (v cm^{-1}) 3700-3100, 1658, 2106. HRMS (ESI) $[M+H]^+$ found: 981.4390 (δ = 1.3 ppm).

Step 3: [4-[[(2S)-2-[[(2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl-[3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl]-dimethyl-ammonium;2,2,2-trifluoroacetate

[830] To a solution of the product from **Step 2** ((21 mg, 2.09 µmol) in DMF (2 mL) were successively added 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid (**P2**) (11.0 mg, 1.74 µmol) as a powder and DIPEA (8.6 µL, 5.22 µmol). The reaction was stirred at room temperature for 8 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the desired product (15 mg, 0.91 mmol). IR: (v cm^{-1}) 3400-3150, 2235, 2105, 1667. ^1H NMR (500 MHz, dmso-d6) δ ppm 7.90 (dl, 1H), 7.76 (d, 2H), 7.68 (s, 1H), 7.58 (dd, 1H), 7.51 (m, 1H), 7.51 (d, 2H), 7.41 (m, 1H), 7.38 (t, 1H), 7.25 (m, 1H), 7.20 (t, 1H), 4.55 (s, 2H), 4.42 (s, 2H), 4.39 (m, 1H), 4.21 (m, 1H), 4.19 (t, 2H), 3.77 (s, 3H), 3.60 (m, 4H), 3.54/3.50 (m+m, 44H), 3.38 (t, 2H), 3.29 (m, 2H), 3.05 (s, 6H), 2.47 (s, 3H), 2.46/2.38 (m+m, 1+1H), 2.16 (quint, 2H), 1.96 (m, 1H), 1.32 (d, 3H), 0.88/0.84 (d+d, 3+3H). ^{13}C NMR (500 MHz, dmso-d6) δ ppm 133.9, 129.7, 126.4, 122.6, 122.1, 120.0, 119.3, 118.1, 115.3, 70.5/70.1, 70.1/67.5, 68.7, 66.2, 57.8, 53.7, 50.6, 49.7, 49.5, 36.4, 35.3, 31.0, 30.9, 23.3, 19.5/18.6, 18.4, 17.7. ^{19}F NMR (500 MHz, dmso-d6) δ ppm -133.8. HRMS (ESI) $[M+H]^+$ found: 1532.6964 (δ = 0.6 ppm).

Method G

Preparation of L19C-P7: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]thiazole-4-carboxylic acid



Step 1: [4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate

[831] To a solution of 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]carbamate (5.0 g, 9.7 mmol) in THF (20 mL) and DCM (10 mL) were successively added paranitrophenyl chlorocarbonate (4.1 g, 20.1 mmol) and pyridine (1.65 mL, 20.4 mmol). The reaction was stirred at room temperature for 15 h. A 10% aqueous solution of citric acid was added and the reaction mixture was extracted twice with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After evaporation under vacuum the solid was dissolved in a minimum amount of AcOEt and ether was added to precipitate the desired compound (5.6 g, 8.22 mmol). IR: (v cm^{-1}) 3350-3200, 1760;1690;1670;1630, 1523;1290. ^1H NMR (400 MHz, dmso-d6) δ ppm 10.07 (m, 1 H), 8.31 (d, 2 H), 8.19 (d, 1 H), 7.89 (d, 2 H), 7.74 (t, 2 H), 7.64 (d, 2 H), 7.57 (d, 2 H), 7.41 (m, 2 H), 7.41 (d, 2 H), 7.4 (m, 1 H), 7.32 (t, 2 H), 5.24 (s, 2 H), 4.43 (m, 1 H), 4.36-4.19 (m, 3 H), 3.92 (dd, 1 H), 2 (m, 1 H), 1.32 (d, 3 H), 0.9/0.87 (2d, 6 H).

Step 2: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[832] To a solution of 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid **(P7)** (366.0 mg, 559 mmol) in DMF (10 mL) were successively added the product from **Step 1** (378 mg, 556 mmol) and DIPEA (368 µL, 2.22 mmol). The reaction mixture was stirred at room temperature for 16 h and then evaporated to dryness. The crude product was purified by silica gel chromatography (gradient of methanol in DCM) to afford the desired compound (15.6 mg, 9.64 µmol).

Step 3: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]thiazole-4-carboxylic acid

[833] To a solution of the product from **Step 2** (424 mg, 366 mmol) in DMF (4 mL) was added piperidine (90 µL, 914 mmol) and the reaction mixture was stirred at room temperature for 1 h. After evaporation to dryness, the crude product was purified by silica gel chromatography (gradient of methanol containing 2% NH_4OH in DCM) to afford the desired compound. IR: (v cm^{-1}) 3270, 3100-2400, 1680, 1520. ^1H NMR (400 MHz, dmso-d6) δ ppm 10.58/10.2 (2*s, 1 H), 8.55/8.28 (2*s, 1 H), 7.9 (d, 1 H), 7.65 (s, 1 H), 7.62 (d, 2 H), 7.52 (d, 1 H), 7.39 (m, 1 H), 7.35-7 (massif, 3 H), 7.32 (d, 2 H), 7.2 (m, 1 H), 5.05 (s, 2 H), 4.48 (m, 1 H), 4.26 (s, 2 H), 4.15 (t, 2 H), 3.71 (s, 3 H), 3.3 (t, 2 H), 3.03 (d, 1 H), 2.9 (s, 3 H), 2.45 (s, 3 H), 2.11 (quint, 2 H), 1.91 (m, 1 H), 1.4-0.7 (br s, 2 H), 1.32 (d, 3 H), 0.88/0.78 (2*d, 6 H). ^{19}F NMR (400 MHz, dmso-d6) δ ppm -134.

Step 4: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]thiazole-4-carboxylic acid

[834] To a solution of 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetic acid (58 mg, 249 µmol) in DMF (1 mL) were successively added TSTU (77 mg, 255 µmol) and DIPEA (190 µL, 1.12 mmol), and the reaction mixture was stirred at room temperature for 2 h. After the addition of the product from **Step 3** (84 mg, 89.6 mmol) in DMF (1.5 mL), the reaction mixture was

stirred at room temperature for 2 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the NH_4HCO_3 to afford the desired compound (64 mg, 55.5 mmol). IR: (v cm^{-1}) 3700-2700, 2104, 1693/1656, 1227/1127. ^1H NMR (400 MHz, dmso-d6) δ ppm 9.76 (s, 1H), 8.16 (dl, 1H), 7.83 (d, 1H), 7.62 (s, 1H), 7.56 (d, 2H), 7.51 (d, 1H), 7.36 (d, 1H), 7.35 (t, 1H), 7.29 (d, 2H), 7.24-7.08 (m, 3H), 7.18 (t, 1H), 5.04 (s, 2H), 4.44 (hept, 1H), 4.28 (dd, 1H), 4.26 (s, 2H), 4.16 (t, 2H), 3.94 (s, 2H), 3.75 (s, 3H), 3.58 (m, 10H), 3.35 (t, 2H), 3.27 (t, 2H), 2.91 (s, 3H), 2.45 (s, 3H), 2.13 (quint, 2H), 2.05 (m, 1H), 1.32 (d, 3H), 0.89/0.84 (2d, 6H). ^{19}F NMR (400 MHz, dmso-d6) δ ppm -133.9. HRMS ESI $[M+H]^+$ found 1152.4207 (δ = 1.5 ppm).

Preparation of L23C-P7: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetyl]amino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]thiazole-4-carboxylic acid

[835] Product was obtained according to **Method G** by replacing 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]carbamate with 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate. IR: (v cm^{-1}) 3687-3060, 2104, very broad - 1656, 1606, 1515, 754 and 725. ^1H NMR (400 MHz, dmso-d6) δ ppm 7.90 (d, 1 H), 7.70 (br s, 1 H), 7.60 (d, 2 H), 7.50 (m, 2 H), 7.40 (t, 1 H), 7.30 (d+m, 3 H), 7.20 (t, 1 H), 7.15 (dd, 1 H), 5.45 (m, 2 H), 4.40 (m, 1 H), 4.30 (m, 1 H), 4.25 (s, 2 H), 4.15 (t, 2 H), 3.95 (s, 2 H), 3.80 (s, 3 H), 3.60/3.30 (2m, 12 H), 3.30 (m, 2 H), 3.00 (2m, 2 H), 2.90 (s, 3 H), 2.45 (s, 3 H), 2.15 (quint, 2 H), 2.00 (m, 1 H), 1.70/1.60 (2m, 2 H), 1.45/1.4 (2m, 2 H), 0.90/0.80 (2d, 6 H). ^{19}F NMR (400 MHz, dmso-d6) δ ppm -134.2. HRMS ESI $[M+H]^+$ found 1238.4675 (δ = 0.4 ppm).

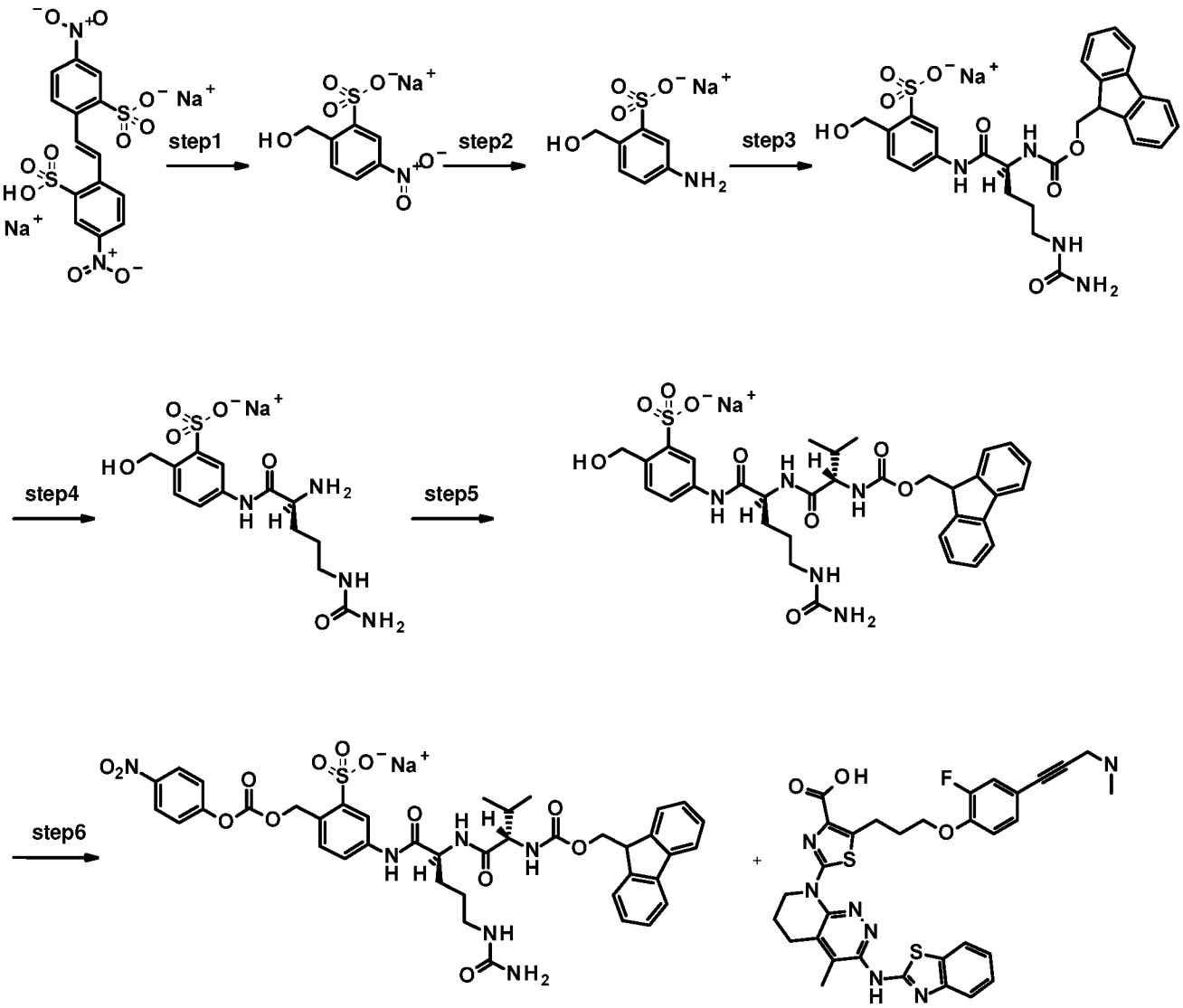
Preparation of L110C-P7: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]thiazole-4-carboxylic acid

[836] Product was obtained according to **Method G** by replacing 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]carbamate with 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-1-[[4-

(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate and 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetic acid with 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid. IR: (v cm^{-1}) 3560-3063, 2100, very broad - 1651, 1608, 1514, 756 and 725. ^{1}H NMR (400 MHz, dmso-d6) δ ppm 7.9 (d, 1 H), 7.7 (br s, 1 H), 7.6 (d, 2 H), 7.5 (m, 1 H), 7.4 (t, 1 H), 7.4-7.1 (m, 3 H), 7.3 (d, 2 H), 7.2 (t, 1 H), 5.4 (m, 2 H), 4.4 (m, 1 H), 4.3 (s, 2 H), 4.25 (m, 1 H), 4.15 (t, 2 H), 3.8 (s, 3 H), 3.65-3.4 (m, 50 H), 3.3 (m, 2 H), 3 (2m, 2 H), 2.9 (s, 3 H), 2.45 (s, 3 H), 2.4 (m, 2 H), 2.1 (quint, 2 H), 2 (m, 1 H), 1.7/1.6 (2m, 2 H), 1.4 (2m, 2 H), 0.85 (2d, 6 H). ^{19}F NMR (400 MHz, dmso-d6) δ ppm -134.4. HRMS (ESI) $[M+H]^{+}$ found 1648.7209 (δ = 1.4 ppm).

Method H

Preparation of L27C-P3: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid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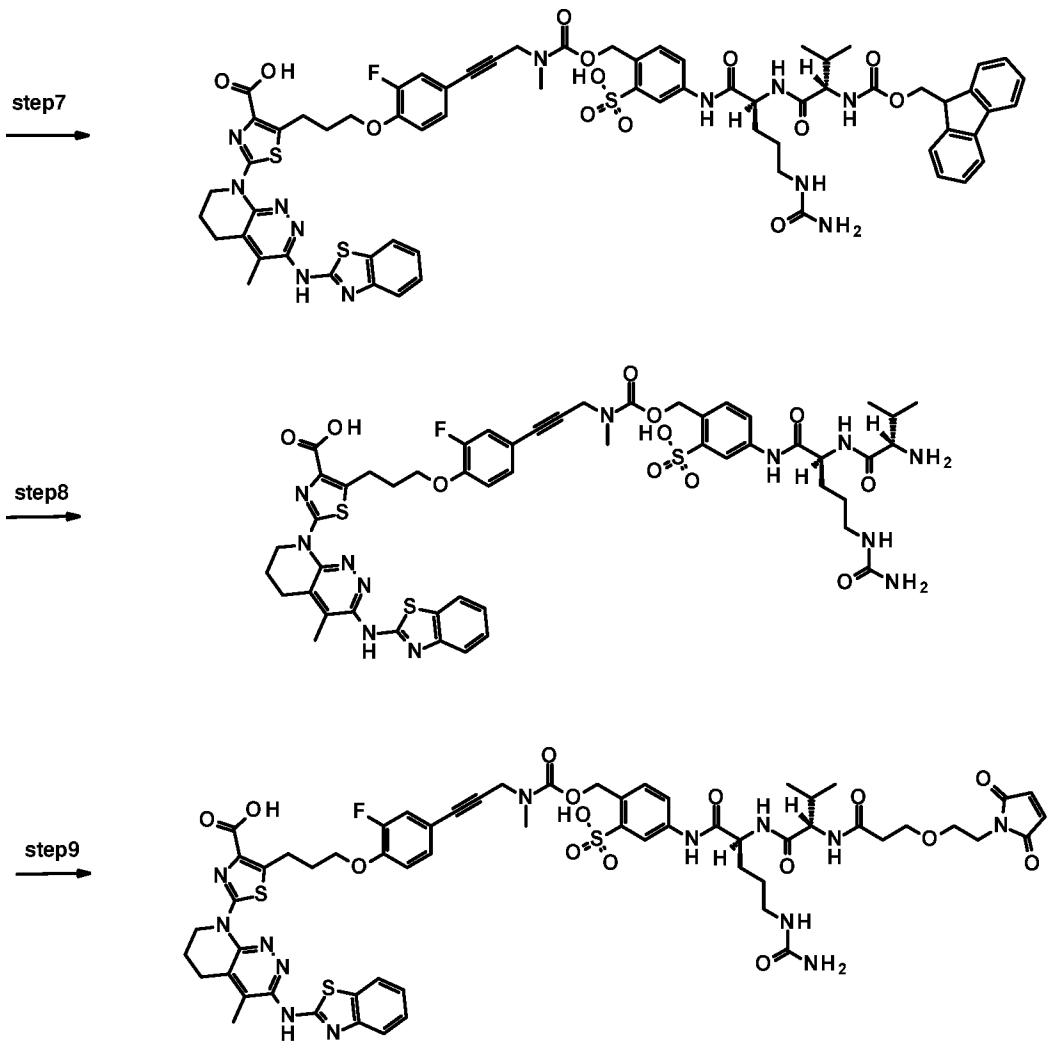


Step 1: sodium;2-(hydroxymethyl)-5-nitro-benzenesulfonate

[837] To a solution of sodium 5-nitro-2-[(E)-2-(4-nitro-2-sulfo-phenyl)vinyl]benzenesulfonate (25.0 g; 52.7 mmol) in water (336 mL) was introduced a stream of ozone for 1.5 h. After the completion of the reaction, the mixture was purged with argon for 30 minutes in order to remove the excess of ozone. Then, sodium carbonate (39.1 g; 7 eq.) and sodium borohydride (3.99 g; 2 eq.) were added and the orange solution was stirred at room temperature for 16 h. The reaction mixture was concentrated to give the desired compound (39.9 g; sup 100%) as a solid (containing residual traces of bore salts). ^{1}H NMR (dmso): δ 4.99 (*d*, 2H, *J* = 3.6 Hz), 5.36 (*t*, 1H, *J* = 5.6 Hz), 7.83 (*d*, 1H, *J* = 8.4 Hz), 8.21 (*d*, 1H, *J* = 8.4 Hz), 8.45 (*s*, 1H).

Step 2: sodium;5-amino-2-(hydroxymethyl)benzenesulfonate

[838] Sodium 2-(hydroxymethyl)-5-nitro-benzenesulfonate (26.9 g; 105 mmol) was solubilized in water (403 mL). Then, the reaction mixture was flushed with argon. Palladium 10% on carbon (2.65 g, 10% wt.) was added then the black suspension was flushed with

argon and then with hydrogen. The reaction mixture was stirred at room temperature for 3.5 days under hydrogen atmosphere. After filtration over Celite® and washing with water and methanol, the filtrate was concentrated to dryness and co-evaporated 3 times with toluene. Purification by column chromatography on silica gel using ethyl acetate / methanol (90/10 to 70/30) as eluent afforded the desired compound (14.29 g; 60%). ^{1}H NMR (dmso): δ 4.52 (*d*, 2H, *J* = 5.2 Hz), 4.95 (*t*, 1H, *J* = 5.2 Hz), 5.04 (*s*, 2H), 6.42 (*d*, 1H, *J* = 7.6 Hz), 6.93 (*d*, 1H, *J* = 7.6 Hz), 7.03 (*s*, 1H).

Step 3: sodium;5-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)benzenesulfonate

[839] To a solution of Fmoc-L-Cit-OH (882 mg; 2.22 mmol) in dimethylformamide (32.5 mL) was added the product from **Step 2** (500 mg; 2.22 mmol), HBTU (1.01 g; 2.66 mmol) and DIPEA (917 µL; 5.55 mmol). The reaction mixture was stirred at room temperature for 16 hours, then was concentrated to dryness and co-evaporated with water (2 x 100 mL). The crude was purified by column chromatography on C18 using acetonitrile /water 2/8 to 8/2 as eluent, to afford the desired compound (1.0 g; 63%). ^{1}H NMR (dmso): δ 1.25-1.28 (*m*, 15H, DIPEA),1.36-1.72 (*m*, 4H), 2.92-3.03 (*m*, 2H), 3.11-3.18 (*m*, 2H, DIPEA), 3.5-3.65 (*m*, 2H, DIPEA), 4.30-4.12 (*m*, 4H), 4.74 (*d,* 2H, *J* = 4.4 Hz), 5.05 (*t*, 1H, *J* = 5.6 Hz), 5.37 (*s*, 2H), 5.97 (*t*, 1H, *J* = 4.8 Hz), 7.34-7.42 (*m*, 4H), 7.62-7.90 (*m*, 7H), 8.15 (*s*, 1H), 10.05 (*s*, 1H).

Step 4: sodium;5-[[(2S)-2-amino-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)benzenesulfonate

[840] To a solution of the product from **Step 3** (11.2 g; 15.73 mmol) in DMF (224 mL) was added piperidine (3.1 mL; 2 eq.). The reaction mixture was stirred at room temperature for 3 hours then water (400 mL) was added. The aqueous layer was extracted with ethyl acetate (2 x 300 mL) and with dichloromethane (300 mL). Sodium carbonate (5.01 g;3 eq.) was added to the aqueous layer and the mixture was stirred at room temperature for 3 h. The mixture was lyophilized in order to give the desired compound (6.01 g;estimated to 100%) as a solid contaminated by sodium salts. ^{1}H NMR (dmso): δ 1.55-1.64 (*m*, 4H), 2.99-3.01 (*m*, 2H), 3.58 (*m*, 1H), 4.75 (*s*, 2H), 5.06 (*s*, 1H), 5.38 (*s*, 2H), 5.98 (*t*, 1H, *J* = 5.6 Hz), 7.38 (*d*, 1H, *J* = 8.4 Hz), 7.72 (*dd*, 1H, *J* = 8.4 & 2.4 Hz), 7.86 (*d*, 1H, *J* = 2.4 Hz,), 10.17 (*s*, 1H).

Step 5: sodium;5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)benzenesulfonate

[841] To a solution of the product from **Step 4** (6.01 g, 15.73 mmol) in dimethylformamide (150 mL) was added Fmoc-L-Val-OSu (6.85 g, 1 eq.). The solution was stirred at room temperature for 3 hours then the reaction mixture was diluted with saturated sodium

hydrogenocarbonate (100 mL) and water (100 mL) and concentrated to dryness. The residue was purified on silica gel using ethyl acetate/methanol 90/10 to 50/50 as eluent to afford the desired compound (4.44 g, 48%). ^{1}H NMR (dmso): 0.85-0.90 (*m*, 6H), 1.31-1.76 (*m*, 4H), 1.95-2.06 (*m*, 1H), 2.91-3.05 (*m*, 2H), 3.95 (*t*, 1H, *J* = 8.4 Hz), 4.24-4.35 (*m*, 3H), 4.37-4.45 (*m*, 1H), 4.76 (*d*, 2H, *J* = 6 Hz), 5.07 (*t*, 1H, *J* = 6.4 Hz,), 5.40 (*s*, 2H), 6.03 (*t*, 1H, *J* = 5.6 Hz), 7.32-7.46 (*m*, 6H), 7.67 (*d*, 1H, *J* = 8 Hz), 7.76 (*t*, 2H, *J* = 7.2 Hz), 7.88-7.91 (*m*, 3H), 8.12 (*d*, 1H, *J* = 7.6 Hz), 10.08 (*s*, 1H). ^{13}C NMR (dmso): 18.25, 19.24, 26.70, 29.56, 30.45, 39.50, 46.67, 53.17, 60.01, 60.96, 65.66, 117.85, 119.15, 120.05, 125.36, 127.06, 127.62, 128.09, 134.39, 136.79, 140.67, 143.89, 145.34, 156.08, 158.82, 170.37, 171.16. LCMS (2-100 ACN/H_2O+0.1% AF): 93.85 % retention time = 8.4 min, Positive mode : 682.15 detected (MH^+), Negative mode : 680.17 detected (MH^-).

Step 6: 5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[(4-nitrophenoxy)carbonyloxymethyl] benzenesulfonate

[842] To a solution of the product from **Step 5** (450 mg, 0.64 mmol) in DMF (6 mL) was added DIPEA (1.34 mL, 7.67 mmol) and bis(4-nitrophenyl)carbonate (778 mg, 2.56 mmol). The solution was stirred at room temperature for 2 h and bis(4-nitrophenyl)carbonate (390 mg, 1.28 mmol) was added. After 1 h, the solution was concentrated under reduced pressure and the residue was purified by silica gel chromatography (gradient of methanol and acetic acid in dichloromethane) to give the desired compound (523 mg).

Step 7: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[843] To a solution of 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid (P3) (70 mg, 109 µmol) in DMF (550µL) were successively added DIPEA (0.19 mL, 1.39 mmol), the product of **Step 6** (111 mg, 131 µmol) and DIEPA (95 µL, 544 µmol). The solution was stirred at room temperature for 15 h and concentrated to give the desired compound, which was used without any further treatment.

Step 8: 5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]-2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]thiazole-4-carboxylic acid

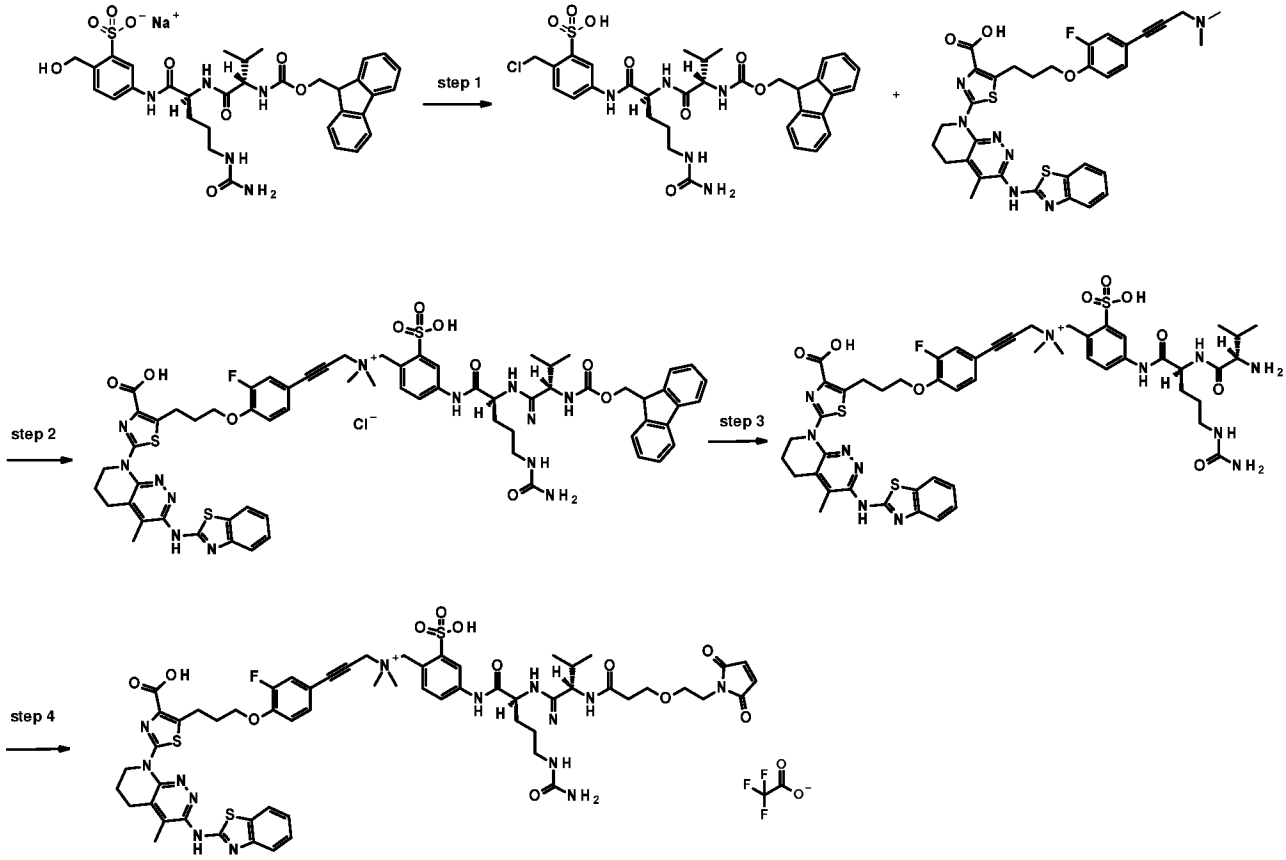
[844] To a solution of the product from **Step 7** (147 mg, 109 µmol) in dioxane (1.1 mL) was added a solution of $LiOHxH_2O$ (13.7 mg, 326 µmol) in water (1.1 mL). The solution was stirred at room temperature for 12 h. A 1 M aqueous solution of HCl was added until pH 7. The reaction mixture was evaporated to dryness and the residue triturated in DCM. The precipitate was washed with water and EtOH to give the desired compound (120 mg).

Step 9: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[845] To a solution of the product from **Step 8** (120 mg, 109 µL) were successively added (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (37.8 mg, 122 µmol) and DIPEA (38.5 µL, 221 µmol). The solution was stirred at room temperature for 1.5 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the desired compound (9 mg). HRMS (ESI) $[M+H]^+$ 1322.3831 (δ=-3.3 ppm).

Method I

Preparation of L27A-P1: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate



Step 1: 2-(chloromethyl)-5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]benzenesulfonic acid

[846] To a solution of 5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)benzenesulfonate (300 mg, 426 µmol) in NMP (6 mL) was added 7 times over 1 h a solution of $SOCl_2$ (31 µL, 426 µmol) in NMP (1 mL). The reaction mixture was stirred at room temperature for 1 h. The product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Oasis column and using the TFA method to give the desired product (225mg). IR: (v cm^{-1}) 3600-2200, 1657, 1250-1100. ^1H NMR (400 MHz, dmso-d6) δ ppm 10.15/8.1/7.42/6 (s+2d+m, 4 H), 7.9 (m, 3 H), 7.75 (m, 3 H), 7.42/7.31 (2m, 5 H), 5.23 (s, 2 H), 4.4 (m, 1 H), 4.3-4.2 (m, 3 H), 3.95 (dd, 1 H), 3 (m, 2 H), 2 (m, 1 H), 1.7/1.6 (2m, 2 H), 1.48/1.37 (2m, 2 H), 0.88 (2d, 6 H). HRMS (ESI) $[M+H]^+$ 700.2199 (δ= -0.5 ppm).

Step 2: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methyl]-dimethyl-ammonium;chloride

[847] To a solution of the product from **Step 1** (55.7 mg, 68.4 µmol) in NMP (0.9 mL) were successively added 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid (P1) (30 mg, 45.6 µmol), DIEPA (63.6 µL, 365 µmol), and TBAI (13 mg, 36.5 µmol). The reaction mixture was stirred at 60°C for 6 h. The desired compound was directly used as a solution in **Step 3**.

Step 3: [4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methyl-[3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl]-dimethyl-ammonium

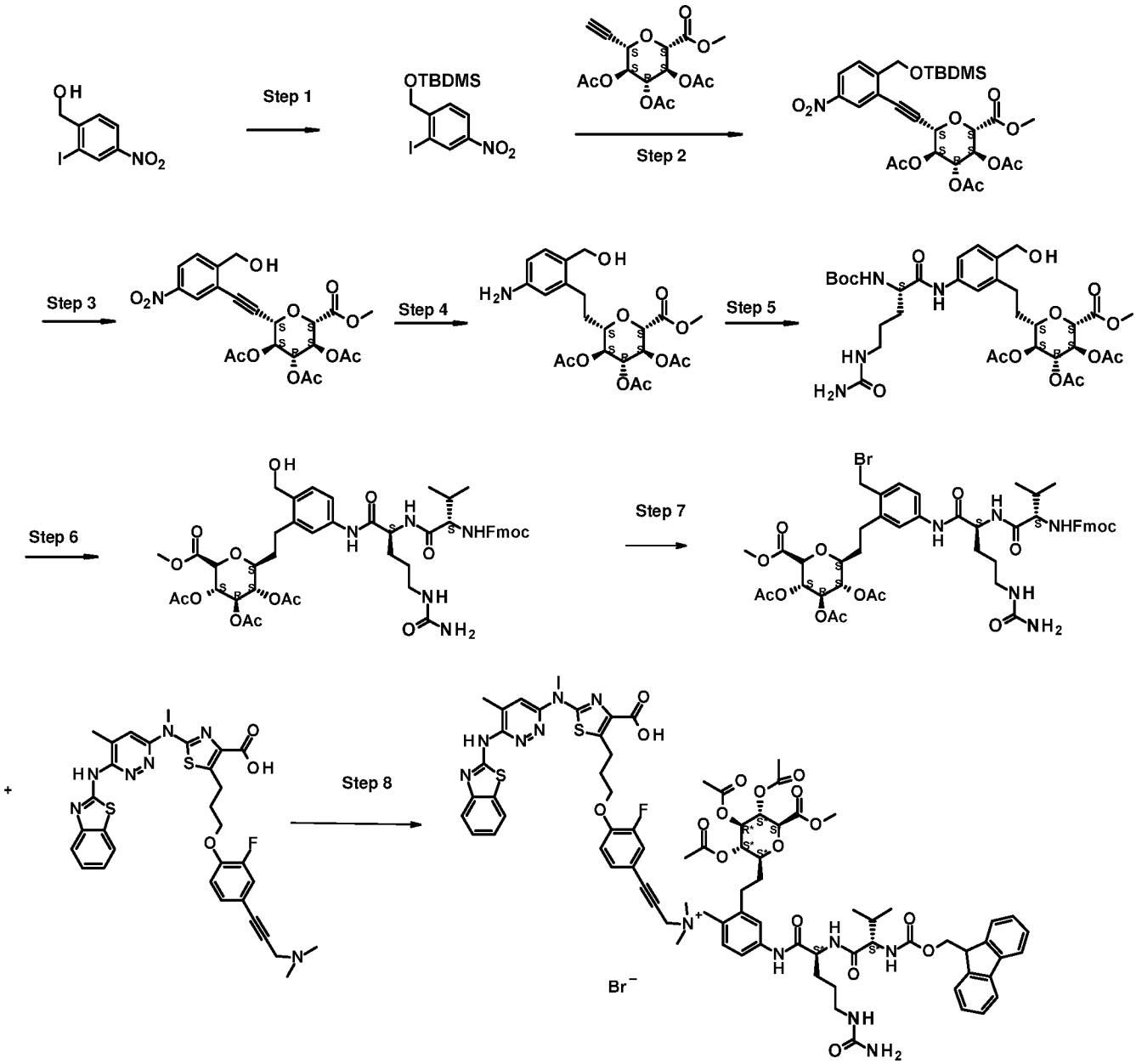
[848] To the NMP solution of the product from **Step 2** (26.5 µmol) was added diethylamine (21.9 µL, 212 µmol). The reaction mixture was stirred at room temperature for 24 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Oasis column and using the NH_4HCO_3 method to give the desired product (18 mg).

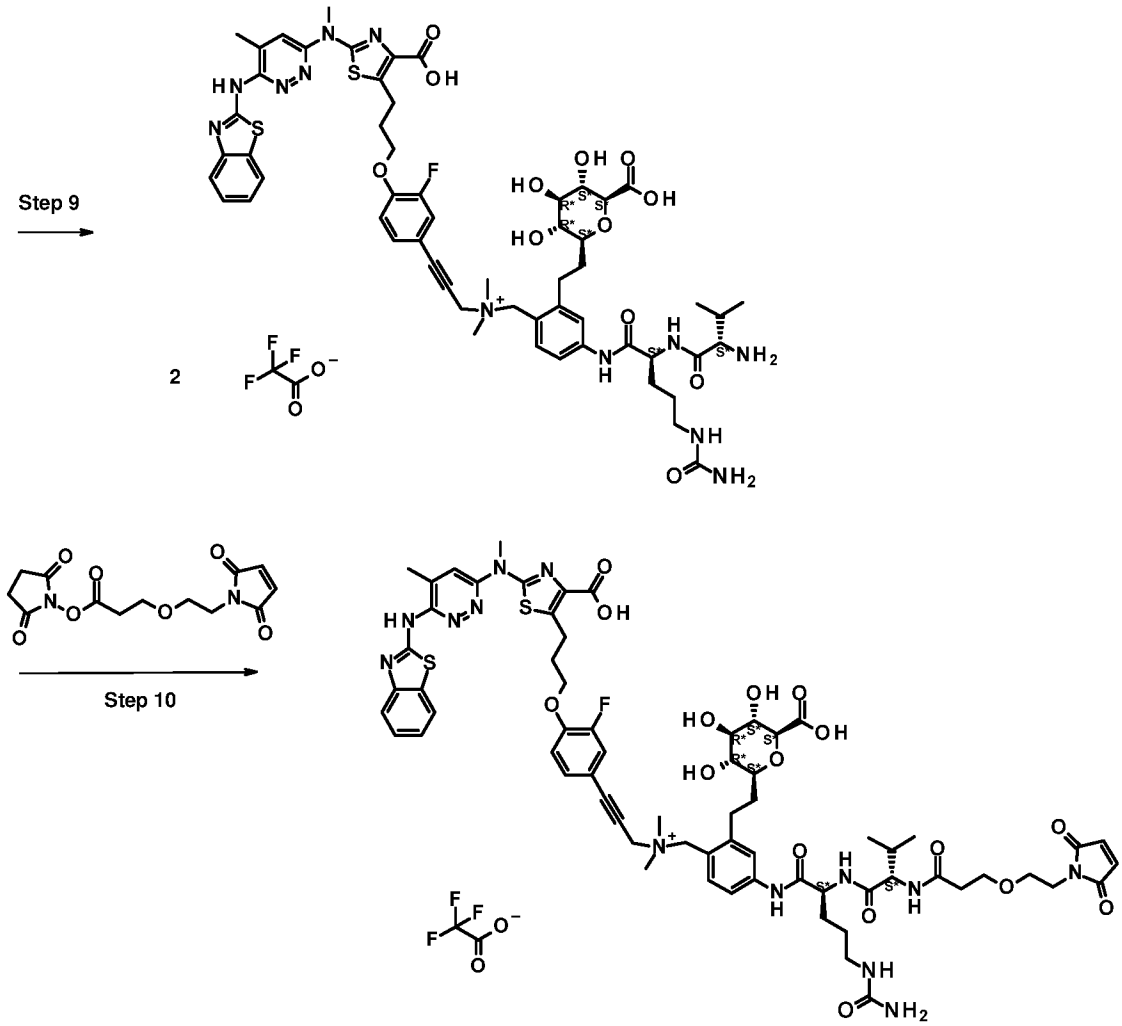
Step 4: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanimidoyl]amino]-5-ureido-pentanoyl]amino]-2-sulfo-phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate

[849] To a solution of the product from **Step 3** (20mg, 18.2 µmol) in DMF (900 µL) were successively added (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (8.5 mg, 27.3 µmol) and DIPEA (9.5 µL, 54.5 µmol). The solution was stirred at room temperature for 1.5 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the NH_4HCO_3 method to give the title compound (15.7 mg). HRMS (ESI) $[M+H]^+$ 1294.4278 δ= 1 ppm.

Method J

Preparation of L21A-P2: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate


Step 1
Step 2
Step 3
Step 4
Step 5
Step 6
Step 7
Step 8
OTBDMS
NHFmoc
Br
Br⁻



Step 1: **tert-*butyl-[(2-iodo-4-nitro-phenyl)methoxy]-dimethyl-silane***

[850] To a solution of (2-iodo-4-nitro-phenyl)methanol (172 g, 61.64 mmol) in dichloromethane (300 mL) was added imidazole (5.04 g, 73.97 mmol). After the mixture was cooled to 0 °C, a solution of *tert*-butyl-chloro-dimethyl-silane (TBDMSCl) (11.15 g, 73.97 mmol) in dichloromethane (300 mL) was added dropwise in 15 min. After stirring at room temperature for 16 h, the reaction mixture was quenched with methanol (20 mL) and concentrated to dryness. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (19.65 g). ^{1}H NMR (400 MHz, dmso-d6): δ 8.57 (s, 1H), 8.31 (d, 1H), 7.66 (d, 1H), 4.67 (s, 2H), 0.92 (s, 9H), 0.14 (s, 6H).

Step 2: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-[[tert-butyl(dimethyl)silyl] oxymethyl]-5-nitro-phenyl]ethynyl]tetrahydropyran-2-carboxylate

[851] To a solution of the product from **Step 1** (3.0 g, 7.63 mmol) in DMF (55 mL) were successively added methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-ethynyl-tetrahydropyran-2-

carboxylate (3.39 g, 9.92 mmol), DIPEA (5.80 mL, 35.09 mmol), copper iodide (145 mg, 0.763 mmol) and dichloro-bis-(triphenylphosphine)palladium(II) (535 mg, 0.763 mmol). The solution was flushed with argon and stirred at room temperature for 16 h. After dilution with water (300 mL), the aqueous layer was extracted with ethyl acetate (2 x 300 mL). The combined organic layers were washed with water (2 x 300 mL), dried, filtered, and concentrated to dryness. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (4.01 g). ^{1}H NMR (400 MHz, dmso-d6): δ 8.32 (dd, 1H), 8.19 (d, 1H), 7.75 (d, 1H), 5.45 (t, 1H), 5.16 (t, 1H), 5.02-5.07 (m, 2H), 4.82 (s, 2H), 4.55 (d, 1H), 3.65 (s, 3H), 1.98-2.07 (m, 9H), 0.92 (m,9H), 0.14 (s, 6H).

Step 3: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-(hydroxymethyl)-5-nitro-phenyl]ethynyl]tetrahydropyran-2-carboxylate

[852] To a solution of the product from **Step 2** (4.01 g, 6.60 mmol) in THF (48 mL) and water (48 mL) was added acetic acid (193 mL, 3.36 mol). The solution was stirred at room temperature for 2 days then diluted with water (300 mL). The aqueous layer was extracted with dichloromethane (2 x 300 mL). The combined organic layers were washed with water (2 x 300 mL) and with a saturated aqueous solution of sodium hydrogen carbonate (400 mL), dried, filtered, and concentrated to dryness. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (2.67 g). ^{1}H NMR (400 MHz, dmso-d6): δ 8.29 (*dd*, 1H), 8.15 (*d*, 1H), 7.79 (*d*, 1H), 5.68 (*t*, 1H), 5.45 (*t*, 1H), 5.16 (*t*, 1H), 5.02-5.07 (*m*, 2H), 4.62 (*d*, 2H), 4.55 (*d*, 1H), 3.65 (*s*, 3H), 1.98-2.07 (*m*, 9H).

Step 4: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-amino-2-(hydroxymethyl)phenyl] ethyl]tetrahydropyran-2-carboxylate

[853] A solution of the product from **Step 3** (2.67 g, 5.41 mmol) in THF (59 mL) was flushed with argon. After adding Platinum on carbon 5% dry (1.34 g, 50%$^{w}/_{w}$), the reaction mixture was successively flushed with argon and with H_2, then stirred under H_2 atmosphere (1 atm) at room temperature for 2 days. The reaction mixture was filtered through a Celite® pad, washed with a solution of ethyl acetate/methanol 9/1 (500 mL), and concentrated to dryness. All the sequence (including addition of platinum on carbon 5% dry (1.34 g, 50% $^{w}/_{w}$), stirring under H_2 (1 atm) at room temperature for 16 h and filtration through a Celite® pad) was repeated to allow the complete conversion. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (1.12 g). ^{1}H NMR (400 MHz, dmso-d6): δ 6.93 (d, 1H). 6.67-6.33 (m, 2H), 5.30 (t,

1H), 4.96 (t, 1H), 4.88 (s, 2H), 4.81 (t, 1H), 4.61 (t, 1H), 4.39 (d, 1H), 4.29-4.24 (m, 2H), 3.78-3.72 (m, 1H), 3.65 (s, 3H), 2.65-2.54 (m, 2H), 2.07-1.98 (m, 9H), 1.79-1.68 (m, 1H), 1.63-1.52 (m, 1H).

Step 5: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-[[(2S)-2-(tert-butoxycarbonyl amino)-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)phenyl]ethyl]tetrahydropyran-2-carboxylate

[854] To a solution of the product from **Step 4** (1.00 g, 2.14 mmol) in DMF (21 mL) were successively added (2S)-2-(*tert*-butoxycarbonylamino)-5-ureido-pentanoic acid (Boc-Cit-OH) (589 mg, 2.14 mmol), DIPEA (707 µl, 4.28 mmol) and HBTU (1.22 g, 3.21 mmol). The reaction mixture was stirred at room temperature for 72 h. After dilution with water (100 mL) and concentration, the crude product was purified by silica gel chromatography (gradient of methanol in dichloromethane) to afford the desired product (1.05 g).^{1}H NMR (400 MHz, dmso-d6): δ 9.82 (s, 1H), 7.35-7.42 (m, 2H), 7.24 (d, 1H), 6.95 (d, 1H), 5.94 (t, 1H), 5.37 (s, 2H), 5.30 (t, 1H), 4.91-4.99 (m, 2H), 4.79 (t, 1H), 4.36-4.42 (m, 3H), 4.01-4.08 (m, 1H), 3.76 (t, 1H), 3.65 (s, 3H), 2.95-3.04 (m, 2H), 2.54-2.65 (m, 2H), 1.98-2.07 (m, 9H), 1.68-1.79 (m, 1H), 1.49-1.63 (m, 3H), 1.30-1.42 (m, 11H).

Step 6: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-(hydroxymethyl)phenyl]ethyl]tetrahydropyran-2-carboxylate

[855] To a solution of the product form **Step 5** (950 mg, 1.31 mmol) in dichloromethane (7.5 mL) was added trifluoroacetic acid (1.9 mL, 25.6 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated to dryness and coevaporated with toluene (2 x 50 mL) to afford the crude compound. To this crude in solution in DMF (13 mL) were successively added (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoic acid (Fmoc-Val-OH) (467 mg, 1.38 mmol), DIPEA (867 µl, 5.24 mmol) and HBTU (845 mg, 2.23 mmol). The reaction mixture was stirred at room temperature for 16 h. A saturated aqueous solution of hydrogenocarbonate (20 mL) was added and the mixture was stirred at room temperature for 1 h, diluted with water (100 mL) and concentrated to dryness. The crude product was purified by silica gel chromatography (gradient of methanol in dichloromethane) and then by reverse phase C18 chromatography using the neutral method to give the desired product (680 mg). LC-MS : MS (ESI) m/z $[M+H]^+$ = 946.3. ^{1}H NMR (400 MHz, dmso-d6): δ 9.90 (s, 1H). 8.07 (d, 2H), 7.89 (d, 2H), 7.74 (t, 2H), 7.44-7.38 (m, 3H), 7.36-7.28 (m, 3H), 7.24 (d, 1H), 5.94 (t, 1H), 5.37 (s, 2H), 5.30 (t, 1H), 4.99-4.92 (m, 2H), 4.79 (t, 1H), 4.42-4.36 (m, 4H), 4.32-4.19 (m, 3H), 3.94-

3.90 (m, 1H), 3.76 (t, 1H), 3.65 (s, 3H), 2.99-2.94 (m, 2H), 2.65-2.54 (m, 2H), 2.07-1.98 (m, 10H), 1.70-1.55 (m, 4H), 1.46-1.36 (m, 2H), 0.89-0.84 (m, 6H). ^{13}C NMR (100 MHz, dmso-d6): δ 171.19, 170.33, 169.58, 169.45, 169.27, 167.77, 158.81, 156.12, 143.89, 143.76, 140.69, 139.48, 137.54, 134.88, 128.44, 127.62, 127.06, 125.35, 120.08, 119.42, 116.65, 75.78, 74.61, 72.65, 71.20, 69.49, 65.68, 60.49, 60.10, 53.14, 52.40, 46.68, 32.32, 30.43, 29.54, 27.19, 26.77, 20.39, 20.34, 20.24, 19.22, 18.25.

Step 7: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-(bromomethyl)-5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]ethyl]tetrahydropyran-2-carboxylate

[856] To a solution of the product from **Step 6** (154 mg, 0.163 mmol) in THF (8.2 mL) were successively added triphenylphosphine (85.4 mg, 0.326 mmol) and 1-bromopyrrolidine-2,5-dione (58.0 mg, 0.326 mmol). The reaction mixture was stirred at room temperature for 2 h. After 5 h, triphenylphosphine (85.4 mg, 0.326 mmol) and 1-bromopyrrolidine-2,5-dione (58.0 mg, 0.326 mmol) were added to the mixture and the reaction was stirred at room temperature for 15 h. The crude product thus obtained was used in the next step. UPLC-MS : MS (ESI) m/z $[M+OMe-Br+H]^+$ = 960.7.

Step 8: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[2-[(2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-methoxycarbonyl-tetrahydropyran-2-yl]ethyl]phenyl]methyl]ammonium; bromide

[857] To the solution of the product from **Step 7** (207.63 mg, 206 µmol) in DMF (5 mL) were successively added 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid (**P2)** (100 mg, 158 µmol) and DIPEA (135 µL, 792 µmol). The reaction mixture was stirred at room temperature for 4 h. The crude product was concentrated and used in the next step without further treatment (246 mg).

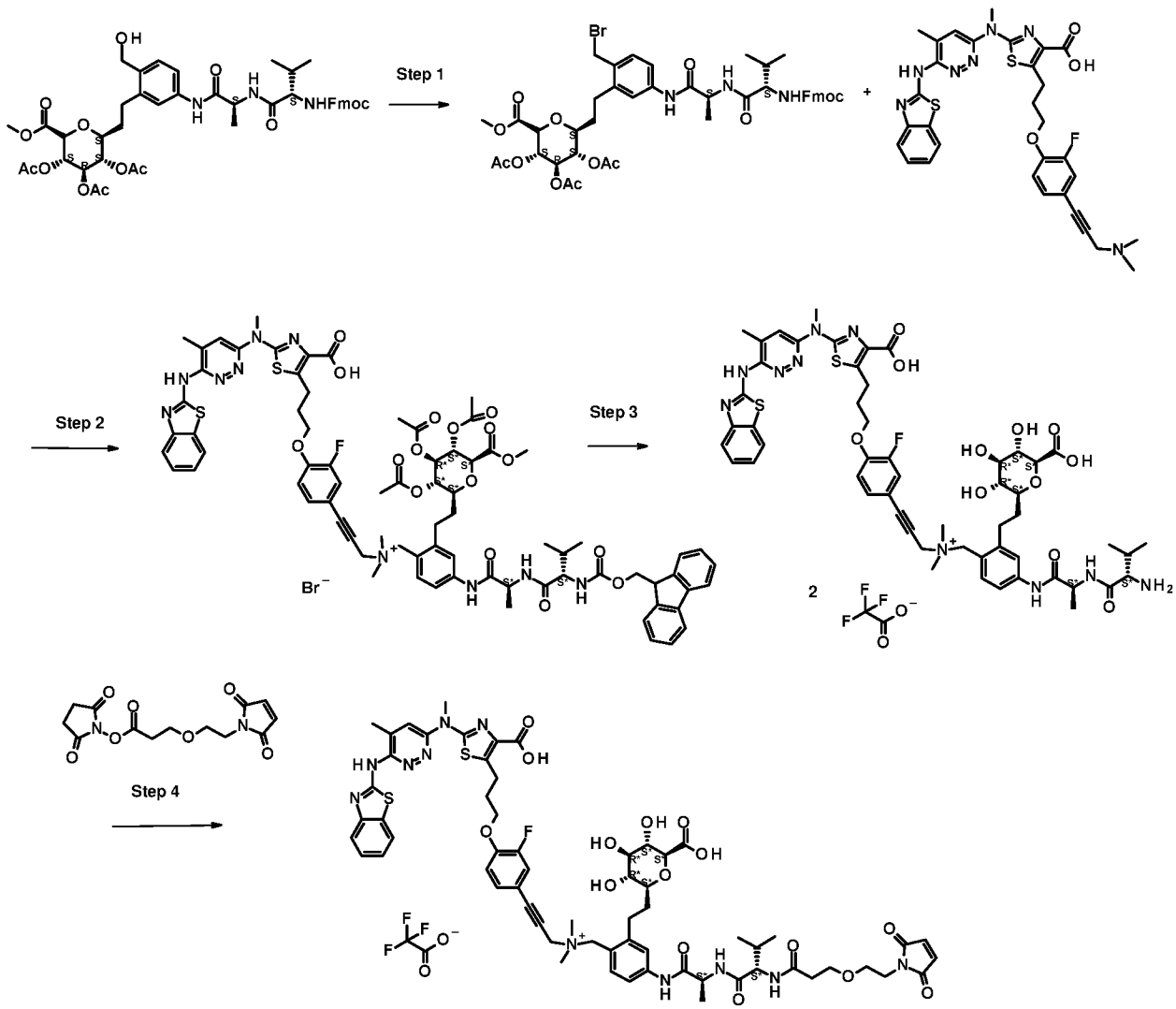
Step 9: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl] ammonium;2,2,2-trifluoroacetate;2,2,2-trifluoroacetic acid

[858] To a solution of the product from **Step 8** (246 mg, 158 µmol) in dioxane (2.0 mL) was added a solution of lithium hydroxide monohydrate (39.7 mg, 946 µmol) in water (2 ml). After the completion of the reaction, a 1 M aqueous solution of HCl was added until pH 6-7. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the expected compound (68 mg).

Step 10: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]ammonium;2,2,2-trifluoroacetate

[859] To a solution of the product from **Step 9** (30 mg, 21.0 µmol) in DMF (1.2 mL) were successively added the solution of (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (12.8 mg, 41.3 µmol) in DMF (500 µL) and DIPEA (18.3 µL, 105 µmol). The reaction mixture was stirred at room temperature for 3 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the title compound (6.5 mg). HRMS (ESI) $[M\text{-}CF_3COO]^+$ found = 1392.5197 (δ= 0.7 ppm).

Preparation of L106A-P2: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate



Step 1: methyl (3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-(bromomethyl)-5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]ethyl]tetrahydropyran-2-carboxylate

[860] To a solution of methyl (3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]-2-(hydroxymethyl)phenyl]ethyl]tetrahydropyran-2-carboxylate (**Preparation of L106C-P7**, Step 16) (255 mg, 297 µmol) in THF (14 mL) were successively added triphenylphosphine (234 mg, 890 µmol) and N-bromosuccinimide (158 mg, 890 µmol). The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was used in the next step without any treatment.

Step 2: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-

butanoyl]amino]propanoyl]amino]-2-[2-[(2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-methoxycarbonyl-tetrahydropyran-2-yl]ethyl]phenyl]methyl]ammonium;bromide

[861] To a suspension of the product from **Step 1** (297 µmol) in THF were successively added a solution 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid (**P2**) (140 mg, 222 µmol) in DMF (3 mL) and DIPEA (116 µL, 665 µmol). The reaction was stirred at room temperature for 60 h. The reaction mixture was evaporated to dryness and used without work-up in the next step.

Step 3: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl]ammonium;2,2,2-trifluoroacetate;2,2,2-trifluoroacetic acid

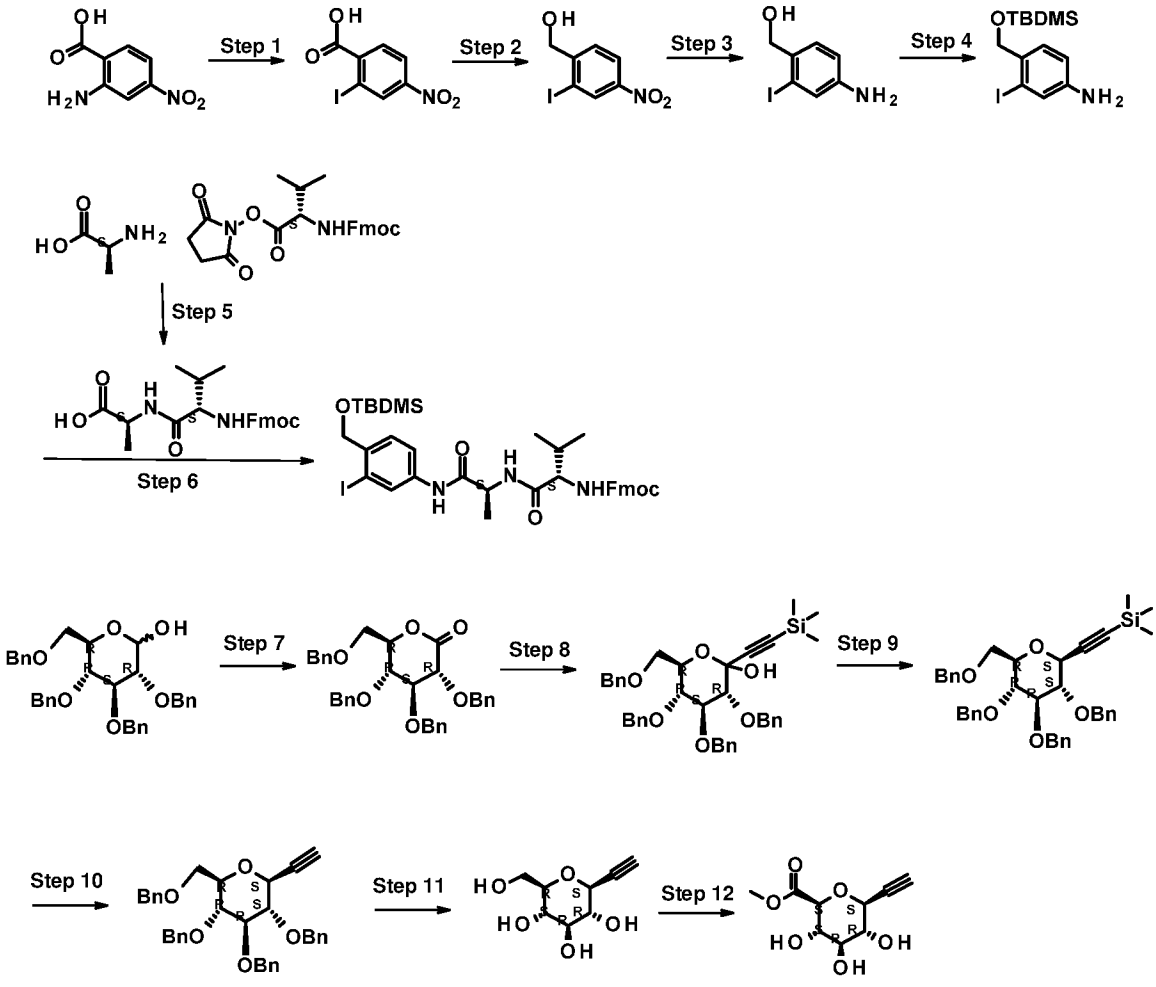
[862] To a solution of the product from **Step 2** (222 µmol) in dioxane (2 mL) was added a solution of $LiOH.H_2O$ (218 mg, 5.20 mmol) in water (2 mL). The solution was stirred at room temperature for 2 h. A 1 M aqueous solution of HCl was added until pH 6-7. The reaction mixture was evaporated to dryness and the crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to afford the expected compound (112 mg). IR: (v cm^{-1}) 3500-2500, 2237, 1667, 1197/1180/1130. ^1H NMR (400/500 MHz, dmso-d6) δ ppm 12.55 (m), 10.35 (s), 8.65 (d), 8.1 (large), 7.89 (d, 1 H), 7.67 (s, 1 H), 7.66 (dd, 1 H), 7.53 (df, 1 H), 7.48 (m, 1 H), 7.4 (m, 1 H), 7.38 (m, 1 H), 7.27 (m, 1 H), 7.24 (t, 1 H), 7.2 (dd, 1 H), 7.19 (m, 1 H), 5.3-4.7 (ml), 4.64/4.54 (2d, 2 H), 4.51 (br s, 2 H), 4.5 (m, 1 H), 4.2 (t, 2 H), 3.78 (s, 3 H), 3.6 (m, 1 H), 3.5 (d, 1 H), 3.32 (t, 1 H), 3.28 (t, 1 H), 3.11 (t, 1 H), 3.1-2.9 (m, 4 H), 3.02 (br s, 6 H), 2.98 (m, 1 H), 2.48 (s, 3 H), 2.2-1.5 (m, 5 H), 1.38 (d, 3 H), 0.98 (d, 6 H).

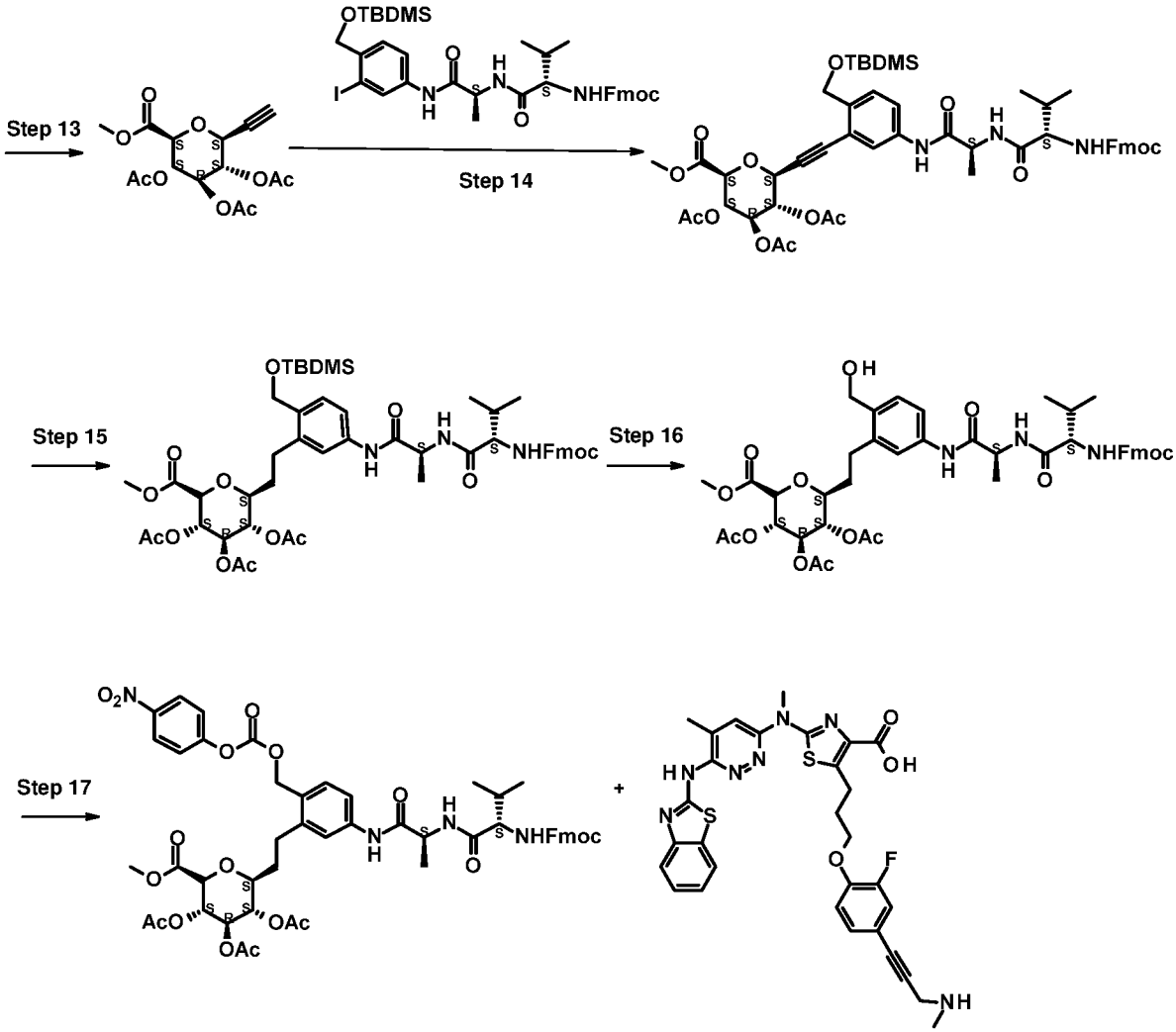
Step 4: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-dimethyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methyl]ammonium; 2,2,2-trifluoroacetate

[863] To a solution of the product from **Step 3** (60mg, 44.8 µmol) in solution in DMF (2.25 mL) were successively added (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (20.9 mg, 67.2 µmol) and DIPEA (23.4 µL, 134 µmol). The solution was stirred at room temperature for 3 h. The crude product was purified using C18 reverse

phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to give the desired product (28.5 mg). IR: (v cm^{-1}) 3600-3100, 2800-2200, 2234, 1705+1687+1614, 1537. ^1H NMR (400 MHz, dmso-d6) δ ppm 12.5 (m, 2H), 10.5/8.20/7.90 (s+2d, 3H), 7.80 (d, 1H), 7.68 (2s, 2H), 7.60-7.40 (m, 4H), 7.40 (m, 2H), 7.20 (2t, 2H), 7.00 (s, 2H), 5.20-5.00 (m, 3H), 4.62/4.53 (2d, 2H), 4.50 (s, 2H), 4.38 (t, 1H), 4.20 (t, 4H), 3.80(s, 3H), 3.60-3.00 (m, 10H), 3.02 (2s, 6H), 2.81 (m, 2H), 2.45 (s, 3H), 2.42/2.30 (2t, 4H), 2.15 (m, 2H), 2.00 (m, 1H), 1.95 (m, 2H), 1.30 (d, 3H), 0.89/0.82 (2d, 6H). HRMS (ESI) $[M\text{-}CF_3CO_2]^+$=1306.4715 (δ=0.6 ppm).

Preparation of L106C-P7: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

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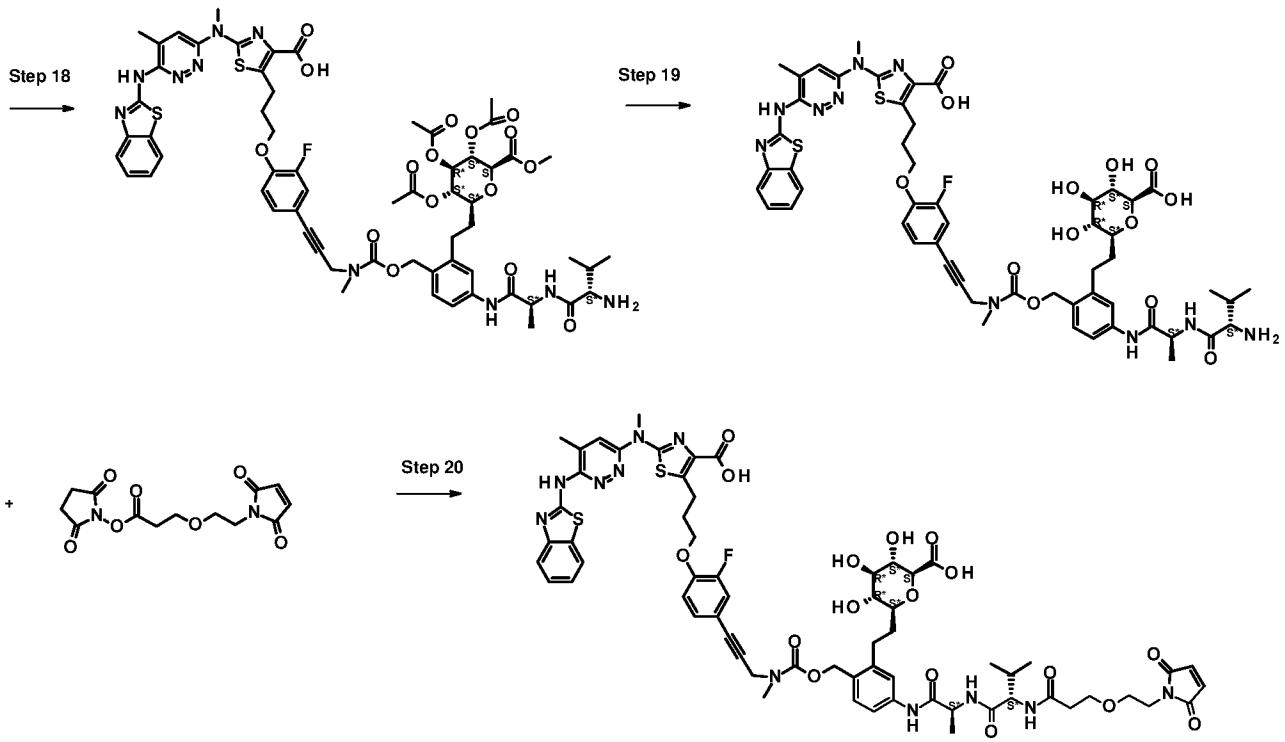
OTBDMS
NHFmoc
Step 14
Step 15
Step 16
Step 17
AcO
OAc
OH
F
NH



Step 1: 2-iodo-4-nitro-benzoic acid

[864] To a solution of 2-amino-4-nitro-benzoic acid (10.0 g, 54.90 mmol) in acetonitrile (280 mL) was added p-toluenesulfonic acid monohydrate (32.0 g, 168.2 mmol). The mixture was stirred at room temperature for 15 min, then a solution containing sodium nitrite (8.00 g, 115.9 mmol) and potassium iodide (24.0 g, 144.6 mmol) in water (140 mL) was added dropwise in 15 min. The reaction mixture was stirred for 19 h. After completion of the reaction, the mixture was quenched with sodium thiosulfate (13.02 g, 82.36 mmol) and acidified with an aqueous solution of hydrogen chloride 3 M (25 mL). The aqueous layer was extracted with ethyl acetate (2 x 250 mL) and the combined organic layers were washed with a 1 M aqueous solution of hydrogen chloride (100 mL), dried over sodium sulfate, filtered and concentrated to dryness. The resulting residue was taken up in dichloromethane (1 L) and washed with a 1 M aqueous solution of HCl (100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give the desired product (15.0 g).^{1}H NMR (400 MHz, dmso-d6): δ 13.8 (br s, 1H), 8.64 (s, 1H), 8.27 (d, 1H), 7.86 (d, 1H).

Step 2: (2-iodo-4-nitro-phenyl)methanol

[865] To a solution of the product from **Step 1** (5.0 g, 17.06 mmol) in THF (70 mL) was added a 1 M solution of borane in THF (85 mL, 85 mmol). The reaction mixture was stirred at 65 °C for 4 h. The reaction mixture was cooled to room temperature and was quenched with the addition of methanol (200 mL). The mixture was stirred at room temperature for 30 min and concentrated to dryness. The crude product was purified by silica gel

chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (3.38 g). ^{1}H NMR (400 MHz, dmso-d6): δ 8.54 (d, 1H), 8.29 (dd, 1H), 7.70 (d, 1H), 5.82 (t, 1H), 4.47 (d, 2H).

Step 3: (4-amino-2-iodo-phenyl)methanol

[866] To a solution of the product from **Step 2** (3.70 g, 13.26 mmol) in ethanol (100 mL) and water (25 mL) were successively added iron (3.70 g, 66.25 mmol) and ammonium chloride (800 mg, 14.96 mmol). The reaction mixture was stirred at 80 °C for 3 h. The reaction mixture was filtered over Celite®, washed with ethanol, and concentrated to dryness. The resulting residue was taken up in ethyl acetate (100 mL) and washed with a saturated solution of sodium hydrogen carbonate (100 mL), dried over sodium sulfate, filtered, and concentrated to dryness to give the desired product (2.48 g). ^{1}H NMR (400 MHz, dmso-d6): δ 7.02-7.10 (m, 2H), 6.57 (d, 1H), 5.16 (s, 2H), 4.97 (t, 1H), 4.28 (d, 2H).

Step 4: 4-[[tert-butyl(dimethyl)silyl]oxymethyl]-3-iodo-aniline

[867] To a solution of the product from **Step 3** (3.51 g, 13.37 mmol) in dichloromethane (150 mL) was added imidazole (0.95 g, 13.95 mmol). The mixture was cooled to 0 °C and a solution of *tert*-butyl-chloro-dimethyl-silane (2.40 mL, 13.85 mmol) in dichloromethane (150 mL) was added dropwise over 15 minutes. After stirring at room temperature for 16 h, the reaction mixture was quenched with methanol (20 mL) and concentrated. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (3.64 g75%). ^{1}H NMR (400 MHz, dmso-d6): δ 7.05 (s, 1H), 7.03 (d, 1H), 6.55 (d, 1H), 5.24 (s, 2H), 4.46 (s, 2H), 0.88 (s, 9H), 0.06 (s, 6H).

Step 5: (2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoic acid

[868] To a solution of (2S)-2-aminopropanoic acid (3.22 g, 36.09 mmol) in water (90 mL) were successively added sodium carbonate (7.29 g, 68.74 mmol) and a solution of (2,5-dioxopyrrolidin-1-yl) (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoate (15.0 g, 34.37 mmol) in dimethoxyethane (90 mL). The reaction mixture was stirred at room temperature for 16 h. After acidification of the reaction until pH=1 with a 1 M aqueous solution of hydrogen chloride, the aqueous layer was extracted with ethyl acetate (3 x 500 mL). The combined organic layers were dried, concentrated, and triturated with diethyl ether (50 mL) to give the desired product (11.25 g). ^{1}H NMR (400 MHz, dmso-d6) δ 12.48 (s, 1H), 8.21 (d, 1H), 7.89 (d, 2H), 7.72-7.79 (m, 2H), 7.28-7.46 (m, 5H), 4.15-4.32 (m, 4H), 3.90 (t, 1H), 1.90-2.02 (m, 1H), 1.28 (d, 3H), 0.86-0.90 (m, 6H).

Step 6: 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-2-[4-[[tert-butyl(dimethyl)silyl]oxymethyl]-3-iodo-anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]carbamate

[869] To a solution of the product from **Step 5** (1.50 g, 3.65 mmol) in dichloromethane (18 mL) and methanol (18 mL) were successively added the product from **Step 4** (1.33 g, 3.65 mmol) and ethyl 2-ethoxy-2H-quinoline-1-carboxylate (EEDQ) (1.36 g, 5.48 mmol). The suspension was stirred at room temperature for 16 h. After concentration, the crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) and then by C18 chromatography (gradient of methanol in water) to give the desired product (1.18 g). ^{1}H NMR (400 MHz, dmso-d6): δ 10.05 (s, 1H). 8.16-8.24 (m, 2H), 7.88 (d, 2H), 7.71-7.77 (m, 2H), 7.55 (d, 1H), 7.37-7.48 (m, 3H), 7.27-7.37 (m, 3H), 4.56 (s, 2H), 4.38 (t, 1H), 4.18-4.33 (m, 3H), 3.91 (t, 1H), 2.08-2.20 (m, 1H), 1.30 (d, 3H), 0.83-0.95 (m, 15H), 0.06 (s, 6H).

Step 7: (3R,4S,5R,6R)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)tetrahydropyran-2-one

[870] A suspension of (3R,4S,5R,6R)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)tetrahydropyran-2-ol (30.0 g, 55.49 mmol) in DMSO (120 mL) was stirred at room temperature for 30 min and treated dropwise with acetic anhydride (90 mL) at room temperature over 15 min. The solution was stirred for 16 h, cooled to 0 °C, and treated with a 1 M aqueous solution of hydrogen chloride (100 mL). The reaction mixture was stirred at room temperature for 20 min and the acetic acid was evaporated. The resulting residue was diluted with water (200 mL) and ethyl acetate (200 mL). The aqueous layer was extracted with ethyl acetate (2 x 200 mL) and the combined organic layers were washed with water (2 x 500 mL) and with a saturated solution of sodium hydrogen carbonate (2 x 500 mL), dried over sodium sulfate, filtered, concentrated, and purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (25.05 g). ^{1}H NMR (400 MHz, dmso-d6): δ 7.19-7.39 (m, 20H), 4.85 (d, 1H), 4.57-4.72 (m, 5H), 4.46-4.56 (m, 3H), 4.36 (d, 1H), 3.98-4.05 (m, 1H), 3.84-3.92 (m, 1H), 3.65-3.76 (m, 2H).

Step 8: (3R,4S,5R,6R)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)-2-(2-trimethylsilylethynyl)tetrahydropyran-2-ol

[871] To a solution of trimethylsilylacetylene (24 mL, 168.6 mmol) in THF (325 mL) was added a 2.5 M solution of butyllithium in hexane (59.41 mL, 148.5 mmol) at -78°C in 20 min. The solution was stirred at -78 °C for 45 min and at 0 °C for 45 min. The reaction mixture was cooled to -78 °C and a solution of the product from **Step 7** (25.0 g, 46.41 mmol) in THF (325 mL) was added dropwise over 45 min. The reaction mixture was stirred at this temperature for 4 h and quenched with water (200 mL). The aqueous layer was extracted

with ethyl acetate (2 x 200 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness to give the desired product (29.56 g) as a mixture of two diastereoisomers in a ratio 4/6. ^{1}H NMR (400 MHz, dmso-d6): δ 7.13-7.43 (m, 20H), 4.87-4.99 (m, 1H), 4.65-4.83 (m, 4H), 3.43-3.57 (m, 3H), 3.70-3.85 (m, 2H), 3.55-3.68 (m, 3H), 3.43-3.53 (m, 2H), 0.11-0.22 (m, 9H).

Step 9: trimethyl-[2-[(2S,3S,4R,5R,6R)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)tetrahydropyran-2-yl]ethynyl]silane

[872] To a solution of the product from **Step 8** (29.56 g, 46.42 mmol) in acetonitrile (83 mL) and dichloromethane (193 mL) was added a solution of triethylsilane (44.98 mL, 278.5 mmol) in a mixture of acetonitrile/dichloromethane (37 mL/18 mL) in 20 min and a solution of boron trifluoride diethyl etherate (23.53 mL, 185.7 mmol) in acetonitrile (37 mL) in 30 min at -15°C. The solution was stirred for 5 h at the same temperature and diluted with water (500 mL). The aqueous layer was extracted with ethyl acetate (2 x 500 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness to give the desired product (28.82 g). ^{1}H NMR (400 MHz, dmso-d6): δ 7.10-7.44 (m, 20H), 4.93 (d, 1H), 4.67-4.86 (m, 4H), 4.43-4.57 (m, 3H), 4.16-4.28 (m, 1H), 3.42-3.68 (m, 6H), 0.15 (s, 9H).

Step 10: (2R,3R,4R,5S,6S)-3,4,5-tribenzyloxy-2-(benzyloxymethyl)-6-ethynyl-tetrahydropyran

[873] To a solution of the product from **Step 9** (28.80 g, 46.39 mmol) in methanol (1.12 L) and dichloromethane (240 mL) was added an 1 M aqueous solution of sodium hydroxide (80 mL). The solution was stirred at room temperature for 1 h, acidified until pH = 1 with a 1 M aqueous solution of hydrogen chloride and diluted with water (500 mL). The methanol was evaporated and the aqueous layer was extracted with ethyl acetate (2 x 1 L). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (20.00 g). ^{1}H NMR (400 MHz, dmso-d6): δ 3.42-3.67 (m, 7H), 4.17 (d, 1H), 4.44-4.56 (m, 3H), 4.67-4.86 (m, 4H), 4.90 (d, 1H), 7.15-7.40 (m, 20H).

Step 11: (2S,3R,4R,5S,6R)-2-ethynyl-6-(hydroxymethyl)tetrahydropyran-3,4,5-triol

[874] To a solution of the product from **Step 10** (20.00 g, 36.45 mmol) in ethanethiol (400 mL) was added boron trifluoride diethyl etherate (147.8 mL, 1166 mmol) dropwise at room temperature over 5 min. The solution was stirred at room temperature for 16 h, cooled to 0°C, equipped with a gas trap containing an aqueous saturated solution of sodium

hypochlorite, and treated dropwise with a saturated aqueous solution of sodium hydrogen carbonate (500 mL) at 0 °C in 1 h. After concentration to dryness, the crude product was purified by silica gel chromatography (gradient of methanol in dichloromethane) to give the desired product (4.05 g). ^{1}H NMR (400 MHz, dmso-d6): δ 5.28 (d, 1H), 4.99 (d, 1H), 4.91 (d, 1H), 4.52 (t, 1H), 3.77 (d, 1H), 3.60-3.69 (m, 1H), 3.35-3.43 (m, 1H), 3.32 (s, 1H), 2.97-3.13 (m, 4H).

Step 12: methyl (2S,3S,4R,5R,6S)-6-ethynyl-3,4,5-trihydroxy-tetrahydropyran-2-carboxylate

[875] To a solution of the product from **Step 11** (4.05 g, 21.52 mmol) in a saturated aqueous solution of sodium hydrogen carbonate (81 mL) and THF (81 mL) was added (2,2,6,6-tetramethylpipéridin-1-yl)oxyl (TEMPO) (168 mg, 1.08 mmol). The suspension was cooled to 0 °C and 1,3-dibromo-5,5-dimethyl-imidazolidine-2,4-dione (12.31 g, 43.04 mmol) was added portionwise in 30 min. The reaction mixture was stirred at 0°C for 4 h and quenched with the addition of methanol (40 mL). After 30 min stirring at this temperature, a saturated aqueous solution of potassium carbonate (10 mL) and dichloromethane (100 mL) were added. After the organic layer was extracted with water (2 x 200 mL), the combined aqueous layers were acidified until pH = 1 with a 3M aqueous solution of hydrogen chloride and concentrated to dryness. The residue was taken up in methanol (100 mL) and in a 3M aqueous solution of hydrogen chloride (20 mL). The mixture was concentrated and co-evaporated several times with methanol (4 x 100 mL). The crude product was purified by silica gel chromatography (gradient of methanol in dichloromethane Cerium developer) to give the desired product (3.00 g). ^{1}H NMR (400 MHz, dmso-d6): δ 5.46 (d, 1H), 5.32 (d, 1H), 5.18 (d, 1H), 3.93-4.00 (m, 1H), 3.75 (dd, 1H), 3.65 (s, 3H), 3.40-3.44 (m, 1H), 3.31 (s, 1H), 3.09-3.19 (m, 2H).

Step 13: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-ethynyl-tetrahydropyran-2-carboxylate

[876] To a solution of the product from **Step 12** (3.00 g, 13.88 mmol) in DMF (37.5 mL) and pyridine (12.5 mL) was added *N,N*-dimethylpyridin-4-amine (DMAP) (84.8 mg, 0.693 mmol). The reaction mixture was cooled to 0 °C and treated with acetic anhydride (20.0 mL, 213 mmol) dropwise over 5 min. The solution was stirred at room temperature for 3 h and diluted with a 1 M aqueous solution of hydrogen chloride (200 mL). The aqueous layer was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were washed with a 1M aqueous solution of hydrogen chloride (2 x 200 mL) and a saturated aqueous solution of potassium carbonate (200 mL), dried over sodium sulfate, filtered, concentrated and purified

by silica gel chromatography (gradient of ethyl acetate in cyclohexane cerium developer) to give the desired product (4.60 g). ^{1}H NMR (400 MHz, dmso-d6): δ 5.33 (t, 1H), 4.93-5.01 (m, 2H), 4.70 (d, 1H), 4.44 (d, 1H), 3.67 (s, 1H), 3.64 (s, 3H), 2.02 (s, 3H), 1.94-2.01 (m, 6H).

Step 14: methyl (2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-[[tert-butyl(dimethyl)silyl]oxymethyl]-5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]phenyl] ethynyl]tetrahydropyran-2-carboxylate

[877] To a solution of the product from **Step 13** (496 mg, 1.45 mmol) in DMF (7.3 mL) were successively added the product from **Step 6** (730 mg, 0.966 mmol), DIPEA (738 µL, 4.47 mmol), copper iodide (18.4 mg, 96.6 mmol), and dichloro-bis-(triphenylphosphine)palladium(II) (67.8 mg, 96.6 mmol). The solution was flushed with argon and stirred at room temperature for 16 h. After dilution with water (100 mL), the aqueous layer was extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with water (2 x 200 mL) and a saturated aqueous solution of ammonium chloride (2 x 200 mL), dried over sodium sulfate, filtered, concentrated, and purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (782 mg). ^{1}H NMR (400 MHz, dmso-d6): δ 10.09 (s, 1H). 8.20 (d, 1H), 7.89 (d, 2H), 7.70-7.78 (m, 3H), 7.55 (d, 1H), 7.32-7.46 (m, 4H), 7.27-7.32 (m, 2H), 5.41 (t, 1H), 4.96-5.14 (m, 3H), 4.67 (s, 2H), 4.51 (d, 1H), 4.36-4.44 (m, 1H), 4.16-4.32 (m, 3H), 3.88-3.95 (m, 1H), 3.64 (s, 3H), 1.94-2.07 (m, 10H), 1.30 (d, 3H), 0.84-0.93 (m, 15H), 0.08 (s, 6H).

Step 15: methyl (3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[2-[[tert-butyl(dimethyl)silyl]oxymethyl]-5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]phenyl] ethyl]tetrahydropyran-2-carboxylate

[878] A solution of the product from **Step 14** (750 mg, 0.773 mmol) in THF (15 mL) was flushed with argon, treated with dry Platinum 5% on carbon (75 mg, 50%$^w/_w$), flushed successively with argon and with H_2, and stirred under H_2 atmosphere (1 atm) at room temperature for 16 h. The reaction mixture was filtered through a Celite® pad, washed with THF, and concentrated to dryness. The complete sequence (including addition of dry platinum 5% on carbon (75 mg, 50%$^w/_w$), stirring under H_2 atmosphere (1 atm) at room temperature for 16 h, and filtration through a Celite® pad) was performed 4 more times. The crude product was purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (470 mg). ^{1}H NMR (400 MHz, dmso-d6): δ 9.90 (s, 1H), 8.16 (d, 1H), 7.89 (d, 2H), 7.70-7.78 (m, 2H), 7.37-7.49 (m, 4H), 7.27-7.32 (m, 3H), 7.23

(d, 1H), 5.29 (t, 1H), 4.95 (t, 1H), 4.78 (t, 1H), 4.60 (s, 2H), 4.34-4.44 (m, 2H), 4.16-4.32 (m, 3H), 3.88-3.95 (m, 1H), 3.72-3.79 (m, 1H), 3.64 (s, 3H), 2.69-2.78 (m, 1H), 2.50-2.60 (m, 1H), 1.92-2.03 (m, 10H), 1.55-1.75 (m, 2H), 1.30 (d, 3H), 0.84-0.93 (m, 15H), 0.05 (s, 6H).

Step 16: methyl (3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]-2-(hydroxymethyl)phenyl]ethyl]tetrahydropyran-2-carboxylate

[879] To a solution of the product from **Step 15** (470 mg, 0.483 mmol) in THF (540 µL) and water (540 µL) was added acetic acid (1.6 mL, 28.28 mmol). The solution was stirred at room temperature for 16 h and diluted with water (100 mL). The aqueous layer was extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with water (2 x 200 mL) and a saturated aqueous solution of sodium hydrogen carbonate (200 mL), dried over sodium sulfate, filtered, concentrated, and purified by silica gel chromatography (gradient of ethyl acetate in cyclohexane) to give the desired product (354 mg). ^{1}H NMR (400 MHz, dmso-d6): δ 9.87 (s, 1H), 8.16 (d, 1H), 7.89 (d, 2H), 7.70-7.78 (m, 2H), 7.37-7.50 (m, 4H), 7.27-7.37 (m, 3H), 7.25 (d, 1H), 5.29 (t, 1H), 4.91-4.98 (m, 2H), 4.78 (t, 1H), 4.34-4.44 (m, 4H), 4.16-4.32 (m, 3H), 3.88-3.95 (m, 1H), 3.72-3.79 (m, 1H), 3.64 (s, 3H), 2.64-2.73 (m, 1H), 2.50-2.60 (m, 1H), 1.92-2.03 (m, 10H), 1.69-1.79 (m, 1H), 1.52-1.65 (m, 1H), 1.30 (d, 3H), 0.84-0.93 (m, 6H).

Step 17: methyl (3S,4R,5S,6S)-3,4,5-triacetoxy-6-[2-[5-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]propanoyl]amino]-2-[(4-nitrophenoxy)carbonyloxymethyl]phenyl]ethyl]tetrahydropyran-2-carboxylate

[880] To a solution of the product from **Step 16** (310 mg, 0.361 mmol) in THF (7.75 mL) were successively added pyridine (146 µL, 1.80 mmol) and 4-nitrophenyl chlorocarbonate (182 mg, 0.901 mmol). The suspension was stirred at room temperature for 16 h, concentrated, and purified by silica gel chromatography (gradient of ethyl acetate in dichloromethane) to give the desired product (257 mg). ^{1}H NMR (400 MHz, dmso-d6): δ 10.04 (s, 1H), 8.31 (d, 2H), 8.20 (d, 1H), 7.89 (d, 2H), 7.66-7.78 (m, 2H), 7.56 (d, 2H), 7.28-7.52 (m, 8H), 5.31 (t, 1H), 5.25 (s, 2H), 4.96 (t, 1H), 4.79 (t, 1H), 4.40 (d, 2H), 4.16-4.32 (m, 3H), 3.88-3.95 (m, 1H), 3.74-3.83 (m, 1H), 3.61 (s, 3H), 2.74-2.84 (m, 1H), 2.60-2.71 (m, 1H), 1.90-2.03 (m, 10H), 1.72-1.83 (m, 1H), 1.58-1.71 (m, 1H), 1.30 (d, 3H), 0.82-0.94 (m, 6H). LC-MS: MS (ESI) m/z $[M+Na]^+$ = 1047.6.

Step 18: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[2-fluoro-4-[3-[methyl-[[4-[[(2S)-2-[[(2S)-2-amino-3-methyl-

butanoyl]amino]propanoyl]amino]-2-[2-[(2S,3S,4R,5S,6S)-3,4,5-triacetoxy-6-methoxycarbonyl-tetrahydropyran-2-yl]ethyl]phenyl]methoxycarbonyl]amino]prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[881] To a solution of the product from **Step 17** (130 mg, 127 µmol) in DMF (1.5 mL) were successively added a solution of 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[2-fluoro-4-[3-(methylamino)prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid **(P7)** (101 mg, 168 µmol) in DMF (1.5 mL) and DIPEA (83 µL, 502 µmol). The reaction mixture was stirred 4 h at room temperature. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the NH_4HCO_3 method to give the desired product (80 mg).

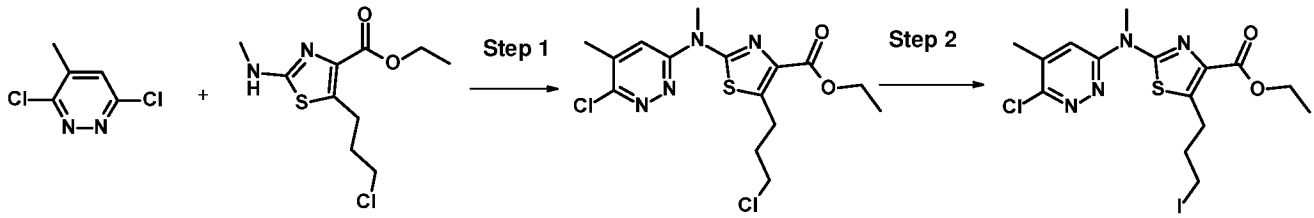
Step 19: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[2-fluoro-4-[3-[methyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl]amino]prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[882] To the solution of the product from **Step 18** (80mg, 62.4µmol) in DMF (2.0 mL) was added and lithium hydroxyde monohydrate (31.5 mg, 750 µmol) in water (500µL). The reaction mixture was stirred at room temperature for 2 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the NH_4HCO_3 method to give the desired product (25 mg).

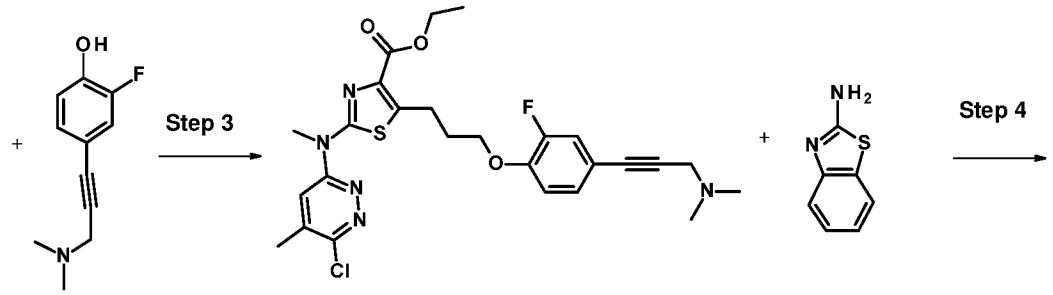
Step 20: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[2-fluoro-4-[3-[methyl-[[2-[2-[(2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]ethyl]-4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl]amino]prop-1-ynyl]phenoxy]propyl]thiazole-4-carboxylic acid

[883] To a solution of the product from **Step 19** (25mg, 21.9µmol) in DMF (1mL) were successively added (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (11.1 mg, 32.9 µmol) and DIPEA (5.4 µL, 32.9 µmol). The solution was stirred at room temperature for 1 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to give the desired product (5 mg). HRMS (ESI) $[M+H]^+$ found = 1336.4453 (δ = 0.3ppm).

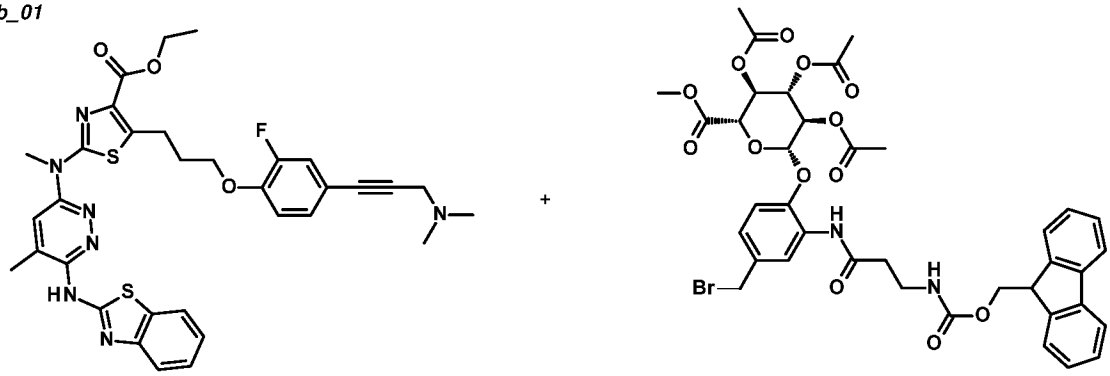
Preparation of L108A-P2: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-3-[3-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]propanoylamino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate



Preparation 3e_01



Preparation 6b_01



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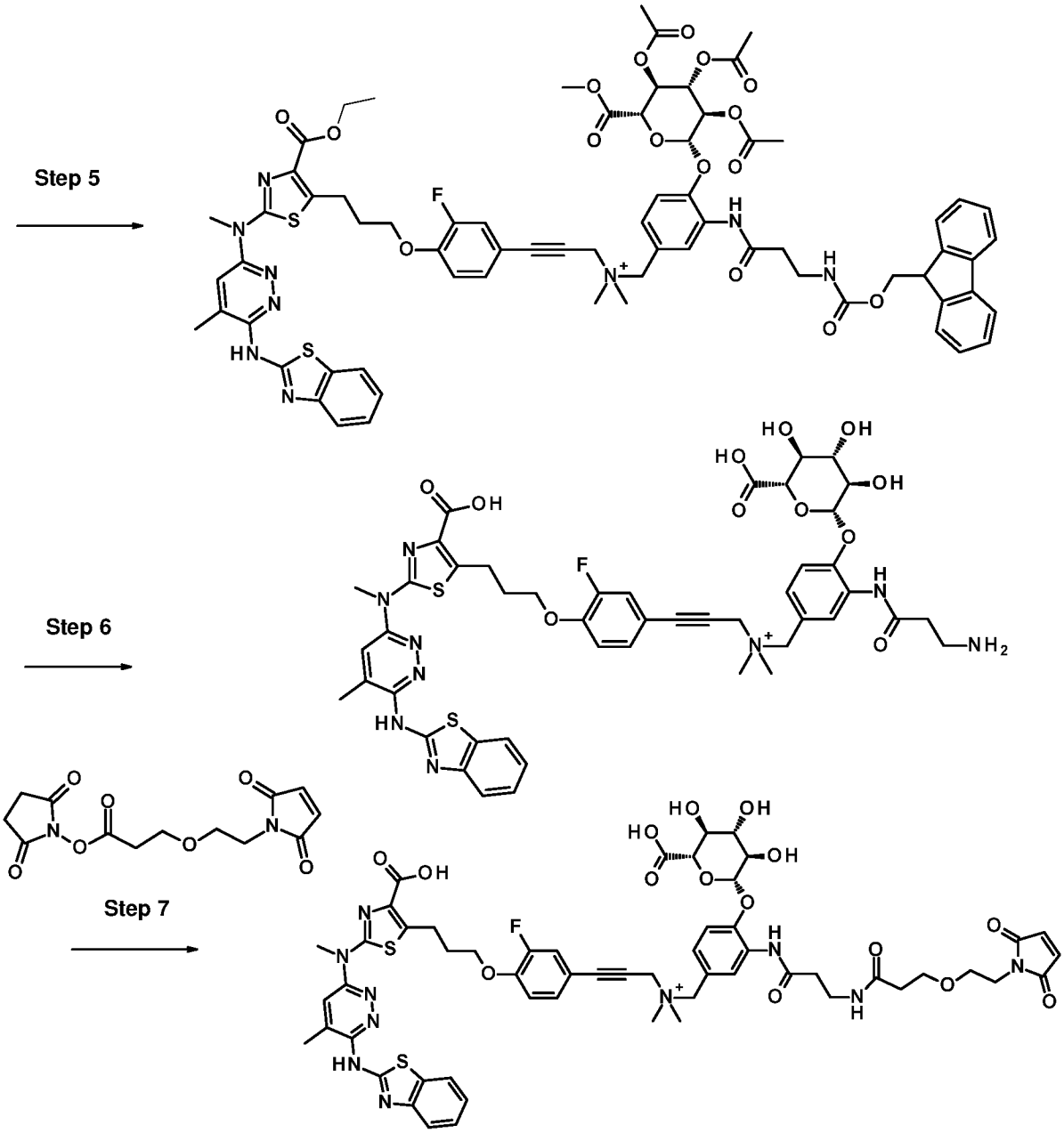


Step 1: ethyl 2-[(6-chloro-5-methyl-pyridazin-3-yl)-methyl-amino]-5-(3-chloropropyl)thiazole-4-carboxylate

[884] To a solution of ethyl 5-(3-chloropropyl)-2-(methylamino)thiazole-4-carboxylate (from **Preparation 3e_01**, 15.44 g, 58.5 mmol) in THF (600 mL) cooled to 0°C was added at 0°C NaH (60% in oil) (2.8 g, 70.6 mmol) in portion over a 0.5h time period. The suspension was stirred at 0°C for 0.5 h. To this suspension was then added dropwise at 0°C a solution of 3,6-dichloro-4-methyl-pyridazine (23.0 g, 141 mmol) in solution in THF (200 mL). The reaction mixture was stirred at room temperature for 15h, cooled to 0°C and then water (25 mL) was slowly added. The aqueous layer was extracted 3 times with AcOEt and the organic layer dried over $MgSO_4$. The crude product was purified by silica gel chromatography (gradient of AcOEt in petroleum ether) to give the desired product (7.0 g, 18.0 µmol). IR: (v

cm^{-1}) 3450, 1698, 1203. ^1H NMR (400 MHz, dmso-d6) δ ppm 7.81 (s, 1 H), 4.3 (quad, 2 H), 3.78 (s, 3 H), 3.31 (t, 2 H), 3.2 (m, 2 H), 2.4 (s, 3 H), 2.12 (quint, 2 H), 1.31 (t, 3 H).

Step 2: ethyl 2-[(6-chloro-5-methyl-pyridazin-3-yl)-methyl-amino]-5-(3-iodopropyl)thiazole-4-carboxylate

[885] To a solution of the product from **Step 1** (7.0 g, 18.0 mmol) in acetone (120 mL) was added sodium iodide (27 g, 178 mmol) and the suspension was heated at reflux (60°C) for 15 h. After the reaction mixture was cooled to room temperature, the precipitate was filtered, washed with acetone and the filtrate was evaporated to dryness. The resulting yellow solid was triturated with ether, filtered and dried over phosphorous pentoxide (P_2O_5) at 35°C for 48 h to give the desired product (7.6 g, 15.8 mmol) as a brown solid. IR: (v cm^{-1}) 1703, 1591. ^1H NMR (400 MHz, dmso-d6) δ ppm 7.82 (df, 1 H), 7.28 (dd, 1 H), 7.2 (dd, 1 H), 7.13 (t, 1 H), 4.26 (q, 2 H), 4.12 (t, 2 H), 3.77 (s, 3 H), 3.41 (s, 2 H), 3.26 (t, 2 H), 2.42 (s, 3 H), 2.22 (s, 6 H), 2.11 (m, 2 H), 1.29 (t, 3 H).

Step 3: ethyl 2-[(6-chloro-5-methyl-pyridazin-3-yl)-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[886] To a solution of product from **Step 2** (3.5 g, 7.28 mmol) in THF (400 mL) were successively added a solution of 4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenol (from **Preparation 6b_01**, 1.74 g, 8.74 mmol) in THF (100 mL) and cesium carbonate (Cs_2CO_3) (4.73 g, 8.74 mmol). The reaction mixture was heated at reflux (70°C) for 15 h. The reaction mixture was cooled to room temperature, poured into water (100 mL) and extracted 3 times with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporate to dryness. The crude product was purified by silica gel chromatography (gradient of methanol in DCM) to afford the desired product (2.40 g, 4.39 mmol). IR: (v cm^{-1}) 1698, ^1H NMR (400/500 MHz, dmso-d6) δ ppm 7.8 (s, 1 H), 4.3 (quad, 2 H), 3.8 (s, 3 H), 3.7 (t, 2 H), 3.2 (m, 2 H), 2.4 (s, 3 H), 2.1 (quint, 2 H), 1.3 (t, 3 H).

Step 4: ethyl 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-(dimethylamino)prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylate

[887] To a solution saturated with argon of the product from **Step 3** (961 mg, 1.76 mmol) and 1,3-benzothiazol-2-amine (317 mg, 2.11 mmol) in NMP (10 mL) were successively added 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) (509 mg, 0.88 mmol) and tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) (12.9 mg, 0.044 mmol). The reaction mixture was again saturated with argon for 15 min, DIEPA (1 mL, 5.28 mmol) was

added and the reaction mixture was stirred at 150°C for 15h. The reaction mixture was cooled to room temperature, water was added, and the aqueous phase was extracted several times with DCM. The organic phases were collected, washed with brine, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by silica gel chromatography (gradient of methanol in DCM) the desired compound (540 mg, 0.818 mmol). **I**R: (v cm^{-1}) 3700-2300, 1706. ^{1}H NMR (400 MHz, dmso-d6) δ ppm 11.55 (m, 1 H), 7.91 (d, 1 H), 7.68 (s, 1 H), 7.53 (d, 1 H), 7.39 (m, 1 H), 7.3 (dd, 1 H), 7.26-7.13 (m, 3 H), 4.26 (q, 2 H), 4.15 (t, 2 H), 3.77 (s, 3 H), 3.4 (s, 2 H), 3.27 (m, 2 H), 2.46 (s, 3 H), 2.21 (s, 6 H).

Step 5: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[3-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propanoylamino]-4-[(2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-methoxycarbonyl-tetrahydropyran-2-yl]oxy-phenyl]methyl]-dimethyl-ammonium

[888] To a solution of the product from **Step 4** (75 mg, 0.119 mmol) in DMF (2 mL) was added DIPEA (40 µL, 0.237 mmol) and methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-[4-(bromomethyl)-2-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propanoylamino]phenoxy] tetrahydropyran-2-carboxylate (WO2017096311A1, 128 mg, 0.158 mmol) and the reaction was stirred at room temperature for 2 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to give the desired compound (88 mg, 51% yield). ^{1}H NMR (400 MHz, dmso-d6) δ ppm 8.9/8.2/7.35 (2s+m, 3 H), 7.9-7.2 (m, 11 H), 7.88 (d, 2 H), 7.68 (d, 2 H), 7.4/7.3 (2t, 4 H), 5.7 (d, 1 H), 5.52 (t, 1 H), 5.21 (t, 1 H), 5.1 (t, 1 H), 4.78 (d, 1 H), 4.52/4.4 (2s, 4 H), 4.3-4.15 (m, 7 H), 3.78 (s, 3 H), 3.62 (s, 3 H), 3.3 (m, 4 H), 3.08 (s, 6 H), 2.55 (m, 2 H), 2.48 (s, 3 H), 2.15 (m, 2 H), 2.01 (3s, 9 H), 1.3 (t, 3 H). LCMS m/z = 660.

Step 6: [3-(3-aminopropanoylamino)-4-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-phenyl]methyl-[3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl]-dimethyl-ammonium

[889] To a solution of the product of **Step 5** (85 mg, 0.06 mmol) in MeOH (4 mL) was added LiOH dihydrate (64 mg, 1.53 mmol) and the reaction was stirred at room temperature for 5 h. The crude product was purified by Porapack® using NH_3/MeOH 7N as an eluent to give the desired compound (55 mg, 91% yield).

Step 7: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxy-3-[3-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]propanoylamino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate

[890] To a solution of product of **Step 6** (50mg, 0.05 mmol) in DMF (6 mL) were successively added DIPEA (30 µL, 0.179 mmol) and (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (28 mg, 0.09 mmol). The solution was stirred at room temperature for 1.5 h. The crude product was purified using C18 reverse phase prep-HPLC by direct deposit of the reaction mixture on the Xbridge® column and using the TFA method to give the desired product (15 mg, 20% yield). ^{1}H NMR (400 MHz, dmso-d6) δ ppm 8.4 (br s, 1 H), 7.9 (m, 1 H), 7.7 (br s, 1 H), 7.6 (dd, 1 H), 7.5 (dl, 1 H), 7.45 (dl, 1 H), 7.4 (td, 1 H), 7.25 (m, 3 H), 7.2 (t, 1 H), 7 (s, 2 H), 5 (d, 1 H), 4.55/4.4 (2 br s, 4 H), 4.2 (t, 2 H), 4 (d, 1 H), 3.8 (s, 3 H), 3.55 (2t, 4 H), 3.45 (m, 2 H), 3.45/3.4 (2m, 3 H), 3.35 (m, 2 H), 3.3 (t, 2 H), 3.1 (br s, 6 H), 2.6 (t, 2 H), 2.45 (s, 3 H), 2.15 (t, 2 H), 2.15 (quint, 2 H). ^{19}F NMR (400 MHz, dmso-d6) δ ppm -133.8. HRMS (ESI) $[M-CF_3CO_2]^+$ found = 1195.3690 (δ = 2.5 ppm)

Preparation of L107C-P7: 2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]propanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[891] Product was synthesized according to **Method G** by replacing 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]acetic acid with 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid. ^{1}H NMR (400 MHz, dmso-d6) δ ppm 12.55 (br s, 1 H), 11.5-10.8 (diffus, 1 H), 9.92 (s, 1 H), 8.16 (d, 1 H), 7.99 (t, 1 H), 7.9 (diffus, 1 H), 7.86 (d, 1 H), 7.67 (br s, 1 H), 7.64 (diffus, 1 H), 7.58 (d, 2 H), 7.38/7.2 (2m, 3 H), 7.35 (m, 1 H), 7.32 (d, 2 H), 7.15 (t, 1 H), 7 (s, 2 H), 5.03 (s, 2 H), 4.39 (quint, 1 H), 4.28 (s, 2 H), 4.2 (dd, 1 H), 4.15 (t, 2 H), 3.77 (s, 3 H), 3.59 (t, 4 H), 3.5 (m, 44 H), 3.36 (t, 2 H), 3.28 (t, 2 H), 3.14 (quad, 2 H), 2.9 (s, 3 H), 2.49 (s, 3 H), 2.45/2.33 (2t, 4 H), 2.13 (quint, 2 H), 1.96 (oct, 1 H), 1.3 (d, 3 H), 0.87/0.83 (2d, 6 H). HRMS (ESI) $[M+H]^+$ found = 1 687.7071 (δ = 0).

Preparation of L107A-P2: 3-[4-[3-[2-[[6-(1,3-benzothiazol-2-ylamino)-5-methyl-pyridazin-3-yl]-methyl-amino]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]propanoylamino]-3-methyl-

butanoyl]amino]propanoyl]amino]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate

[892] The desired product was obtained using **Method A.** (2S)-2-amino-N-[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide and (2,5-dioxopyrrolidin-1-yl) 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate was used in **Step 1**, and **P2** was used as the appropriate payload in **Step 3**. ^{1}H NMR (400 MHz, dmso-d6) δ ppm 10.2 (s), 8.23 (d), 7.99 (t), 7.89 (large, 1 H), 7.85 (d), 7.76 (d, 2 H), 7.67 (s, 1 H), 7.56 (d, 1 H), 7.5 (d, 2 H), 7.4 (t, 1 H), 7.38 (m, 2 H), 7.24 (t, 1 H), 7.2 (t, 1 H), 6.99 (s, 2 H), 4.55 (s, 2 H), 4.41 (s, 2 H), 4.39 (m, 1 H), 4.2 (m, 1 H), 4.19 (m, 2 H), 3.77 (s, 3 H), 3.65-3.33 (m, 24 H), 3.59 (m, 2 H), 3.29 (t, 2 H), 3.14 (quad, 2 H), 3.05 (s, 6 H), 2.46 (s, 3 H), 2.39 (m, 2 H), 2.33 (t, 2 H), 2.15 (m, 2 H), 1.96 (m, 1 H), 1.32 (d, 3 H), 0.89/0.84 (2d, 6 H). ^{13}C NMR (400 MHz, dmso-d6) δ ppm 134.7, 134.2, 126, 122.9, 122.2, 119.8, 119.7, 119.4, 118.3, 115.5, 70.4/69.2/67.2, 69, 66.8, 58.1, 53.9, 49.9, 49.9/40.4, 39, 36.4, 35.4, 34.6, 34.6, 31.1, 31.1, 23.6, 20.1, 18.2, 18.1. HRMS (ESI) $[M+H]^+$ found = 1 657.7339 (δ = 0.4).

Preparation of L9C-P59: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-hydroxypropyl)amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[893] Using **Method C** and **P59** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1288.4656 (δ = -4.5 ppm).

Preparation of L9C-P3: 2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-5-[3-[4-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-methyl-amino]prop-1-ynyl]-2-fluoro-phenoxy]propyl]thiazole-4-carboxylic acid

[894] Using **Method C** and **P3** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1244.4473 (δ = 1.7 ppm).

Preparation of L9C-P60: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[[(3S)-3,4-dihydroxybutyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-

5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[895] Using **Method C** and **P60** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1394.6300 (δ = -3.6 ppm).

Preparation of L9A-P61: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[896] Using **Method A** and **P61** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1316.6347 (δ = -3.8 ppm).

Preparation of L9A-P62: 2-[[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(3-hydroxypropyl)-methyl-ammonium; 2,2,2-trifluoroacetate

[897] Using **Method B** and **P62** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1362.6748 (δ = -5.0 ppm).

Preparation of L9A-P63: 3-[1-[[(5SR,7RS)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(5-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[898] Using **Method A** and **P63** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1362.6585 (δ = -2.3 ppm).

Preparation of L9A-P64: 3-[1-[[(5RS,7SR)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[4-methyl-3-[(6-methyl-1,3-benzothiazol-2-

yl)amino]-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[899] Using **Method A** and **P64** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1358.6809 (δ = -4.3 ppm).

Preparation of L9A-P65: 3-[1-[[(5SR,7RS)-3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-6-[3-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[900] Using **Method A** and **P65** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1362.6557 (δ = -4.3 ppm).

Preparation of L9A-P66: 3-[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-dimethyl-ammonium; 2,2,2-trifluoroacetate

[901] Using **Method A** and **P66** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1316.6703 (δ = -4.4 ppm).

Preparation of L9A-P67: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[2-[4-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-4-methyl-piperazin-4-ium-1-yl]ethoxy]-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[902] Using **Method A** and **P67** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1345.6582 (δ = -6.0 ppm).

Preparation of L9A-P68: 3-[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-

pentanoyl]amino]phenyl]methyl]-(3-hydroxypropyl)-methyl-ammonium; 2,2,2-trifluoroacetate

[903] Using **Method A** and **P68** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1360.6941 (δ = -6.0 ppm).

Preparation of L9C-P69: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[3-[[(3S)-3,4-dihydroxybutyl]-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[904] Using **Method C** and **P69** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1420.6913 (δ = 3.0 ppm).

Preparation of L9A-P48: 2-[[(5SR,7RS)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-(carboxymethyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[905] Using **Method A** and **P48** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1362.6399 (δ = -3.9 ppm).

Preparation of L9A-P70: 2-[[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-(2-carboxyethyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[906] Using **Method A** and **P70** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1376.6548 (δ = -4.4 ppm).

Preparation of L9C-P71: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[2-carboxyethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[907] Using **Method C** and **P71** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1406.6280 (δ = -5.0 ppm).

Preparation of L9C-P72: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(4-hydroxybutyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[908] Using **Method C** and **P72** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ calculated = 1404.6927

Preparation of L9A-P49: 3-[(5SR,7RS)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(2-hydroxyethyl)-methyl-ammonium; 2,2,2-trifluoroacetate

[909] Using **Method A** and **P49** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1346.6794 (δ = -5.4 ppm).

Preparation of L9C-P51: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[3-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl-(3-methoxypropyl)amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[910] Using **Method C** and **P51** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1404.6889 (δ = -2.3 ppm).

Preparation of L9A-P50: 3-[(5SR,7RS)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-(3-methoxypropyl)-methyl-ammonium; 2,2,2-trifluoroacetate

[911] Using **Method A** and **P50** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1374.7111 (δ = -5.0 ppm).

Preparation of L9A-P52: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5RS,7SR)-3-[3-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]azepan-1-ium-1-yl]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid; 2,2,2-trifluoroacetate

[912] Using **Method A** and **P52** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1370.7281 (δ = 3.7 ppm).

Preparation of L9C-P53: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[3-[carboxymethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[913] Using **Method C** and **P53** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1390.6301 (δ = -7.2 ppm).

Preparation of L9A-P55: 3-[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-(carboxymethyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[914] Using **Method A** and **P55** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1360.6561 (δ = -7.2 ppm).

Preparation of L9C-P54: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[3-[2-carboxyethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]propyl]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[915] Using **Method C** and **P54** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1404.6464 (δ = -6.7 ppm).

Preparation of L9C-P47: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[(5SR,7RS)-3-[2-[carboxymethyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methoxycarbonyl]amino]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxylic acid

[916] Using **Method C** and **P47** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M+H]+ found = 1392.6186 (δ = -0.6 ppm).

Preparation of L9A-P56: 3-[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-(2-carboxyethyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[917] Using **Method A** and **P56** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1374.6740 (δ = -5.5 ppm).

Preparation of L9A-P58: 3-[(5RS,7SR)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]propyl-(3-carboxypropyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[918] Using **Method A** and **P58** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1388.6891 (δ = -5.9 ppm).

Preparation of L9A-P57: 2-[[(5SR,7RS)-3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-carboxy-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl-(3-carboxypropyl)-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]-methyl-ammonium; 2,2,2-trifluoroacetate

[919] Using **Method A** and **P57** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1390.6692 (δ = -5.3 ppm).

Preparation of L9A-P73: (2S)-N-[4-[[1-[2-[[3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-(hydroxymethyl)-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl]pyrrolidin-1-ium-1-yl]methyl]phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide; 2,2,2-trifluoroacetate

[920] Using **Method B** and **P73** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1330.6754 (δ=-12.3 ppm).

Preparation of L9A-P74: (2S)-N-[4-[[1-[2-[[3-[[4-[6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-2-(pyrrolidine-1-carbonyl)-3-pyridyl]-5-methyl-pyrazol-1-yl]methyl]-5,7-dimethyl-1-adamantyl]oxy]ethyl]pyrrolidin-1-ium-1-yl]methyl]phenyl]-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide; 2,2,2-trifluoroacetate

[921] Using **Method B** and **P74** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1397.7343 (δ=0.2 ppm).

Preparation of L9A-P75: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]-N-isopropyl-pyridine-2-carboxamide; 2,2,2-trifluoroacetate

[922] Using **Method B** and **P75** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1385.7328 (δ = -0.8 ppm).

Preparation of L9A-P76: 6-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-3-[1-[[3-[2-[1-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl]pyrrolidin-1-ium-1-yl]ethoxy]-5,7-dimethyl-1-adamantyl]methyl]-5-methyl-pyrazol-4-yl]pyridine-2-carboxamide; 2,2,2-trifluoroacetate

[923] Using **Method B** and **P76** as the appropriate payload, the desired product was obtained. **HRMS (ESI)** [M]+ found = 1343.6874 (δ=0.3 ppm).

Preparation of L112A-P1: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]propyl]phenyl]methyl]-dimethyl-ammonium; 2,2,2-trifluoroacetate

Step A*: *9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-1-[[4-(hydroxymethyl)-3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]propyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate

[924] The title compound was synthesized according to the experimental procedure described in **WO2020/236817A2, Preparation of L26-P1** using 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol as the starting material. **^{1}H NMR** (400 MHz, dmso-d6): δ 9.88 (s, 1H), 8.07 (d, 1H), 7.89 (d, 2H), 7.72-7.76 (m, 2H), 7.37-7.45 (m, 5H), 7.30-7.34 (m, 2H), 7.25 (d, 1H), 5.95 (t, 1H), 5.38 (s, 2H), 4.95 (t, 1H), 4.45 (d, 2H), 4.38-4.42 (m, 1H), 4.20-4.32 (m, 3H), 3.90-3.94 (m,1H), 3.45-3.55 (m, 94H), 3.38-3.43 (m, 4H), 3.23 (s, 3H), 2.89-3.03 (m, 2H), 2.56-2.62 (m, 2H), 1.94-2.04 (m, 1H), 1.54-1.76 (m, 4H), 1.29-1.49 (m, 2H), 0.84-0.89 (m, 6H). **UPLC-MS:** MS (ESI)m/z [M/2 + Na]+ found = 888.

Step B*: *9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-1-[[4-(chloromethyl)-3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate

[925] To the product from *Step A* (50 mg, 0.0288 mmol) in THF (0.7 ml) was added equivalent amount of thionyl chloride (0.35 M solution in THF) in every 10 min until no starting material was observed. The mixture was concentrated, and the crude product was used in the next step without further purification.

***Step C**: 9H-fluoren-9-ylmethyl N-[(1S)-1-[[(1S)-1-[[4-(iodomethyl)-3-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate*

[926] After stirring the mixture of the product from *Step B* (44 mg, 0.025mmol) and sodium iodide (2 eq) in butan-2-one (30 mL/mmol) for 5 h, the reaction was concentrated and the crude product was used in the next step without further purification.

***Step D**: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]propyl]phenyl]methyl]-dimethyl-ammonium*

[927] After stirring the mixture of payload **P1** (15 mg, 0.023 mmol), the product from *Step C* (46.18 mg, 0.025 mmol) and DIPEA (5 eq) in DMF (0.7 mL) for 44 h, the crude product was concentrated and used in the next step without further purification.

***Step E**: [4-[[(2S)-2-[[(2S)-2-amino-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]propyl]phenyl]methyl-[3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl]-dimethyl-ammonium*

[928] After stirring the mixture of the product from *Step D* (54 mg, 0.023 mmol) and *N*-ethylethanamine (10 eq) in DMF (0.7 mL) for 1 h, the crude product was purified by preparative HPLC to give the desired compound (22 mg). **UPLC-MS:** MS (ESI) m/z [(M+2)/2] found = 1075.

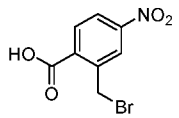
***Step F**: 3-[4-[3-[2-[3-(1,3-benzothiazol-2-ylamino)-4-methyl-6,7-dihydro-5H-pyrido[2,3-c]pyridazin-8-yl]-4-carboxy-thiazol-5-yl]propoxy]-3-fluoro-phenyl]prop-2-ynyl-[[4-[[(2S)-2-[[(2S)-2-[3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propyl]phenyl]methyl]-dimethyl-ammonium;2,2,2-trifluoroacetate*

[929] After stirring the mixture of the product from *Step E* (22 mg, 0.0097 mmol), DIEA (2 eq) and (2,5-dioxopyrrolidin-1-yl) 3-[2-(2,5-dioxopyrrol-1-yl)ethoxy]propanoate (1.1 eq) in DMF (0.3 mL) for 15 h, the crude product was purified using preparative HPLC and using the TFA method to give **L112A-P1** (5.5 mg). **HR-ESI+:** m/z [M-CF3COO]+ found = 2344.

Example 4. Synthesis and Characterization of Additional Linkers, Linker-Payloads, and Precursors thereof.

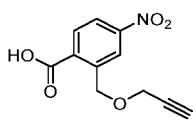
[930] Exemplary linkers, linker-payloads, and precursors thereof were synthesized using exemplary methods described in this example.

Synthesis of 2-(bromomethyl)-4-nitrobenzoic acid



[931] To a stirred solution of 2-methyl-4-nitrobenzoic acid (300 g, 1.5371 mol) in CCl_4 (3000 mL) was added NBS (300.93 g, 1.6908 mol) and AIBN (37.86 g, 0.2305 mol) at RT. The reaction mixture was stirred at 80°C for 16 h. Reaction mixture was monitored by TLC analysis. The reaction mixture was diluted with sat. $NaHCO_3$ solution (2 L) and extracted with ethyl acetate (2 x 2 L). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude compound was purified by column chromatography on silica gel using 2-3% of ethyl acetate in petroleum-ether as an eluent and 2-(bromomethyl)-4-nitrobenzoic acid was obtained. ^1H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, J=2.0 Hz, 1H), 8.20 (q, J=8.8, 2.4 Hz, 1H), 8.12 (d, J=8.8 Hz, 1H), 4.97 (s, 2H), 4.00 (s, 3H).

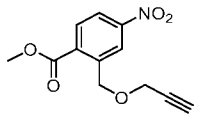
Synthesis of 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid



[932] To the mixture of 2-(bromomethyl)-4-nitrobenzoic acid (250 g, 0.9122 mol) in MeCN (5000 mL) was added prop-2-yn-1-ol (255.68 g, 265.50 mL, 4.5609 mol, d=0.963 g/mL) and

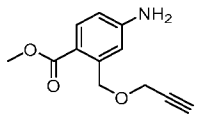
Cs_2CO_3 (743.03 g, 2.2805 mol) at RT. The resulting mixture was heated to 80°C for 16 h. The reaction mixture was filtered through celite pad washed with ethyl acetate (2 L). The filtrate was concentrated under reduced pressure. The obtained crude compound was added sat. $NaHCO_3$ solution (1 L) and the aqu layer was acidified to pH 2 by using 2N HCl (2 L). After filtration vacuum drying 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid was obtained. ^{1}H NMR (400 MHz, DMSO): δ 13.61 (brs, 1H), 8.37 (d, J=2.4 Hz, 1H), 8.23 (dd, J=2.4, 8.4 Hz, 1H), 8.10 (d, J=8.8 Hz, 1H), 4.95 (s, 2H), 4.37 (d, J=2.4 Hz, 2H), 3.52 (t, J=2.4 Hz, 1H)

Synthesis of methyl 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate



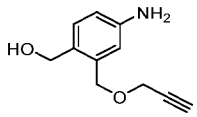
[933] To a stirred solution of 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid (130 g, 0.5527 mol) in MeOH (1300 mL) was added $SOCl_2$ (526.08 g, 320.78 mL, 4.4219 mol, d=1.64 g/mL) slowly at 0°C. The reaction stirred at 70°C for 4 h. The reaction solvent was evaporated under reduced pressure. The obtained residue was dissolved in ethyl acetate (1000 mL) and washed with sat. $NaHCO_3$ (600 mL), water (500 mL) and brine solution (500 mL). The separated organic layer was dried over sodium sulphate, filtered and evaporated under reduced pressure to yield methyl 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (t, J=0.8 Hz, 1H), 8.18 – 8.09 (m, 2H), 5.03 (s, 2H), 4.35 (d, J=2.4 Hz, 2H), 3.96 (s, 3H), 2.49 (t, J=2.4 Hz, 1H).

Synthesis of methyl 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate



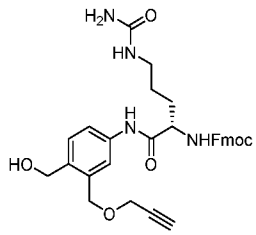
[934] To a solution of methyl 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate (110 g, 0.4414 mol) in a mixture of EtOH (1100 mL) and H_2O (550 mL) was added Fe powder (197.21 g, 3.5310 mol) and NH_4Cl (188.88 g, 3.5310 mol) at RT. The resulting mixture was heated at 80°C for 16 h. The reaction mixture was cooled to RT and filtered through celite® and washed with ethyl acetate (2 L). The filtrate was concentrated under reduced pressure up to half of the volume. To the residue, ethyl acetate (1.5 L) was added and separated the two layers and the aqueous layer was extracted with ethyl acetate (2 L). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain crude product. Purification by SiO_2 column chromatography (15-20% of ethyl acetate in petroleum-ether) yielded methyl 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, J=8.8 Hz, 1H), 6.78 (t, J=1.6 Hz, 1H), 6.48 (q, J=8.4, 2.4 Hz, 1H), 4.79 (s, 2H), 4.25 (d, J=2.4 Hz, 2H), 3.70 (d, J=4.0 Hz, 3H), 3.42 (t, J=2.4 Hz, 1H).

Synthesis of (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol



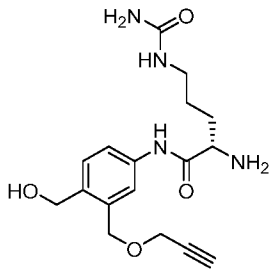
[935] To a stirred solution of THF (1000 mL) was added LiAlH4 (1 M in THF) (21.23 g, 798.2 mmol, 798.2 mL) slowly at 0°C. A solution of methyl 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate (70 g, 319.3 mmol) in THF (800 mL) was added slowly at 0°C. The reaction was stirred at RT for 4 h. The reaction mixture was cooled to 0°C, then was added water (22 mL) very slowly and followed by the addition of 20% NaOH (22 mL) and water (66 mL). The reaction mixture was stirred at 0°C for 30 min. Anhydrous sodium sulfate was added to absorb excess of water. The mixture was filtered through celite®. The filter cake was washed with ethyl acetate (1000 mL) and 10% MeOH/DCM (500 mL). The filtrate was concentrated under reduced pressure. The resulting crude compound was purified by SiO_2 column chromatography (35-40% of ethyl acetate in petroleum-ether as an eluent) to give yield (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.98 (d, J=8.0 Hz, 1H), 6.56 (d, J=2.4 Hz, 1H), 6.43 (dd, J=2.4, 8.0 Hz, 1H), 4.98 (s, 2H), 4.64 (t, J=5.2 Hz, 1H), 4.47 (s, 2H), 4.34 (d, J=5.6 Hz, 2H), 4.15 (d, J=2.4 Hz, 2H), 3.46 (t, J=2.4 Hz, 1H).

Synthesis of (9H-fluoren-9-yl)methyl (S)-(1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)carbamate



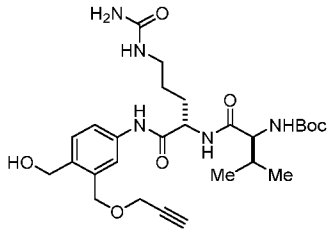
[936] To a solution of (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol (1.92 g, 10.04 mmol, 1.0 equiv.), (9H-fluoren-9-yl)methyl (S)-(1-amino-1-oxo-5-ureidopentan-2-yl)carbamate (3.99 g, 10.04 mmol, 1.0 equiv.), and (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (4.20 g, 11.04 mmol, 1.1 equiv.) in DMF (10 mL) was added N,N-diisopropylethylamine (2.62 mL, 15.06 mmol, 1.5 equiv.). After stirring at ambient temperature for 1 h, the mixture was poured into water (200 mL). The resulting solids were filtered, rinsed with water, and dried under vacuum, and (9H-fluoren-9-yl)methyl (S)-(1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)carbamate was obtained . LCMS: MH+=571.5; Rt=0.93 min (2 min acidic method).

Synthesis of (S)-2-amino-N-(4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)-5-ureidopentanamide



[937] To (9H-fluoren-9-yl)methyl (S)-(1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)carbamate (6.08 g, 10.65 mmol, 1.0 equiv.) was added dimethylamine (2 M in THF, 21.31 mL, 42.62 mmol, 4 equiv.). After stirring at ambient temperature for 1.5 hours, the supernatant solution was decanted from the gum-like residue that had formed. The residue was triturated with ether (3 x 50 mL) and the resulting solids were filtered, washed with ether, and dried under vacuum. (S)-2-amino-N-(4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)-5-ureidopentanamide was obtained. LCMS: MH+ 349.3; Rt=0.42 min (2 min acidic method).

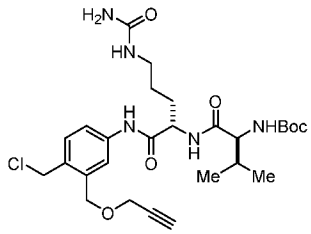
Synthesis of tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



[938] To a solution of (S)-2-amino-N-(4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)-5-ureidopentanamide (3.50 g, 10.04 mmol, 1.0 equiv.), (tert-butoxycarbonyl)-L-valine (2.62 g, 12.05 mmol, 1.2 equiv.), and (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (4.58 g, 12.05 mmol, 1.2 equiv.) in DMF (10 mL) was added N,N-diisopropylethylamine (3.50 mL, 20.08 mmol, 2.0 equiv.). After stirring at ambient temperature for 2 h, the mixture was poured into water (200 mL) and the resulting suspension was extracted with EtOAc (3x100 mL). The combined organic layers were dried over sodium sulfate and concentrated under vacuum. After purification by ISCO SiO_2 chromatography (0-20% methanol / dichloromethane), tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate was obtained. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.00 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.55 (dq, J = 4.9, 2.2 Hz, 2H, aryl), 7.32 (d, J = 8.9 Hz, 1H, aryl), 6.76 (d, J = 8.9 Hz, 1H), 5.95 (t, J = 5.8 Hz, 1H), 5.38 (s, 2H), 5.01 (t, J = 5.5 Hz, 1H), 4.54 (s, 2H), 4.45 (dd, J = 25.2, 5.3 Hz, 3H), 4.20 (d, J = 2.4 Hz, 2H), 3.83 (dd, J

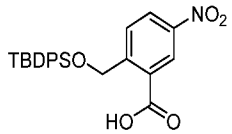
= 8.9, 6.7 Hz, 1H), 3.49 (t, J = 2.4 Hz, 1H), 2.97 (dh, J = 26.0, 6.5 Hz, 2H), 1.96 (h, J = 6.6 Hz, 1H), 1.74 - 1.50 (m, 2H), 1.39 (m, 11H), 0.84 (dd, J = 16.2, 6.7 Hz, 6H). LCMS: M+Na 570.5; Rt=0.79 min (2 min acidic method).

Synthesis of tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



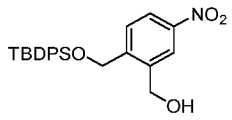
[939] To a solution of tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (2.00 grams, 3.65 mmol, 1.0 equiv.) in acetonitrile (13.3 mL) at 0 °C was added thionyl chloride (0.53 mL, 7.30 mmol, 2.0 equiv.). After stirring in the ice bath for one hour the solution was diluted with water (40 mL) and the resulting white precipitate was collected by filtration, air drying and drying under high vacuum to yield tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate. LCMS: M+Na 588.5; Rt=2.17 min (5 min acidic method).

Synthesis of 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzoic acid



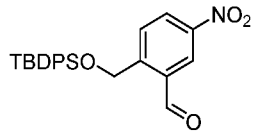
[940] To a solution of 6-nitroisobenzofuran-1(3H)-one (90 g, 502.43 mmol, 1.00 equiv.) in MeOH (1000 mL) and KOH (28.19 g, 502.43 mmol, 1.00 equiv.) in H_2O (150 mL) was added. The brown mixture was stirred at 25°C for 1.5 h. The brown mixture was concentrated under reduced pressure to give a residue and dissolved in DCM (2000 mL). To the mixture was added tert-Butyldiphenylchlorosilane (296.91 g, 1.08 mol, 277.49 mL, 2.15 equiv.) and imidazole (171.03 g, 2.51 mol, 5.00 equiv.) and stirred at 25°C for 12 h. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate=1/0, 1/1) and 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzoic acid was obtained as a white solid. ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 1.13 (s, 9 H) 5.26 (s, 2 H) 7.34 - 7.48 (m, 6 H) 7.68 (br d, J=8 Hz, 4 H) 8.24 (br d, J=8 Hz, 1 H) 8.46 (br d, J=8 Hz, 1 H) 8.74 (s, 1 H).

Synthesis of (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)methanol



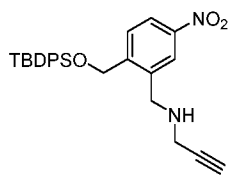
[941] To a mixture of 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzoic acid (41 g, 94.14 mmol, 1 equiv.) in THF (205 mL) was added BH_3. THF (1 M, 470.68 mL, 5 equiv.). The yellow mixture was stirred at 60°C for 2h. The mixture was added MeOH (400mL), and concentrated under reduced pressure to give a residue. Then addition of H_2O (200mL) and DCM(300mL), extracted with DCM (3 ×200 mL), washed with brine (300mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate=1/0, 1/1). (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)methanol was obtained as a white solid. ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 1.10 (s, 9 H) 4.58 (s, 2 H) 4.89 (s, 2 H) 7.32 - 7.51 (m, 6 H) 7.68 (dd, J=8, 1.38 Hz, 4 H) 7.76 (d, J=8 Hz, 1 H) 8.15 (dd, J=8 2.26 Hz, 1 H) 8.30 (d, J=2 Hz, 1 H).

Synthesis of 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzaldehyde



[942] To a solution of (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)methanol (34 g, 80.65 mmol, 1 equiv.) in DCM (450 mL) was added MnO_2 (56.09 g, 645.22 mmol, 8 equiv.). The black mixture was stirred at 25°C for 36 h. The mixture was added MeOH (400mL), and concentrated under reduced pressure to give a residue. Then addition of H_2O (200mL) and DCM (300mL), extracted with DCM (3 ×200 mL), washed with brine (300mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (CH_2Cl_2=100%). 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzaldehyde was obtained as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.14 (s, 9 H) 5.26 (s, 2 H) 7.34 - 7.53 (m, 6 H) 7.60 - 7.73 (m, 4 H) 8.13 (d, J=8Hz, 1 H) 8.48 (dd, J=8, 2.51 Hz, 1 H) 8.67 (d, J=2 Hz, 1 H) 10.16 (s, 1 H).

Synthesis of N-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)prop-2-yn-1-amine

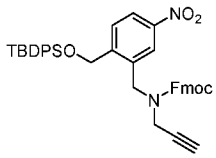


[943] To a solution of 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzaldehyde (12.6 g, 30.03 mmol, 1 equiv.) in DCM (130 mL) was added prop-2-yn-1-amine (4.14 g, 75.08 mmol, 4.81 mL, 2.5 equiv.) and $MgSO_4$ (36.15 g, 300.33 mmol, 10 equiv.) then the suspension

mixture was stirred at 25°C for 24hr. Taking a little reaction solution and treating with $NaBH_4$, the TLC showed one new spot was formed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. (E)-N-[[2-[[tert-butyl(diphenyl)silyl]oxymethyl]-5-nitro-phenyl]methyl]prop-2-yn-1-imine was obtained as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.11 (s, 9 H) 2.48 (t, J=2.38 Hz, 1 H) 4.52 (t, J=2.13 Hz, 2 H) 5.09 (s, 2 H) 7.35 - 7.49 (m, 6 H) 7.63 - 7.72 (m, 4 H) 7.79 (d, J=8.53 Hz, 1 H) 8.25 (dd, J=8.53, 2.51 Hz, 1 H) 8.68 (d, J=2.26 Hz, 1 H) 8.84 (t, J=1.88 Hz, 1 H).

[944] (E)-N-[[2-[[tert-butyl(diphenyl)silyl]oxymethyl]-5-nitro-phenyl]methyl]prop-2-yn-1-imine (12 g, 26.28 mmol, 1 equiv.) was dissolved in MeOH (100 mL) and THF (50 mL) , then $NaBH_4$ (1.49 g, 39.42 mmol, 1.5 equiv.) was added and the yellow mixture was stirred at -20°C for 2hr. LCMS showed desired compound was detected. The reaction mixture was quenched by addition MeOH (200 mL) at -20 °C, and then concentrated under reduced pressure to give a residue. The residue was dissolved with EtOAc (500 mL) washed with brine (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (Eluent of 0-10% Ethyl acetate/Petroleum ether gradient). N-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)prop-2-yn-1-amine was obtained as a pale yellow oil. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.12 (s, 9 H) 2.13 (t, J=2.38 Hz, 1 H) 3.33 (d, J=2.51 Hz, 2 H) 3.80 (s, 2 H) 4.93 (s, 2 H) 7.36 - 7.49 (m, 6 H) 7.69 (dd, J=7.91, 1.38 Hz, 4 H) 7.77 (d, J=8.53 Hz, 1 H) 8.16 (dd, J=8.41, 2.38 Hz, 1 H) 8.24 (d, J=2.26 Hz, 1 H).

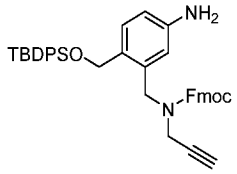
Synthesis of (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(prop-2-yn-1-yl)carbamate



[945] To a solution of N-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)prop-2-yn-1-amine (9 g, 19.62 mmol, 1 equiv.) and Fmoc-OSu (7.28 g, 21.59 mmol, 1.1 equiv.) in dioxane (90 mL) was added sat. $NaHCO_3$ (90 mL) and the white suspension was stirred at 20°C for 12 h. The reaction mixture was diluted with H_2O (150 mL) and extracted with EtOAc (150 mL x 2). The combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (Eluent of 0-30% Ethyl acetate/Petroleum ether). (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(prop-2-yn-1-yl)carbamate (7.7 g, 11.08 mmol, 56.48% yield, 98% purity) was

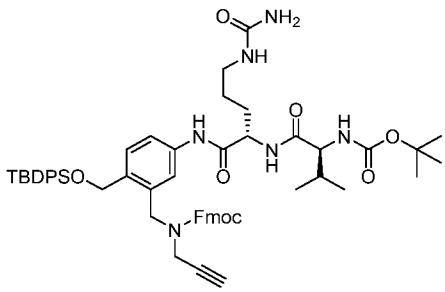
obtained as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 1.12 (s, 9 H) 2.17 (br d, J=14.31 Hz, 1 H) 3.87 - 4.97 (m, 9 H) 6.98 - 8.28 (m, 21 H).

Synthesis of (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate



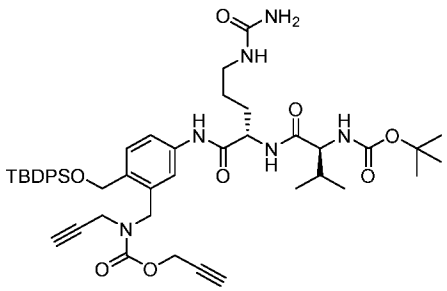
[946] To an ice bath cooled solution of (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(prop-2-yn-1-yl)carbamate (5.0 g, 7.34 mmol, 1.0 equiv.) in 10% AcOH/CH_2Cl_2 (100 mL) was added Zn (7.20 g, 110 mmol, 15 equiv.). The ice bath was removed, and the resulting mixture stirred for 2 hours at which time it was filtered through a pad of celite®. The volatiles were removed in vacuo and the residue was dissolved in EtOAc, was washed with $NaHCO_3$(sat.), NaCl(sat.), dried over $MgSO_4$, filtered, concentrated and after ISCO SiO_2 chromatography (0-75% EtOAc/Heptane) (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=651.6; Rt=3.77 min (5 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate



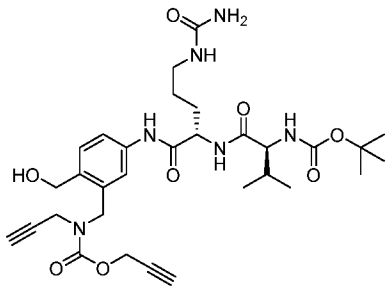
[947] To (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate (2.99 g, 4.59 mmol, 1.0 equiv.) and (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (1.72 g, 4.59 mmol, 1.0 equiv.) in CH_2Cl_2 (40 mL) was added ethyl 2-ethoxyquinoline-1(2H)-carboxylate (2.27 g, 9.18 mmol, 2.0 equiv.). After stirring for 10 min, MeOH (1 mL) was added and the solution became homogeneous. The reaction was stirred for 16 h, the volatiles were removed in vacuo and after purification by ISCO SiO_2 chromatography (0-15% MeOH/CH2Cl2) (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=1008.8; Rt=3.77 min (5 min acidic method).

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate



[948] To (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate (1.60 g, 1.588 mmol, 1.0 equiv.) was added 2M dimethylamine in MeOH (30 mL, 60 mmol, 37 equiv.) and THF (10 mL). After standing for 3 h, the volatiles were removed in vacuo and the residue was triturated with Et_2O to remove FMOC deprotection byproducts. To the resulting solid was added CH_2Cl_2 (16 mL) and pyridine (4 mL) and to the heterogeneous solution was added propargyl chloroformate (155 uL, 1.588 mmol, 1.0 equiv.). After stirring for 30 minutes additional propargyl chloroformate (155 uL, 1.588 mmol, 1.0 equiv.) was added. After stirring for an additional 20 min, MeOH (1 mL) was added to quench remaining chloroformate and the volatiles were removed in vacuo. Upon purification by ISCO SiO_2 chromatography (0-15% MeOH/CH_2Cl_2) prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=867.8; Rt=3.40 min (5 min acidic method).

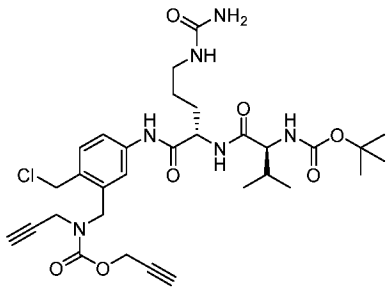
Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(prop-2-yn-1-yl)carbamate



[949] To a solution of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(prop-2-yn-1-yl)carbamate (984 mg, 1.135 mmol, 1.0 equiv.) in THF (7.5 mL) was added 1.0 M TBAF in THF (2.27 mL, 2.27 mmol, 2.0 equiv.).

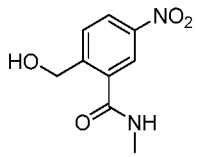
After standing for 6 h, the volatiles were removed in vacuo, the residue was purified by ISCO SiO_2 chromatography (0-40% MeOH/CH2Cl2) and prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=629.6; Rt=1.74min (5 min acidic method).

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(prop-2-yn-1-yl)carbamate



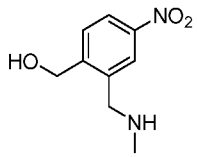
[950] To prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(prop-2-yn-1-yl)carbamate (205 mg, 0.326 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) was added pyridine (158 uL, 1.96 mmol, 5 equiv.). The heterogeneous mixture was cooled in a 0 °C ice bath and thionyl chloride (71 uL, 0.98 mmol, 3 equiv.). After stirring in the ice bath for 3 hours the reaction was directly purified by ISCO SiO_2 chromatography (0-30% MeOH/CH_2Cl_2) and prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=647.6; Rt=2.54 min (5 min acidic method).

Synthesis of 2-(hydroxymethyl)-N-methyl-5-nitrobenzamide



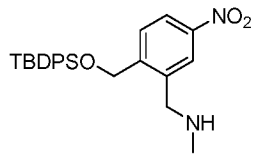
[951] To a stirred suspension of 6-nitroisobenzofuran-1(3H)-one (500 g, 2.79 mol) in MeOH (1500 mL) was added $MeNH_2$ (3.00 kg, 29.94 mol, 600 mL, 31.0% purity) at 25 °C and stirred for 1 h. The solid was filtered and washed with water twice (600 mL) and dried under high vacuum to get a residue. The product 2-(hydroxymethyl)-N-methyl-5-nitrobenzamide was obtained as white solid. LCMS: Rt = 0.537 min, MS m/z = 193.2. 1H NMR: 400 MHz DMSO δ 8.57 (br d, J = 4.4 Hz, 1H), 8.31 (dd, J = 2.4, 8.6 Hz, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 5.54 (t, J = 5.6 Hz, 1H), 4.72 (d, J = 5.5 Hz, 2H), 2.78 (d, J = 4.4 Hz, 3H).

Synthesis of (2-((methylamino)methyl)-4-nitrophenyl)methanol



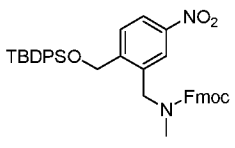
[952] To a solution of 2-(hydroxymethyl)-N-methyl-5-nitrobenzamide (560 g, 2.66 mol) in THF (5000 mL) was cooled to 0 °C, then added BH_3-Me_2S (506 g, 6.66 mol) (2.0 M in THF) dropwise for 60 min and heated to 70 °C for 5 h. LCMS showed the starting material was consumed. After completion, 4M HCl (1200 mL) in Methanol was added to reaction mixture at 0 °C and heated at 65 °C for 8 h. The reaction mixture was cooled to 0 °C, the solid was filtered and concentrated under reduce pressure. The product (2-((methylamino)methyl)-4-nitrophenyl)methanol (520 g) was obtained as a white solid. LCMS: Rt = 0.742 min, MS m/z = 197.1 [M+H]+. ^{1}H NMR: 400 MHz DMSO δ 9.25 (br s, 2H), 8.37 (d, J = 2.4 Hz, 1H), 8.14 (dd, J = 2.4, 8.5 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 5.72 (br s, 1H), 4.65 (s, 2H), 4.15 (br s, 2H), 2.55 - 2.45 (m, 3H)

Synthesis of 1-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)-N-methylmethanamine



[953] To a solution of (2-((methylamino)methyl)-4-nitrophenyl)methanol (520 g, 2.65 mol) and imidazole (721 g, 10.6 mol) in DCM (2600 mL) was cooled to 0°C was added TBDPS-CL (1.09 kg, 3.98 mol, 1.02 L) drop wise and stirred for 2 h. The mixture was poured in ice cold water (1000 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under vacuum to give crude product. The crude product was purified by chromatography on a silica gel eluted with Ethyl acetate: Petroleum ether (from 10/1 to 1) to give a residue. The product 1-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)-N-methylmethanamine was obtained as yellow liquid. LCMS: product: Rt = 0.910 min, MS m/z = 435.2 [M+H]+. ^{1}H NMR: 400 MHz CDCl3 δ 8.23 (d, J=2.4 Hz, 1H), 8.15 (dd, J=2.4, 8.4 Hz, 1H), 7.76 (d, J=8.4 Hz, 1H), 7.71 - 7.66 (m, 4H), 7.50 - 7.37 (m, 6H), 4.88 (s, 2H), 3.65 (s, 2H), 2.39 (s, 3H), 1.12 (s, 9H)

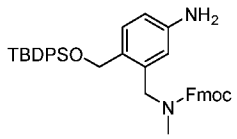
Synthesis of (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(methyl)carbamate



[954] To a solution of 1-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrophenyl)-N-methylmethanamine (400 g, 920.3 mmol) in THF (4000 mL) was added Fmoc-OSu (341.5 g,

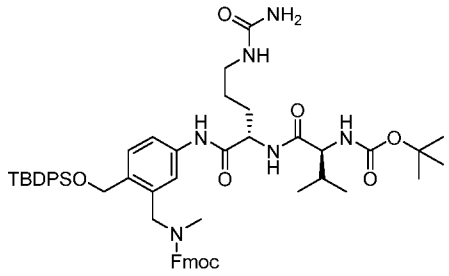
1.01 mol) and Et_3N (186.2 g, 1.84 mol, 256.2 mL), the mixture was stirred at 25 °C for 1 h. The mixture was poured into water (1600 mL) and extracted with ethyl acetate (1000 mL x 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under vacuum to give crude product. The crude product was purified by chromatography on a silica gel eluted with petroleum ether: ethyl acetate (from 1/0 to 1/1) to give (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(methyl)carbamate as white solid. LCMS: Rt = 0.931 min, MS m/z = 657.2 [M+H]+. ^{1}H NMR: EW16000-26-P1A, 400 MHz CDCl3 δ 8.21 - 7.96 (m, 1H), 7.87 - 7.68 (m, 3H), 7.68 - 7.62 (m, 4H), 7.62 - 7.47 (m, 2H), 7.47 - 7.28 (m, 9H), 7.26 - 7.05 (m, 2H), 4.81 (br s, 1H), 4.62 - 4.37 (m, 4H), 4.31 - 4.19 (m, 1H), 4.08 - 3.95 (m, 1H), 2.87 (br d, J = 5.2 Hz, 3H), 1.12 (s, 9H).

Synthesis of (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate



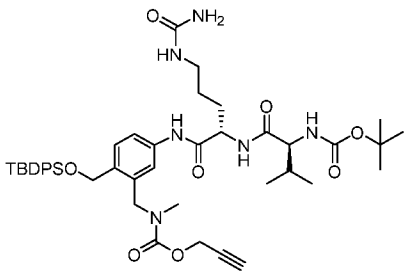
[955] A solution of (9H-fluoren-9-yl)methyl (2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)(methyl)carbamate (3.0 g, 4.57 mmol, 1.0 equiv.) in MeOH (90 mL) and EtOAc (30 mL) was degassed and purged to a balloon of N_2 via three way stopcock. After repeating degas/N_2 purge 2x, 10% Pd/C deGussa type (0.486 g, 0.457 mmol, 0.1 equiv.) was added. The resulting mixture was degassed and purged to a balloon of 2 H_2 via three-way stopcock. After repeating degas/H_2 purge 2x, the reaction stirred under the balloon pressure of H_2 for 4 hours. The reaction was degassed and purged to N_2, filtered through a pad of celite eluting further with MeOH. After removal of the volatiles in vacuo and pumping on high vacuum (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate was obtained. LCMS: MH+=627.7; Rt=1.59 min (2 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate



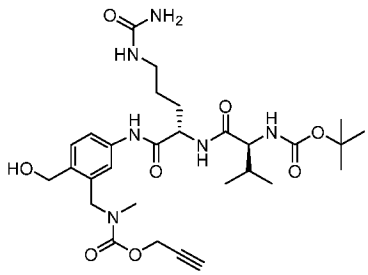
[956] To (9H-fluoren-9-yl)methyl (5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate (2.86 g, 4.56 mmol, 1.0 equiv.) and (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (1.71 g, 4.56 mmol, 1.0 equiv.) in 2:1 CH_2Cl_2/MeOH (60 mL) was added ethyl 2-ethoxyquinoline-1(2H)-carboxylate (2.256 g, 9.12 mmol, 2.0 equiv.). The homogeneous solution was stirred for 16 hours at which time additional (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (0.340 g, 0.2 equiv.) and ethyl 2-ethoxyquinoline-1(2H)-carboxylate (0.452 g, 0.4 equiv.) were add to drive the reaction to completion. After stirring for an adiditonal 5 hours the volatiles were removed in vacuo and after purification by ISCO SiO_2 chromatography (0-5% MeOH/CH_2Cl_2) (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate was obtained. LCMS: MH+=984.1; Rt=1.54 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate



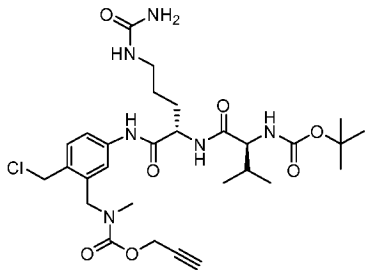
[957] To (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate (2.05 g, 2.085 mmol, 1.0 equiv.) in THF (10 mL) was added 2.0 M dimethyl amine in MeOH (10.42 mL, 20.85 mmol, 10 equiv.). After stirring for 16 hours the volatiles were removed in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL) and DIEA (0.533 mL, 4.17 mmol, 2 equiv.) and propargyl chloroformate (0.264 mL, 2.71 mmol, 1.3 equiv.) were added. After stirring at RT for 16 hours the reaction was diluted with CH_2Cl_2 (20 mL), was washed with $NaHCO_3$ (sat.), NaCl(sat.), dried over $MgSO_4$, filtered, concentrated and purified by ISCO SiO_2 chromatography (0-15% MeOH/CH_2Cl_2) to yield prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate . LCMS: MH+=843.8; Rt=1.35 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate



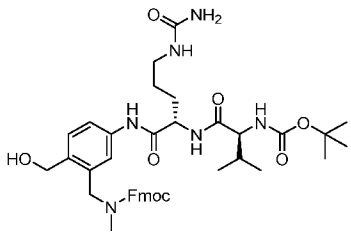
[958] To a 0 °C solution of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate (1.6 g, 1.90 mmol, 1.0 equiv.) in THF (10.0 mL) was added 1.0 M TBAF in THF (3.80 mL, 3.80 mmol, 2.0 equiv.). After warming to RT and stirring for 16 h the volatiles were removed in vacuo, the residue was dissolved in EtOAc, was washed with $NaHCO_3$(sat.), with NaCl(sat.), dried over $MgSO_4$, filtered, concentrated and the residue was purified by ISCO SiO_2 chromatography (0-30% MeOH/CH2Cl2) to yield prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate. LCMS: MH+=605.7; Rt=0.81 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(methyl)carbamate



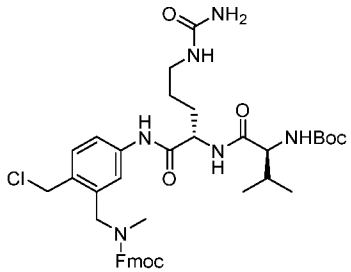
[959] To prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate (350 mg, 0.579 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) was added pyridine (0.278 mL, 3.47 mmol, 6 equiv.). The heterogeneous mixture was cooled in a 0 °C ice bath and thionyl chloride (0.126 mL, 1.73 mmol, 3 equiv.). After stirring in the ice bath for 3 h, the reaction was purified by ISCO SiO_2 chromatography (0-30% MeOH/CH_2Cl_2) and prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(prop-2-yn-1-yl)carbamate was obtained. LCMS: MH+=623.7; Rt=2.19 min (5 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate



[960] To (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl)(methyl)carbamate (2.6 g, 2.64 mmol, 1.0 equiv.) dissolved in THF (20 mL) was added acetic acid (0.757 mL, 13.22 mmol, 5.0 equiv.) and 1.0 M TBAF in THF (2.91 mL, 2.91 mmol, 1.1 equiv.). The solution was stirred for 72 hours at which time the volatiles were removed in vacuo. After purification by ISCO SiO_2 chromatography (0-30% MeOH/CH_2Cl_2) (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate was obtained. LCMS: MH+=745.5; Rt=1.07 min (2 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(methyl)carbamate

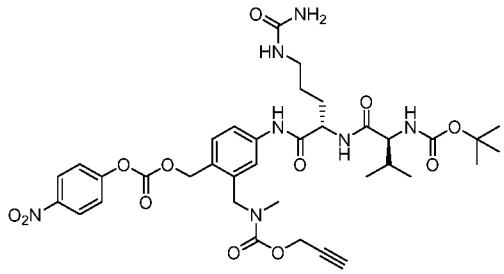


[961] To (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate (1.0 gram, 1.342 mmol) in THF(20 mL) was added $NaHCO_3$ (677 mg, 8.05 mmol)(6eq), then cooled to 0°C in ice-water bath, followed by adding thionyl chloride (0.245 mL, 3.36 mmol) (2.5eq) slowly. The mixture was stirred at 0°C for 15 min, then at RT for 1h. The reaction was paritioned between EtOAc and NaHCO3 (sat.), separated, washed with NaCl (sat.), dried over MgSO4 and the volatiles were removed in vacuo. The residue was purified by ISCO SiO_2 chromatography (0-30% iPrOH/CH_2Cl_2) to yield (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-

(chloromethyl)benzyl)(methyl)carbamate was obtained. LCMS: MH+=763.2; Rt=1.18 min (2 min acidic method).

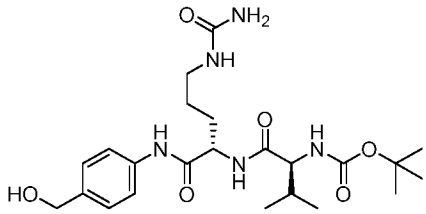
GENERAL PROCEDURE 1

Synthesis of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate



[962] A solution of prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate (249 mg, 0.412 mmol) and bis(4-nitrophenyl)carbonate (356 mg, 1.24 mmol, 3.0 equiv.) in DMF (2 mL) was swirled until homogeneous and sat for 16 hours. The solution was diluted with DMSO (6 mL) and was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H_2O, no modifier). Upon lyophilization, prop-2-yn-1-yl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate was obtained. LC/MS MH+=770.7, Rt=2.45 min (5 min acidic method).

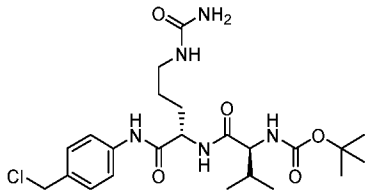
Synthesis of tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



[963] To a suspension of (4-aminophenyl)methanol (450.0 mg, 3.65 mmol) and (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (1368.0 mg, 3.65 mmol, 1.0 equiv.) in DCM (4.0 mL) was added EEDQ (2259.0 mg, 9.13 mmol, 2.5 equiv.). The mixture was stirred for 16 hours at RT, after which the reaction was purified by ISCO SiO_2 chromatography (0-30% MeOH/CH_2Cl_2) and tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate was obtained. LC/MS MH+=480.6, Rt=0.75 min (2 min acidic method). ^1H NMR (400 MHz, DMSO-d6) δ 9.97 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.60 - 7.48 (m, 2H), 7.29 - 7.19 (m, 2H), 6.76 (d, J = 8.9 Hz, 1H), 5.96 (t, J = 5.8 Hz, 1H), 5.40 (s, 2H), 5.09 (t, J = 5.7

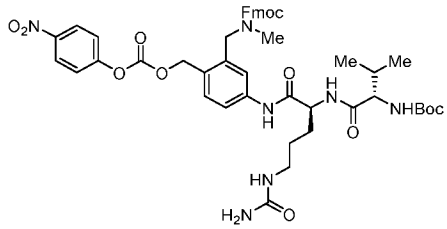
Hz, 1H), 4.43 (d, J = 5.7 Hz, 3H), 3.83 (dd, J = 8.9, 6.7 Hz, 1H), 2.98 (dp, J = 30.3, 6.6 Hz, 2H), 1.95 (p, J = 6.7 Hz, 1H), 1.80 - 1.54 (m, 2H), 1.38 (s, 11H), 0.84 (dd, J = 15.9, 6.8 Hz, 6H).

Synthesis of tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



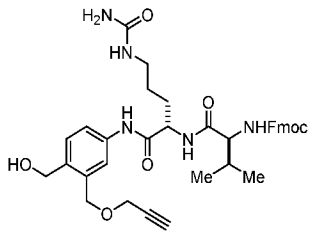
[964] To tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (500.0 mg, 1.043 mmol) in DCM (20.0 mL) was added pyridine (0.506 mL, 6.26 mmol, 6.0 equiv.). The heterogeneous mixture was cooled in an 0 °C ice bath and thionyl chloride (0.228 mL, 3.13 mmol, 3 equiv.) was added. After stirring in the ice bath for 4 hours, the mixture was warmed up to RT for 15 min. The reaction was purified by ISCO SiO2 chromatography (0-30% MeOH/CH2Cl2) and tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate was obtained. LC/MS MH+=498.1, Rt=2.02 min (5 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate



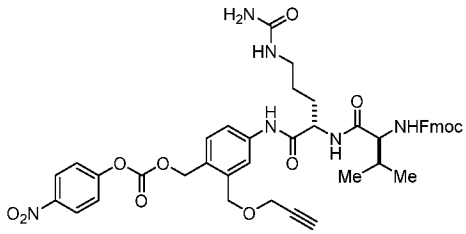
[965] Following **GENERAL PROCEDURE 1** with (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl)(methyl)carbamate (100.0 mg, 0.134 mmol), (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate was obtained. LC/MS MH+=910.5, Rt=1.24 min (2 min acidic method). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.19 (s, 1H), 8.26 (s, 2H), 8.00 (d, J = 7.7 Hz, 1H), 7.93 - 7.58 (m, 4H), 7.42 (td, J = 33.3, 32.9, 13.8 Hz, 9H), 7.14 (s, 1H), 6.72 (d, J = 9.0 Hz, 1H), 6.01 (s, 1H), 5.27 (d, J = 23.7 Hz, 2H), 4.58 (s, 2H), 4.48 - 4.13 (m, 4H), 3.89 - 3.78 (m, 1H), 2.92 (t, J = 35.0 Hz, 5H), 2.00 - 1.86 (m, 1H), 1.54 (s, 3H), 1.37 (m, 11H, incl. Boc), 0.82 (dd, J = 15.4, 6.7 Hz, 6H).

Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



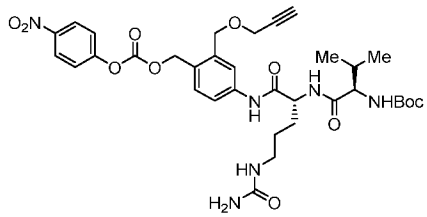
[966] To a solution of (S)-2-amino-N-(4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)-5-ureidopentanamide (3.64 g, 10.45 mmol), (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoic acid (3.55 g, 10.54 mmol, 1.0 equiv.) and 1-((dimethylamino)(dimethyliminio)methyl)-1H-[1,2,3]triazolo[4,5-b]pyridine 3-oxide hexafluorophosphate(V) (3.97 g, 10.54 mmol, 1.0 equiv.) in DMF (10.0 mL) was added DIPEA (3.64 mL, 20.90 mmol, 2.0 equiv.). The mixture was stirred for 45 min. at RT. Diluted with 100 mL water, stirred for 5min. and filtered the precipitate which was dried under reduced vacuo. Upon drying, (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate was obtained. LC/MS MH+=670.3, Rt=0.96 min (2 min acidic method).

Synthesis of (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate



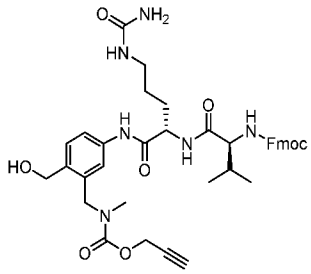
[967] Following **GENERAL PROCEDURE 1** with (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (200.0 mg, 0.299 mmol), (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate was obtained. LC/MS MH+= 835.7, Rt=1.19 min (2 min acidic method).

Synthesis of tert-butyl ((R)-3-methyl-1-(((R)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate



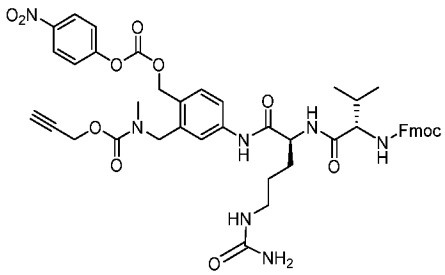
[968] Following **GENERAL PROCEDURE 1** with tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (200.0 mg, 0.365 mmol), tert-butyl ((R)-3-methyl-1-(((R)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate was obtained. LC/MS MH+=713.6, Rt=1.08 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate



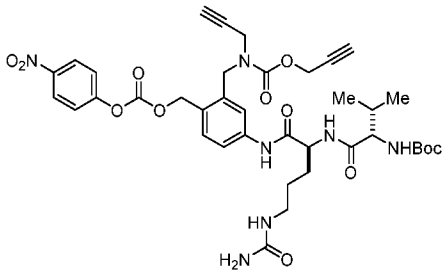
[969] To a solution of prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (48.0 mg, 0.079 mmol) in DCM (1.0 mL) at 0 °C was added TFA (0.2 mL). The mixture was stirred for 1 hour at this temperature. Afterwards the solvents were removed under vacuo. The residue was dissolved in DMF (1.0 mL), followed by adding DIPEA (0.138 mL, 0.794 mmol, 10 equiv.) and (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (40.2 mg, 0.119 mmol, 1.5 equiv.). The mixture was stirred for 18 hours at RT. Reaction was purified by RP-HPLC ISCO gold chromatography (0-100% MeCN/H2O, no modifier). Upon lyophilization, prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate was obtained. LC/MS MH+=727.3, Rt=2.28 min (5 min acidic method). 1H NMR (400 MHz, DMSO-d6) δ 10.01 (s, 1H), 8.09 (d, J = 7.6 Hz, 1H), 7.89 (d, 2H), 7.74 (t, J = 8.2 Hz, 2H), 7.62 (s, 1H), 7.45 - 7.36 (m, 3H), 7.35 - 7.15 (m, 4H), 5.95 (t, J = 5.9 Hz, 1H), 5.36 (s, 2H), 5.03 (s, 1H), 4.70 (d, J = 14.8 Hz, 2H), 4.54 - 4.36 (m, 5H), 4.35 - 4.19 (m, 3H), 3.96 - 3.87 (m, 1H), 3.50 (d, J = 26.0 Hz, 1H), 2.97 (dp, J = 20.1, 6.6 Hz, 2H), 2.82 (s, 3H), 1.98 (q, J = 6.8 Hz, 1H), 1.73 - 1.50 (m, 2H), 1.51 - 1.30 (m, 2H), 0.86 (dd, J = 10.2, 6.7 Hz, 6H).

Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(methyl)carbamate



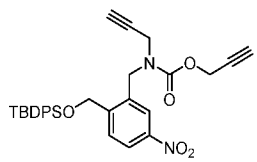
[970] Following **GENERAL PROCEDURE 1** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (77.6 mg, 0.107 mmol), prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(methyl)carbamate was obtained. LC/MS MH+= 892.4, Rt=1.14 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate



[971] Following **GENERAL PROCEDURE 1** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(prop-2-yn-1-yl)carbamate (250.0 mg, 0.398 mmol), prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS MH+= 794.9, Rt=1.07 min (2 min acidic method).

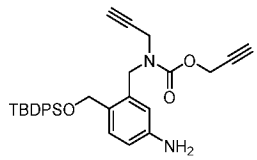
Synthesis of prop-2-yn-1-yl 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl(prop-2-yn-1-yl)carbamate



[972] To a solution of N-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl)prop-2-yn-1-amine (1.348 g, 2.94 mmol) in DCM (10.0 mL) was added pyridine (2.0 mL) followed by

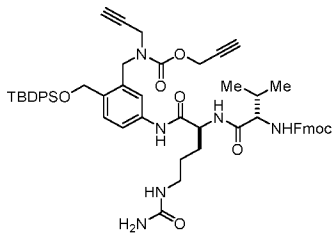
prop-2-yn-1-yl carbonochloridate (0.574 mL, 5.88 mmol, 2.0 equiv.) and the mixture was stirred for 30 min. at RT. Reaction was quenched with MeOH, diluted with CH_2Cl_2 (20 mL), then washed with water, NaCl(sat.), dried over Na_2SO_4, filtered, concentrated and purified by ISCO SiO_2 chromatography (0-50% EtOAc/heptane), prop-2-yn-1-yl 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS MH+= 541.6, Rt=1.47 min (2 min acidic method). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.18 (dd, J = 8.4, 2.4 Hz, 1H, Ar), 8.10 (d, J = 2.3 Hz, 1H, Ar), 7.72 - 7.63 (m, 4H, Ph), 7.54 - 7.35 (m, 7H, Ph + Ar), 4.86 (s, 2H), 4.80 - 4.53 (m, 4H), 4.02 (d, J = 22.3 Hz, 2H), 2.76 (d, J = 4.7 Hz, 1H), 2.17 (t, J = 2.4 Hz, 1H), 1.13 (d, J = 3.1 Hz, 9H).

Synthesis of prop-2-yn-1-yl 5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate



[973] To a solution of prop-2-yn-1-yl 2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-nitrobenzyl(prop-2-yn-1-yl)carbamate (1.66 g, 2.07 mmol) in DCM (9.0 mL) and AcOH (1.0 mL) at 0 °C was added zinc (3.01 g, 46.1 mmol, 15.0 equiv.) and the mixture was stirred for 40 min. at this temperature. Reaction was filtered through celite and rinsed with DCM. Filtrate was washed with $NaHCO_3$ (sat.), water and NaCl(sat.), dried over Na_2SO_4, filtered, concentrated and purified by ISCO SiO_2 chromatography (0-100% EtOAc/heptane), prop-2-yn-1-yl 5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS M+Na= 533.2, Rt=1.35 min (2 min acidic method).

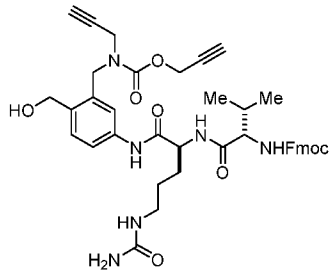
Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate



[974] Suspended prop-2-yn-1-yl 5-amino-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate (1.19 g, 2.33 mmol) and (S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (1.157 g, 2.33 mmol, 1.0 equiv.) in DCM (10.0 mL) and MeOH (5.0 mL), added EEDQ (0.691 g, 2.80 mmol, 1.2 equiv.) and stirred for 3 hours at RT. Solvents were removed in vacuo, residue dissolved in DMSO (3.0 mL) and purified by RP-HPLC

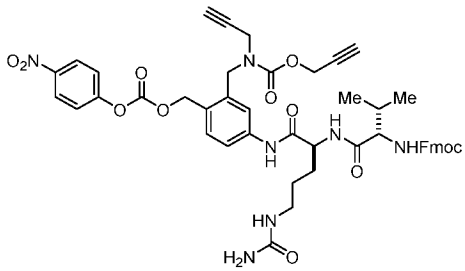
ISCO gold chromatography (0-100% MeCN/H2O, 0.05 % TFA modifier). Upon lyophilization, prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS M+H= 990.0, Rt=1.47 min (2 min acidic method).

Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(prop-2-yn-1-yl)carbamate



[975] To a solution of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((tert-butyldiphenylsilyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate (732 .0 mg, 0.740 mmol) in THF (5.0 mL) was added acetic acid (0.127 mL, 2.220 mmol, 3.0 equiv.) and 1.0 M TBAF in THF (1.48 mL, 1.480 mmol, 2.0 equiv.). The mixture was stirred at RT for 20 hours. LCMS indicated some start material left. Added 1.0 M TBAF in THF (0.75 mL, 0.750 mmol, 1.0 equiv.) and stirred at RT for 20 hours. Solvent was removed in vacuo, the material was purified by ISCO SiO_2 chromatography (0-50% MeOH/CH_2Cl_2) and prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS M+H= 751.6, Rt=0.99 min (2 min acidic method).

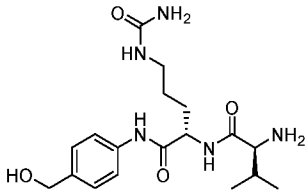
Synthesis of prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate



[976] Following **GENERAL PROCEDURE 1** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(hydroxymethyl)benzyl(prop-2-yn-1-yl)carbamate (556.0 mg, 0.740 mmol), prop-2-yn-1-yl 5-

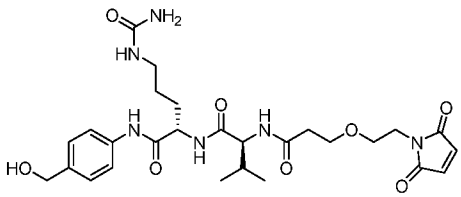
((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate was obtained. LC/MS M+H= 916.8, Rt=1.16 min (2 min acidic method).

Synthesis of (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide



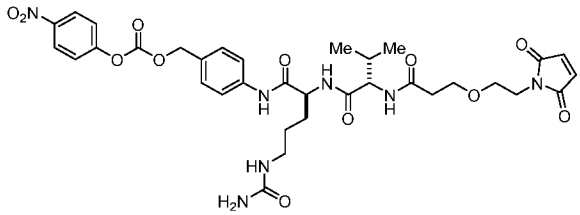
[977] Following **GENERAL PROCEDURE 4** described below with tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (2.00 g, 4.17 mmol), (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide was obtained. LC/MS M+H= 380.6, Rt=0.40 min (2 min acidic method).

Synthesis of (S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide



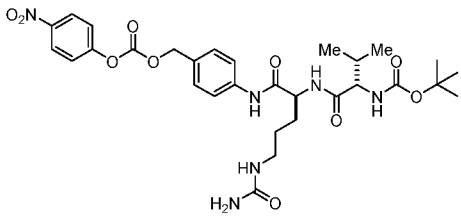
[978] Following **GENERAL PROCEDURE 5** described below with 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (100.0 mg, 0.322 mmol) and (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide (175.0 mg, 0.355 mmol, 1.1 equiv.), (S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide was obtained. LC/MS M+H= 575.4, Rt=0.61 min (2 min basic method).

Synthesis of 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate



[979] Following **GENERAL PROCEDURE 1** with (S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide (126.0 mg, 0.219 mmol), 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate was obtained. LC/MS M+H= 575.4, Rt=0.61 min (2 min basic method).

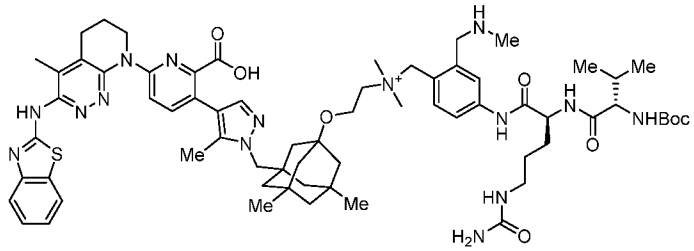
Synthesis of tert-butyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate



[980] Following **GENERAL PROCEDURE 1** with tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (200.0 mg, 0.417 mmol), tert-butyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate was obtained. LC/MS M+H= 645.5, Rt=1.02 min (2 min acidic method).

GENERAL PROCEDURE 2

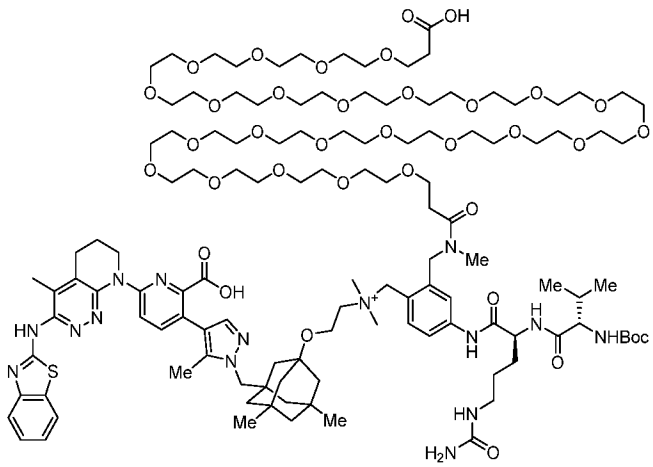
Synthesis of 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)-N,N-dimethylethan-1-aminium



[981] To a suspension of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-(dimethylamino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (25 mg, 0.033 mmol), (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(methyl)carbamate (25 mg, 0.033 mmol, 1.0 equiv.) and TBAI (12 mg, 0.033 mmol, 1.0 equiv.) in DMSO (1 mL) was added DIPEA (0.03 mL, 0.164 mmol, 5.0 equiv.) and stirred for 16 hours at RT. 2.0 M dimethylamine in THF (0.164 mL, 0.328 mmol, 10 equiv.) was added. After standing for 1.5 hours, the solution was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)-N,N-dimethylethan-1-aminium was obtained. HRMS: M+= 1266.3000; Rt=1.85 min (5 min acidic method).

GENERAL PROCEDURE 3

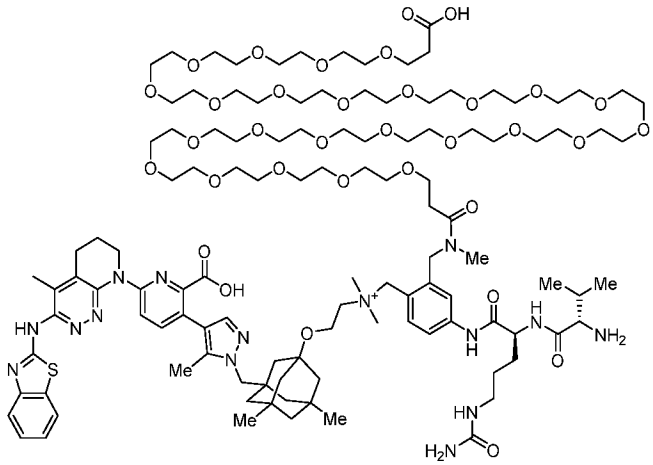
Synthesis of 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-N,N-dimethylethan-1-aminium



[982] To a solution of 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)-N,N-dimethylethan-1-aminium (42 mg, 0.027 mmol) and 79-((2,5-dioxopyrrolidin-1-yl)oxy)-79-oxo-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-pentacosaoxanonaheptacontanoic acid (42 mg, 0.032 mmol, 1.2 equiv.) in DMF (0.5 mL) was added DIPEA (0.023 mL, 0.133 mmol, 5.0 equiv.) and stirred for 5 hours at RT. DMSO (2 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-N,N-dimethylethan-1-aminium was obtained. HRMS: M+= 2465.7800; Rt=2.15 min (5 min acidic method).

GENERAL PROCEDURE 4

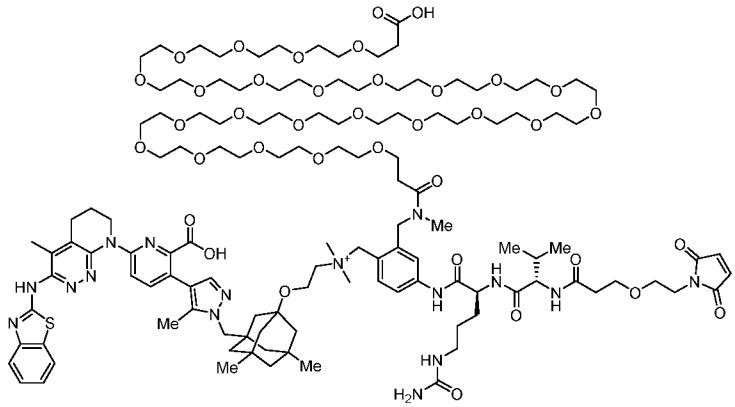
Synthesis of N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N,N-dimethylethan-1-aminium



[983] To a solution of 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-N,N-dimethylethan-1-aminium (28 mg, 0.011 mmol) in CH_2Cl_2 (0.75 mL) at 0 °C in an ice bath was added trifluoroacetic acid (0.25 mL). The mixture was stirred for 1 hour in the ice bath, at which time the volatiles were removed in vacuo. DMSO (1.5 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N,N-dimethylethan-1-aminium was obtained. HRMS: M+= 2367.3101; Rt=1.86 min (5 min acidic method). For this general procedure, in some cases the amine was taken on as is without RP-HPLC purification.

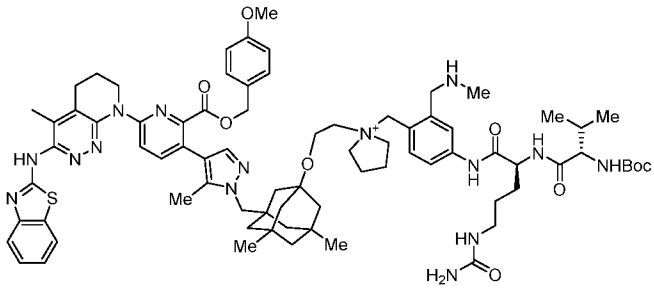
GENERAL PROCEDURE 5

Synthesis of 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylethan-1-aminium (L11A-P27)



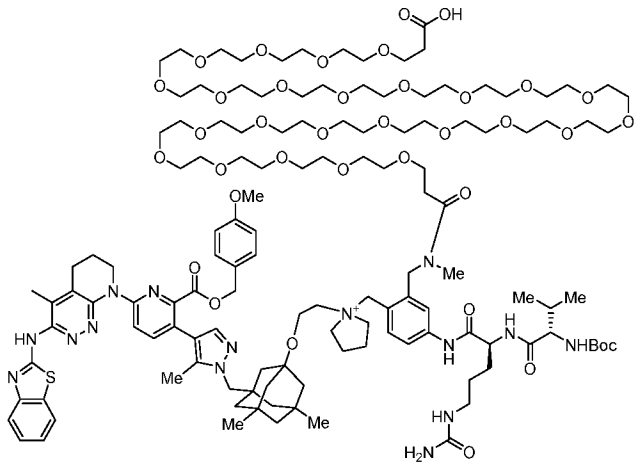
[984] To a solution of N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N,N-dimethylethan-1-aminium (10.0 mg, 0.004 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (1.4 mg, 0.005 mmol, 1.2 equiv.) in DMF (0.5 mL) was added DIPEA (6.7 µL, 0.039 mmol, 10.0 equiv.). The mixture was stirred for 3.5 hours at RT. DMSO (1.5 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, 2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-(2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylethan-1-aminium was obtained. HRMS: M+= 2562.3401; Rt=2.04 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium



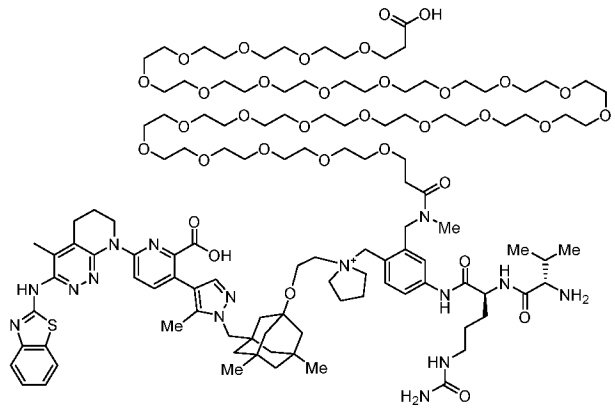
[985] Following **GENERAL PROCEDURE 2** with 4-methoxybenzyl 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3R,5S,7s)-3,5-dimethyl-7-(2-(pyrrolidin-1-yl)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate (30.0 mg, 0.033 mmol) and (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl)(methyl)carbamate (25.2 mg, 0.033 mmol, 1.0 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 1412.7600; Rt=2.22 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)pyrrolidin-1-ium



[986] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (42.0 mg, 0.026 mmol) and 79-((2,5-dioxopyrrolidin-1-yl)oxy)-79-oxo-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-pentacosaoxanonaheptacontanoic acid (40.4 mg, 0.031 mmol, 1.2 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2613.4199; Rt=2.38 min (5 min acidic method).

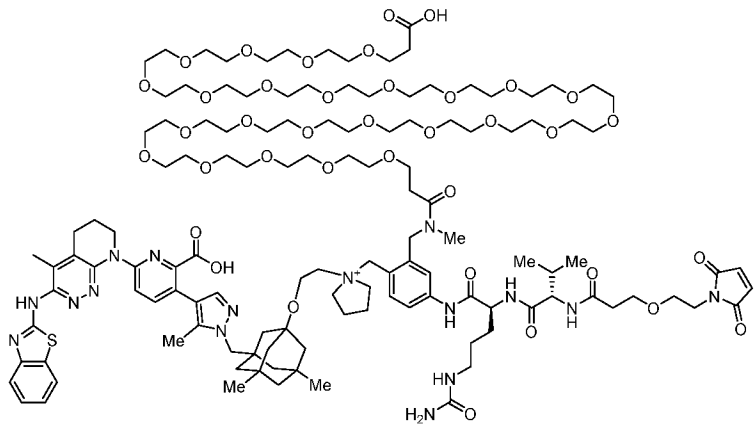
Synthesis of 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium



[987] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)pyrrolidin-1-ium (68.0 mg, 0.26 mmol), 1-(4-((S)-2-((S)-2-amino-3-

methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2393.3301; Rt=1.85 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium (L11A-P21)

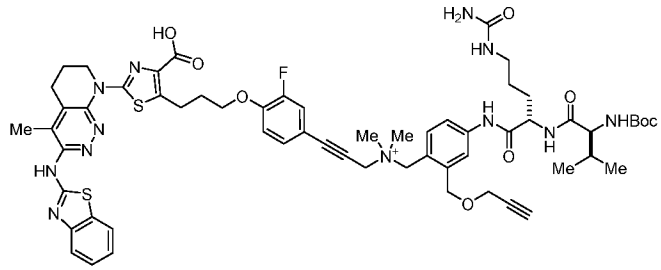


[988] Following **GENERAL PROCEDURE 5** with 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium (26.1 mg, 0.011 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (5.1 mg, 0.016 mmol, 1.5 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2588.3899; Rt=2.05 min (5 min acidic method).

GENERAL PROCEDURE 6

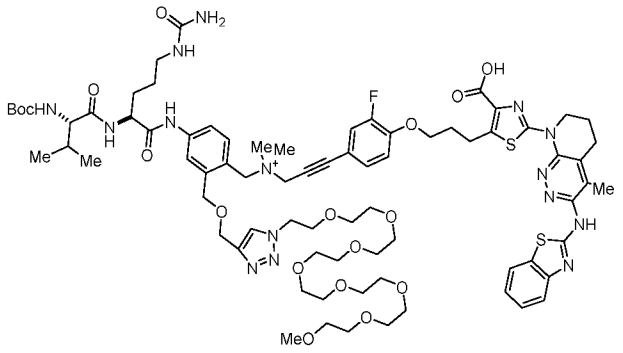
Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[989] To a suspension of 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (75.0 mg, 0.114 mmol) and tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (103.0 mg, 0.182 mmol, 1.6 equiv.) in DMSO (2.0 ml) was added TBAI (67.4 mg, 0.182 mmol, 1.6 equiv.) and DIPEA (0.16 mL, 0.912 mmol, 9.0 equiv.). The mixture went into solution and was stirred for 2 hours at RT. After this time the solution was purified by RP-HPLC ISCO gold chromatography (10-70% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1187.6; Rt=0.93 min (2 min acidic method).

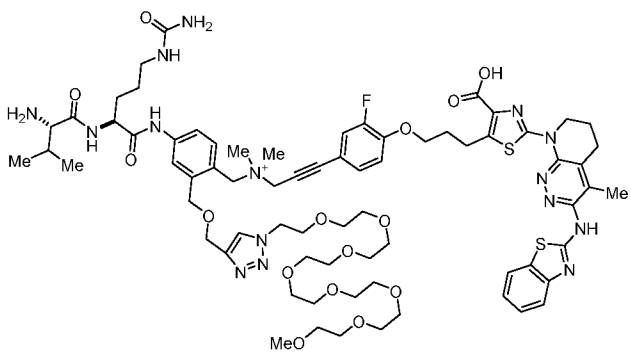
GENERAL PROCEDURE 7

Synthesis of N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium



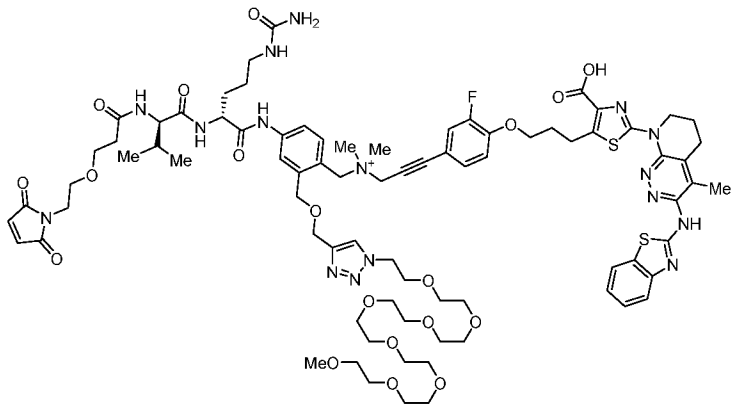
[990] After a flask with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (50.0 mg, 0.042 mmol), 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (34.5 mg, 0.084 mmol, 2.0 equiv.), sodium (R)-2-((S)-1,2-dihydroxyethyl)-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate (12.5 mg, 0.63 mmol, 1.5 equiv.) and copper(II) sulfate pentahydrate (2.1 mg, 0.008 mmol, 0.2 equiv.) was sealed and evacuated / purge with N_2 3x, tert.-butanol (5.0 mL) and water (0.5 mL) were added via syringe. The mixture was stirred for 2 hours at RT. DMSO (1 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (0-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1596.7531; Rt=1.18 min (2 min acidic method). For this general procedure, in some cases instead of tert.-butanol, DMF or DMSO was used.

Synthesis of N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium



[991] Following **GENERAL PROCEDURE 4** with N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (30.0 mg, 0.019 mmol), N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1497.2; Rt=1.94 min (5 min acidic method).

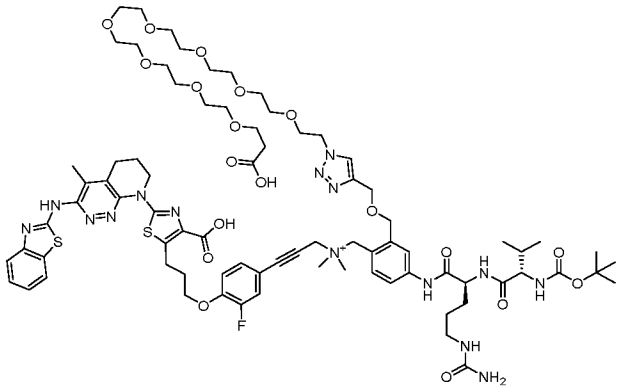
Synthesis of N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((R)-2-((R)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (L8A-P1)



[992] Following **GENERAL PROCEDURE 5** with N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (24.0 mg, 0.016 mmol), N-(2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((R)-2-((R)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-

fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1691.7500; Rt=4.35 min (5 min acidic method).

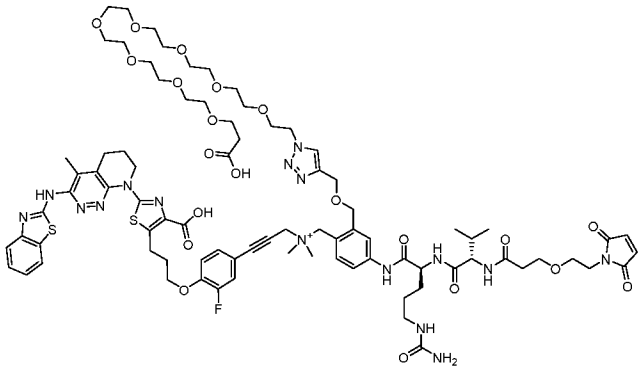
Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[993] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (50.0 mg, 0.042 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (39.4 mg, 0.084 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: 1/2M+= 828.1; Rt=0.71 min (2 min acidic method).

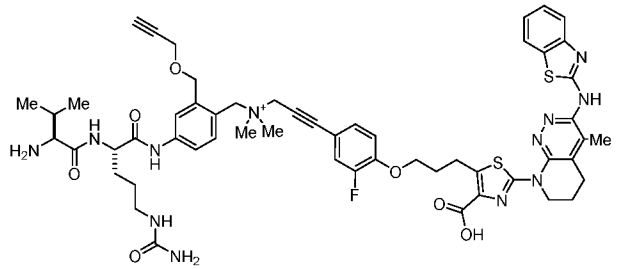
GENERAL PROCEDURE 8

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L7A-P1)



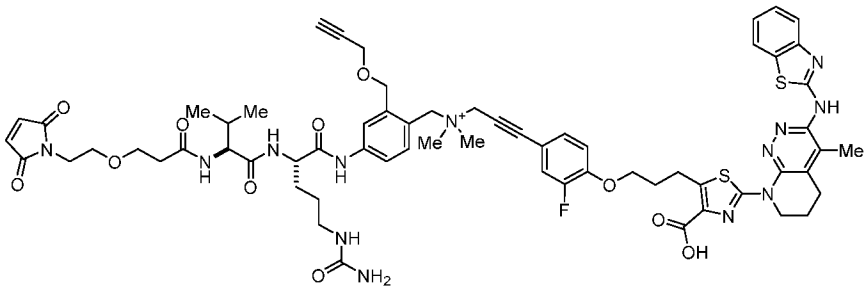
[994] A solution of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (32.2 mg, 0.019 mmol) in DCM/TFA (3:1, 2.6 mL) was cooled to 0 °C and stirred for 1 hour at this temperature. After the mixture was evaporated under reduced pressure to yield crude de-Boc intermediate, crude was solved in DMF (0.5 mL) and followed by adding 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (12.1 mg, 0.039 mmol, 2.0 equiv.) and DIPEA (0.1 mL, 0.584 mmol, 30.0 equiv.). Mixture was stirred for 30 min. at RT. The solution was purified by RP-HPLC ISCO gold chromatography (0-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilisation, 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1749.7400; Rt=2.51 min (5 min acidic method).

Synthesis of N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium



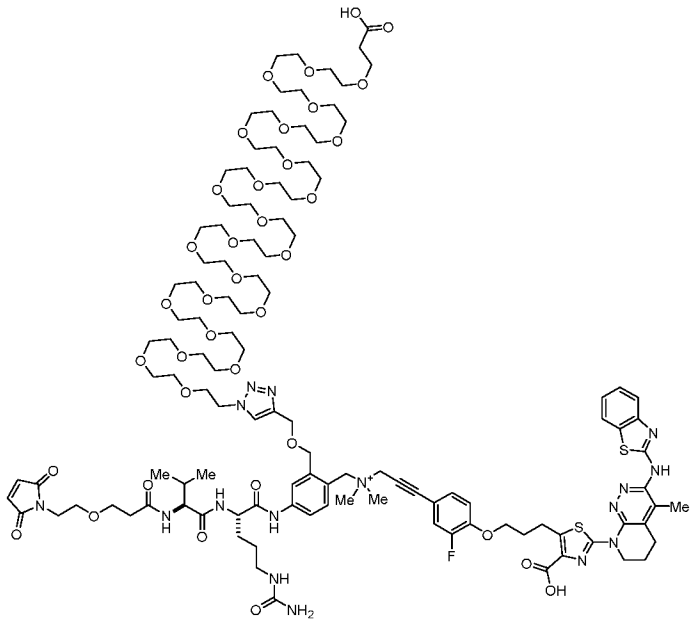
[995] Following **GENERAL PROCEDURE 4** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (263.0 mg, 0.221 mmol), N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1087.2700; Rt=1.85 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



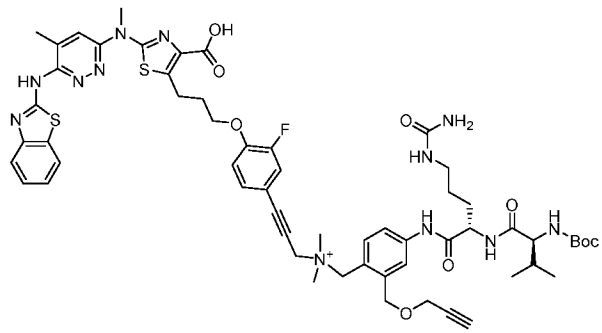
[996] Following **GENERAL PROCEDURE 5** with N-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (77.0 mg, 0.050 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (23.2 mg, 0.075 mmol, 1.5 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1282.4800; Rt=2.15 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(74-carboxy-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxatetraheptacontyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L109A-P1)



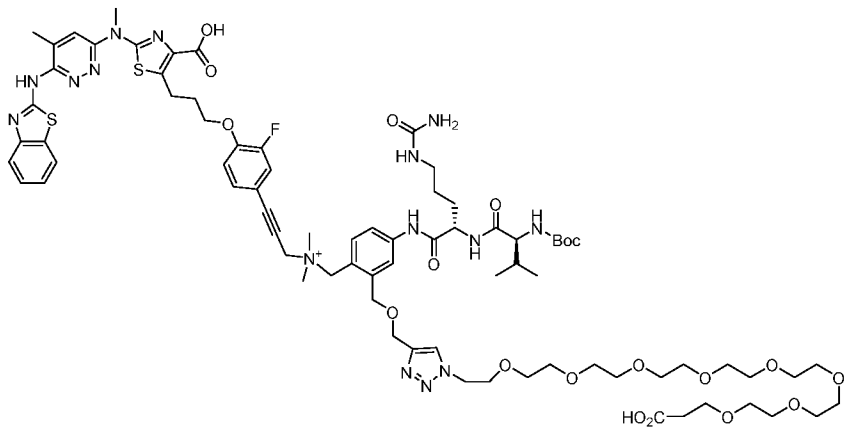
[997] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (51.8 mg, 0.037 mmol) and 1-azido-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxapentaheptacontan-75-oic acid (87.0 mg, 0.074 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(74-carboxy-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxatetraheptacontyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2453.8899; Rt=2.17 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



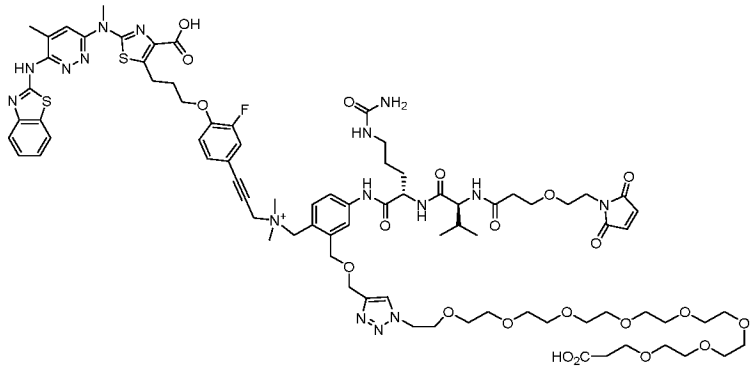
[998] Following **GENERAL PROCEDURE 6** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (50.0 mg, 0.079 mmol) and tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (71.7 mg, 0.127 mmol, 1.6 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1162.2; Rt=0.94 min (2 min basic method).

Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[999] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (40.0 mg, 0.034 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (25.8 mg, 0.055 mmol, 1.6 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M/2+= 815.4; Rt=0.99 min (2 min acidic method).

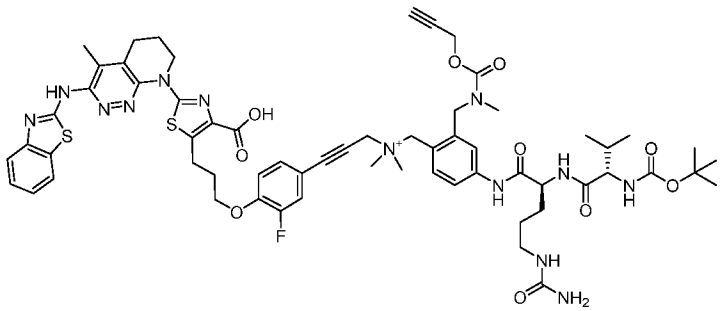
Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L7A-P2)



[1000] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-

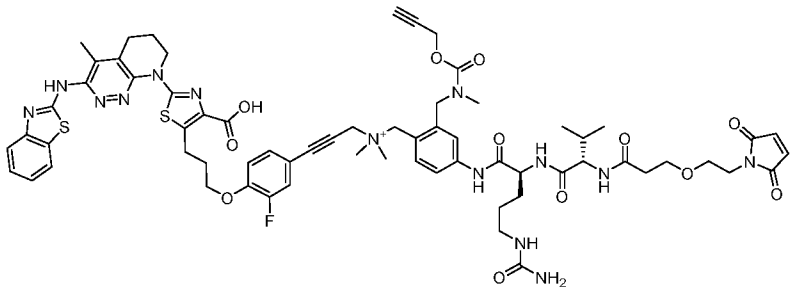
((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (37.0 mg, 0.023 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (10.6 mg, 0.034 mmol, 1.5 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M-= 1722.9; Rt=0.91 min (2 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



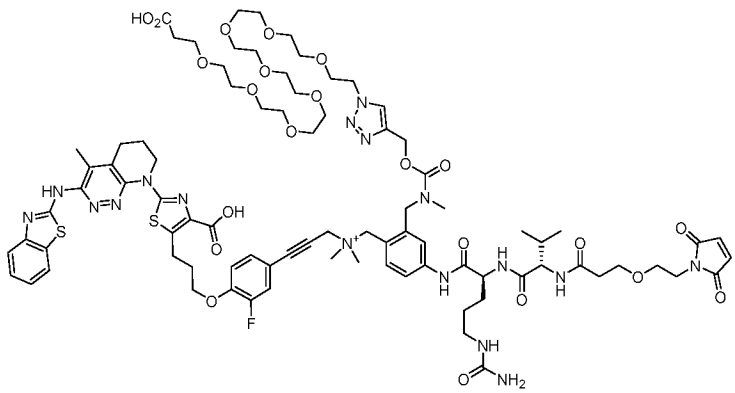
[1001] Following **GENERAL PROCEDURE 6** with 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (118.0 mg, 0.170 mmol) and prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl(methyl)carbamate (127.0 mg, 0.204 mmol, 1.2 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1244.5100; Rt=2.42 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



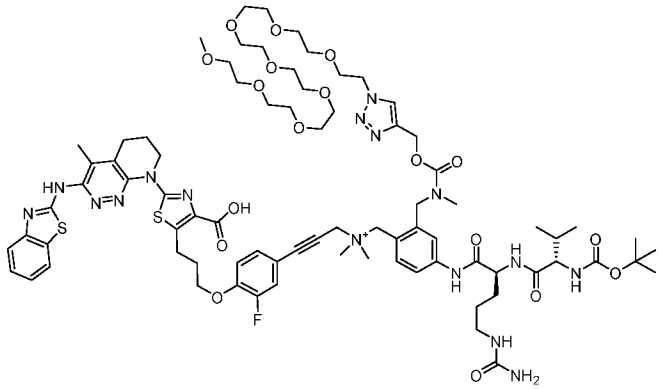
[1002] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (65.0 mg, 0.052 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (32.4 mg, 0.104 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1341.1; Rt=2.20 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L3A-P1)



[1003] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (65.0 mg, 0.049 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (45.4 mg, 0.097 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1806.7700; Rt=2.05 min (5 min acidic method).

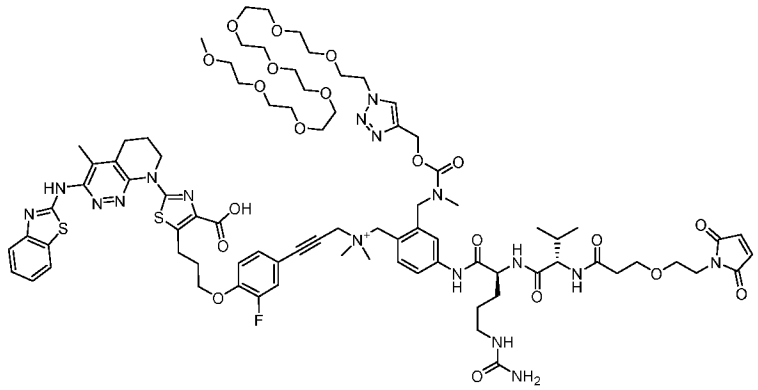
Synthesis of N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium



[1004] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (36.0 mg, 0.029 mmol) and 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (23.7 mg, 0.058 mmol, 2.0 equiv.), N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-

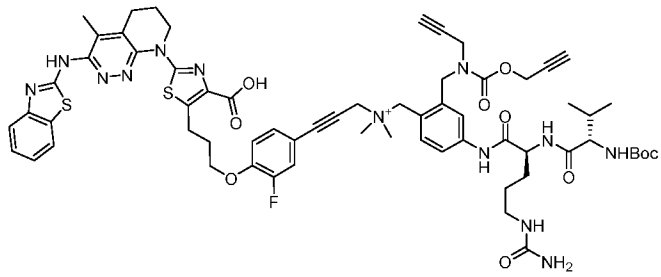
dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1653.7500; Rt=2.29 min (5 min acidic method).

Synthesis of N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (L4A-P1)



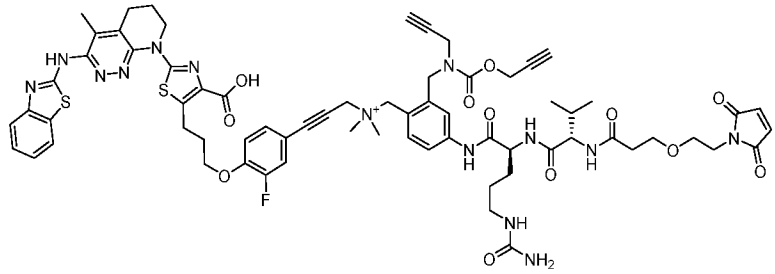
[1005] Following **GENERAL PROCEDURE 8** with N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (19.6 mg, 0.012 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (7.4 mg, 0.024 mmol, 2.0 equiv.), N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1748.7600; Rt=2.15 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[1006] Following **GENERAL PROCEDURE 6** with 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (50.0 mg, 0.076 mmol) and prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl(prop-2-yn-1-yl)carbamate (73.8 mg, 0.114 mmol, 1.5 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1269.2; Rt=2.24 min (5 min basic method).

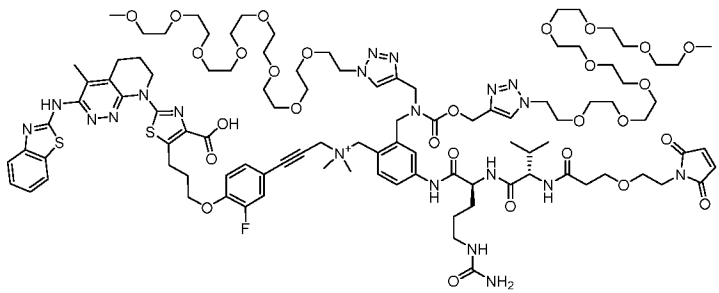
Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[1007] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (45.9 mg, 0.036 mmol) and 2,5-dioxo-2,5-dihydro-1H-

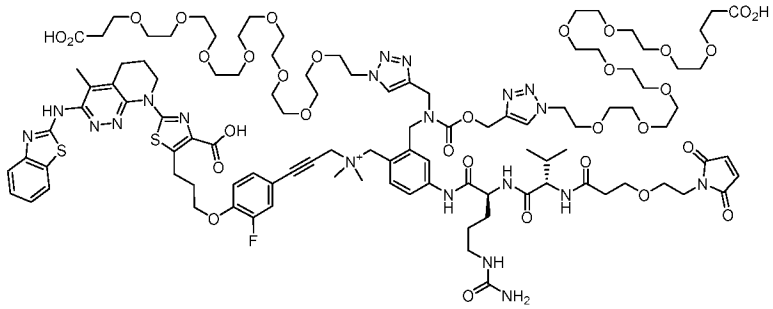
pyrrol-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (22.3 mg, 0.072 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1363.5100; Rt=2.26 min (5 min acidic method).

Synthesis of N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (L1A-P1)



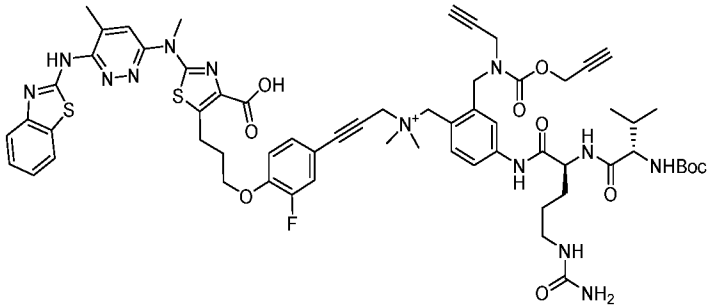
[1008] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (21.9 mg, 0.016 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxahexacosane (51.0 mg, 0.120 mmol, 7.5 equiv.), N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2181.9800; Rt=2.31 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L10A-P1)



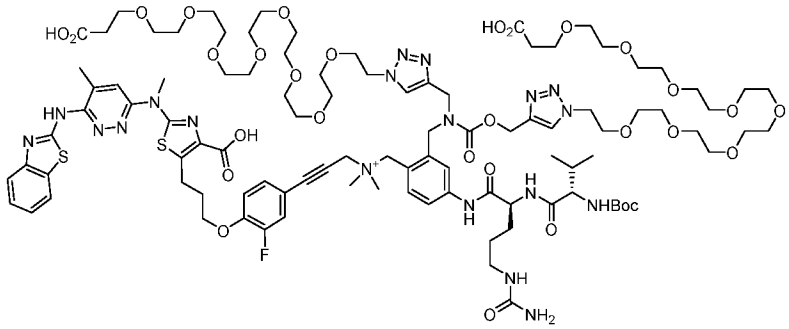
[1009] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (20.0 mg, 0.015 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (51.4 mg, 0.110 mmol, 7.5 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2298.0100; Rt=2.44 min (5 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)dimethylammonio)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate



[1010] Following **GENERAL PROCEDURE 6** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (50.0 mg, 0.079 mmol) and prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(chloromethyl)benzyl(prop-2-yn-1-yl)carbamate (61.5 mg, 0.095 mmol, 1.2 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)dimethylammonio)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate was obtained. LCMS: M+= 1243.2; Rt=2.27 min (5 min acidic method).

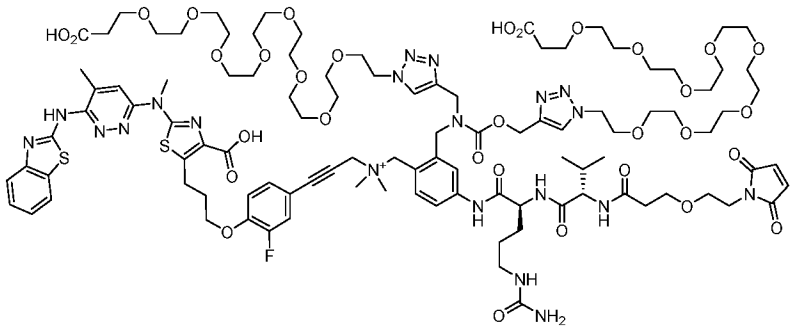
Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[1011] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (21.8 mg, 0.018 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (32.8 mg, 0.070 mmol, 4.0 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-

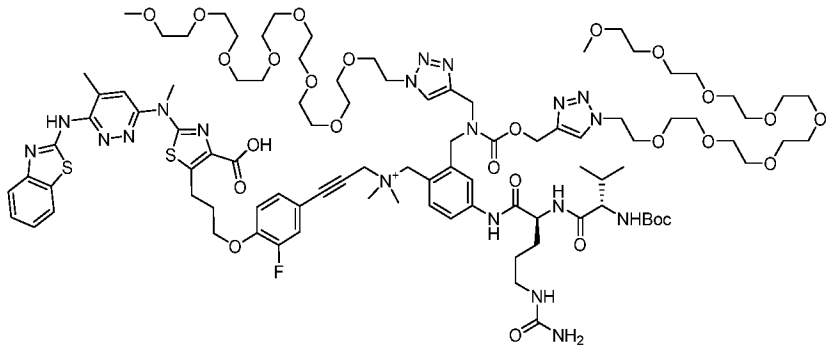
methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2176.8301; Rt=2.25 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L10A-P2)



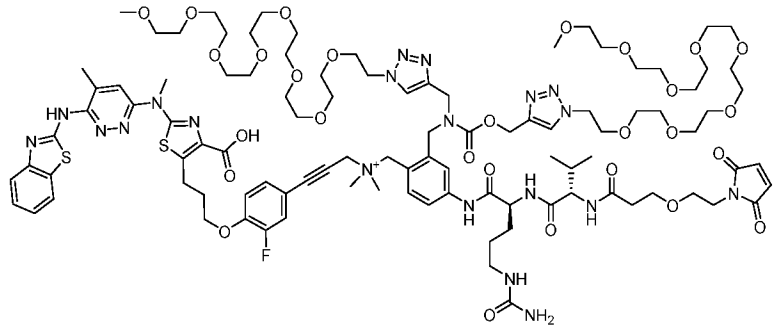
[1012] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (17.8 mg, 0.008 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (10.2 mg, 0.033 mmol, 4.0 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2271.8186; Rt=2.12 min (5 min acidic method).

Synthesis of N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium



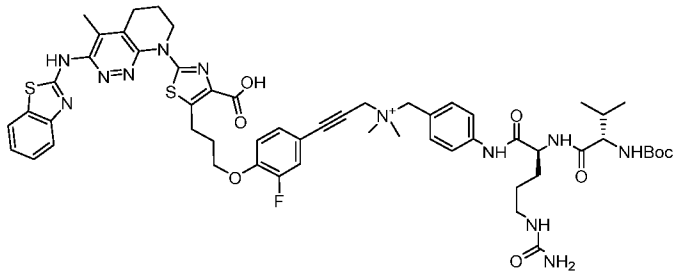
[1013] Following **GENERAL PROCEDURE 7** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)-N,N-dimethylprop-2-yn-1-aminium (22.6 mg, 0.018 mmol) and 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (29.8 mg, 0.073 mmol, 4.0 equiv.), N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M/2+= 1032.3; Rt=2.25 min (5 min acidic method).

Synthesis of N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (L1A-P2)



[1014] Following **GENERAL PROCEDURE 8** with N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium (23.0 mg, 0.011 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (10.4 mg, 0.033 mmol, 3.0 equiv.), N-(2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 2155.8176; Rt=2.23 min (5 min acidic method).

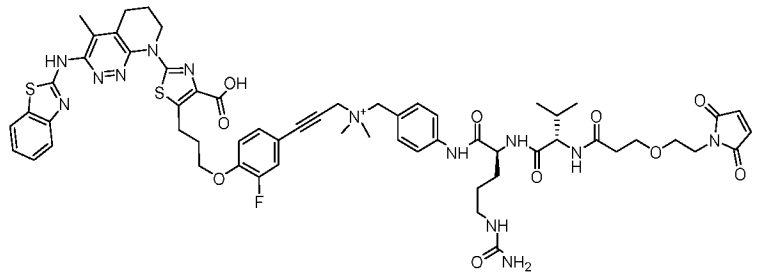
Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[1015] Following **GENERAL PROCEDURE 6** with 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (21.5 mg, 0.033 mmol) and tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (21.2 mg, 0.042 mmol, 1.3 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-

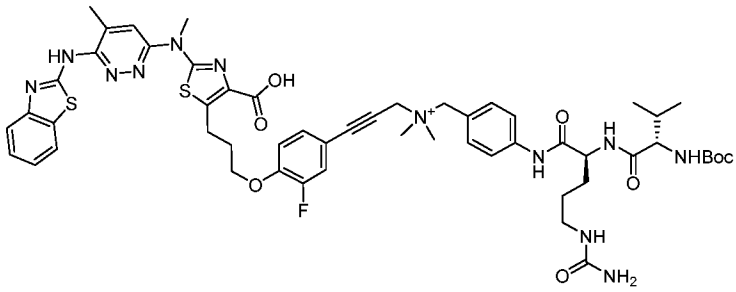
ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1119.3; Rt=2.15 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L9A-P1)



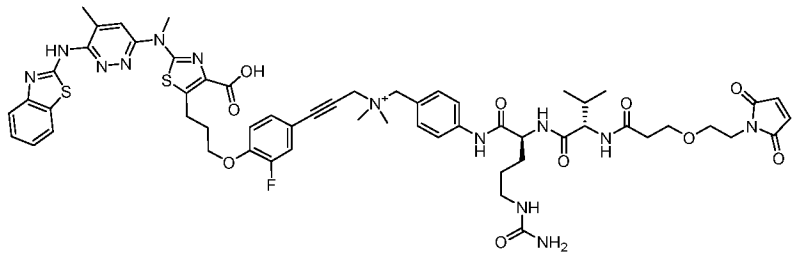
[1016] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (36.6 mg, 0.033 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (20.3 mg, 0.065 mmol, 2.0 equiv.), 3-(4-(3-(2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1214.4700; Rt=2.10 min (5 min acidic method).

Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium



[1017] Following **GENERAL PROCEDURE 6** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-(dimethylamino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (25.0 mg, 0.040 mmol) and tert-butyl ((S)-1-(((S)-1-((4-(chloromethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (25.6 mg, 0.051 mmol, 1.3 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. LCMS: M+= 1094.1; Rt=2.14 min (5 min acidic method).

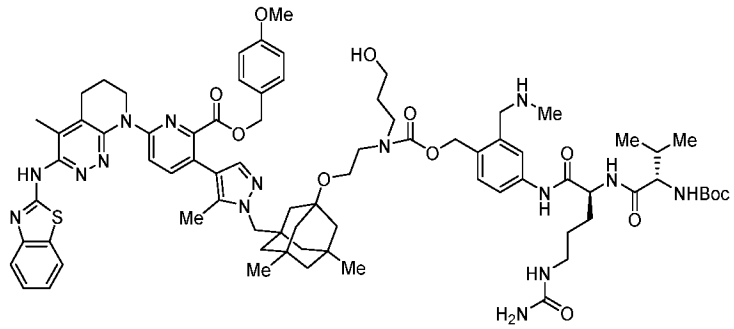
Synthesis of 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (L9A-P2)



[1018] Following **GENERAL PROCEDURE 8** with 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium (31.6 mg, 0.029 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (26.9 mg, 0.087 mmol, 3.0 equiv.), 3-(4-(3-(2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-4-carboxythiazol-5-yl)propoxy)-3-fluorophenyl)-N-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)-N,N-dimethylprop-2-yn-1-aminium was obtained. HRMS: M+= 1188.4500; Rt=2.07 min (5 min acidic method).

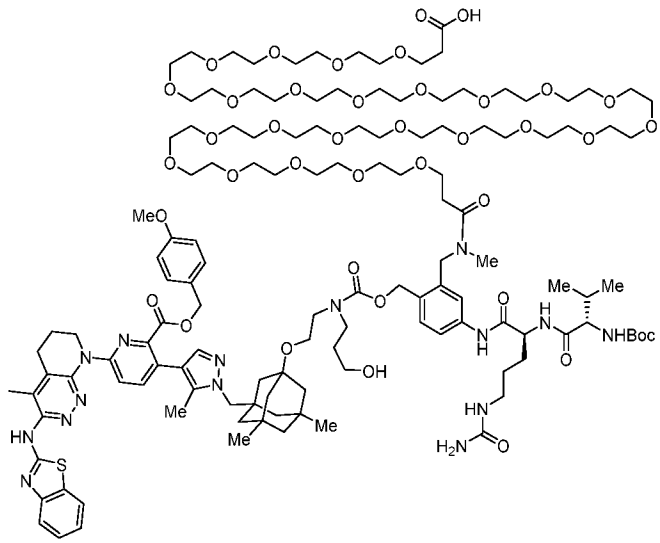
GENERAL PROCEDURE 9

Synthesis of 4-methoxybenzyl 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate



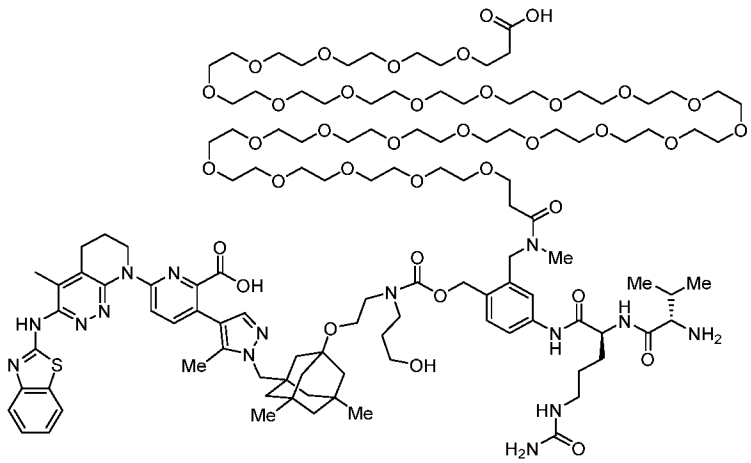
[1019] To a solution of 4-methoxybenzyl 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate (30.0 mg, 0.033 mmol) and (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate (35.9 mg, 0.039 mmol, 1.2 equiv.) in DMF (1.0 mL) was added DIPEA (0.03 mL, 0.164 mmol, 5.0 equiv.) and the mixture was stirred for 16 hours at RT. After the carbamate formation, 2M dimethylamine in THF (0.164 mL, 0.329 mmol, 1.0 equiv.) was added and stirred mixture for 1.5 hours. DMSO (2.0 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (10-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilization, 4-methoxybenzyl 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate was obtained. HRMS: M+= 1460.7500; Rt=2.31 min (5 min acidic method).

Synthesis of 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azahenoctacontan-81-oic acid



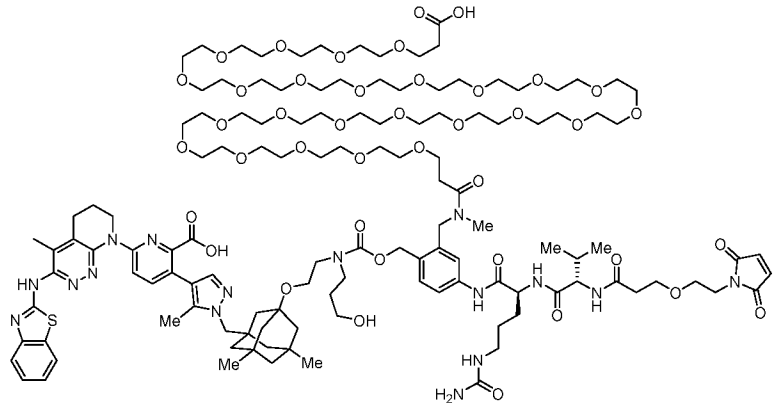
[1020] Following **GENERAL PROCEDURE 3** with 4-methoxybenzyl 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate (32.0 mg, 0.022 mmol) and 79-((2,5-dioxopyrrolidin-1-yl)oxy)-79-oxo-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-pentacosaoxanonaheptacontanoic acid (43.3 mg, 0.033 mmol, 1.5 equiv.), 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azahenoctacontan-81-oic acid was obtained. HRMS: M-H+2Na= 2705.3601; Rt=2.63 min (5 min acidic method).

Synthesis of 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid



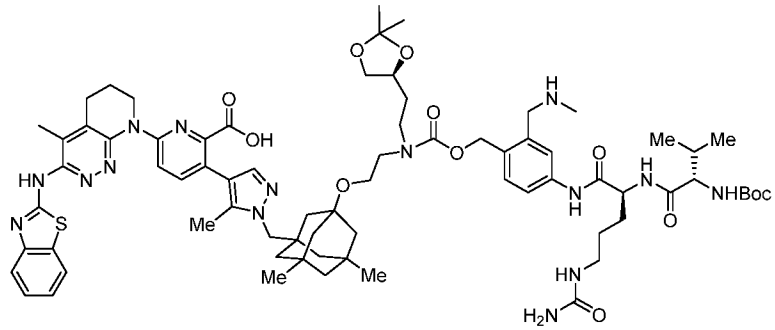
[1021] Following **GENERAL PROCEDURE 4** with 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azahenoctacontan-81-oic acid (35.1 mg, 0.013 mmol), 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid was obtained. HRMS: M-H+2Na= 2485.2700; Rt=2.02 min (5 min acidic method).

Synthesis of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (L11C-P25)



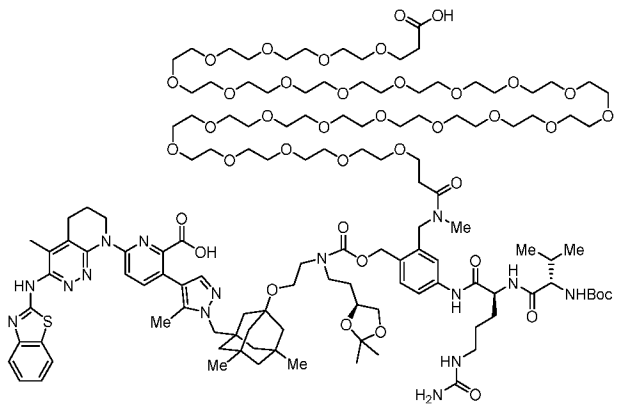
[1022] Following **GENERAL PROCEDURE 5** with 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid (17.3 mg, 0.007 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (2.6 mg, 0.009 mmol, 1.2 equiv.), 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid was obtained. HRMS: M+H= 2636.3701; Rt=1.73 min (5 min acidic method).

Synthesis of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid



[1023] Following **GENERAL PROCEDURE 9** with 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (24.0 mg, 0.028 mmol) and (9H-fluoren-9-yl)methyl (5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate (27.9 mg, 0.031 mmol, 1.1 equiv.), 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid was obtained. HRMS: M+H= 1410.7300; Rt=2.24 min (5 min acidic method).

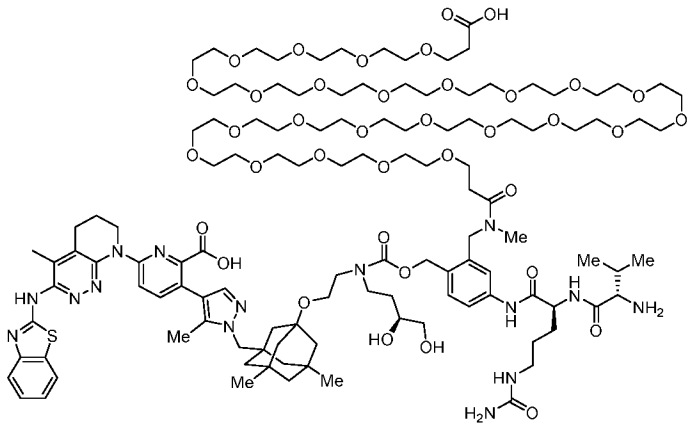
Synthesis of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid



[1024] Following **GENERAL PROCEDURE 3** with 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (19.0 mg, 0.012 mmol) and 79-((2,5-dioxopyrrolidin-1-yl)oxy)-79-oxo-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-pentacosaoxanonaheptacontanoic acid (24.6 mg, 0.019 mmol, 1.5 equiv.), 6-(3-

(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid was obtained. HRMS: M-H+2Na = 2655.3701; Rt=2.59 min (5 min acidic method).

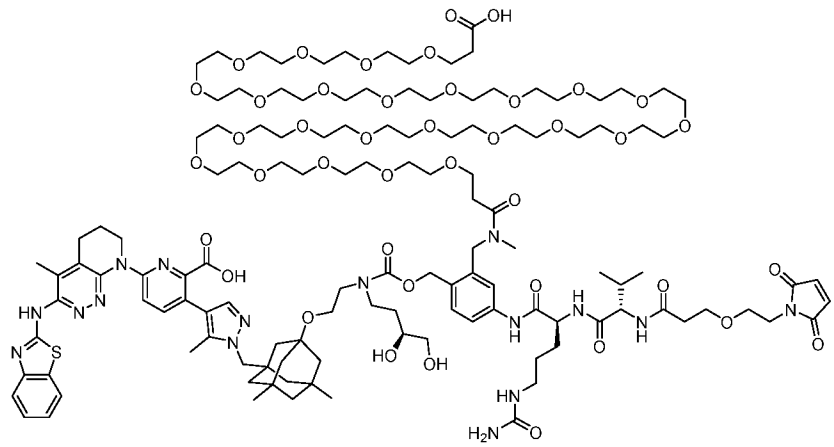
Synthesis of 3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid



[1025] Following **GENERAL PROCEDURE 4** with 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)(2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (28.4 mg, 0.011 mmol), 3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-

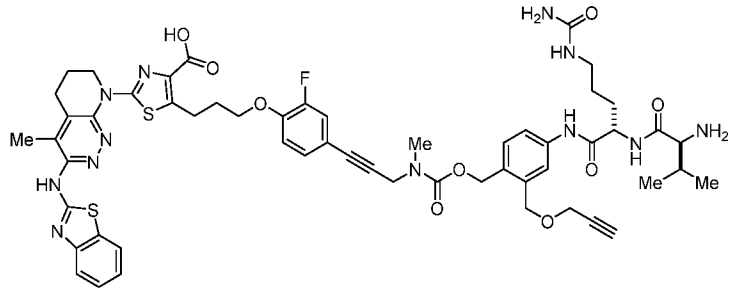
4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid was obtained. HRMS: M+H = 2471.3301; Rt=1.97 min (5 min acidic method).

Synthesis of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (L11C-P19)



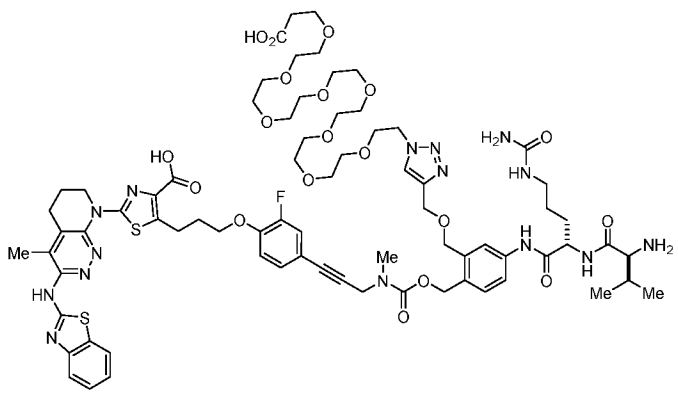
[1026] Following **GENERAL PROCEDURE 5** with 3-(1-(((1S,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid (35.6 mg, 0.014 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (10.7 mg, 0.034 mmol, 2.5 equiv.), 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1S,3s,5R,7S)-3-(2-((((2-(80-carboxy-2-methyl-3-oxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-pentacosaoxa-2-azaoctacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)((S)-3,4-dihydroxybutyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid was obtained. HRMS: M+H = 2666.3701; Rt=2.19 min (5 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



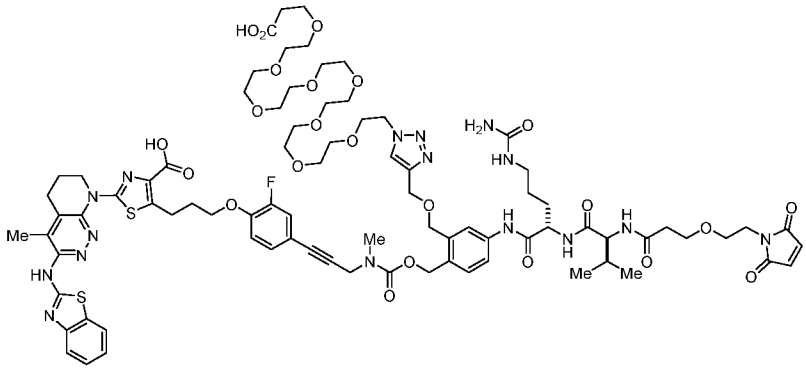
[1027] Following **GENERAL PROCEDURE 9** with 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (40.0 mg, 0.062 mmol) and (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (57.1 mg, 0.068 mmol, 1.1 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1117.8; Rt=0.84 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1028] Following **GENERAL PROCEDURE 7** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (18.2 mg, 0.016 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (9.1 mg, 0.020 mmol, 1.2 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 793.1; Rt=1.17 min (2 min acidic method).

Synthesis of 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (L7C-P3)

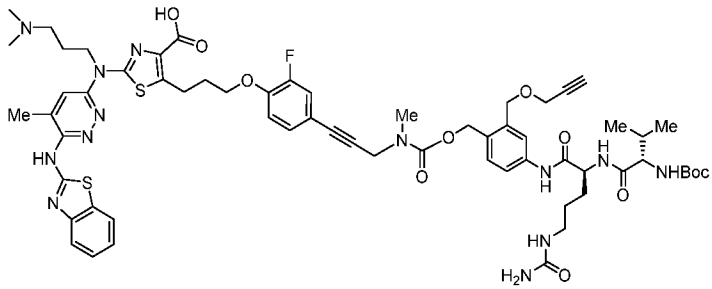


[1029] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (10.5 mg, 0.007 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (2.5 mg, 0.08 mmol, 1.2 equiv.), 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-

2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 891.2; Rt=2.56 min (5 min acidic method).

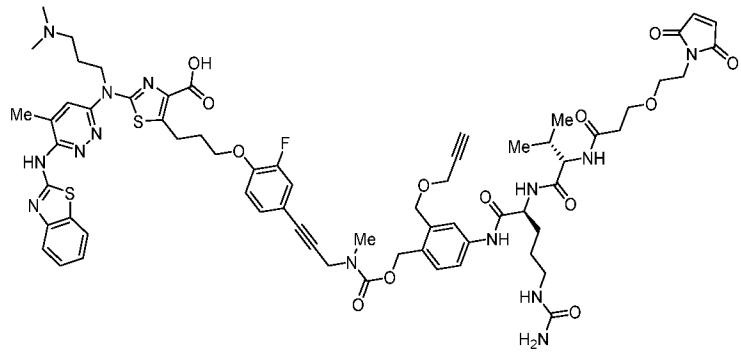
GENERAL PROCEDURE 10

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid



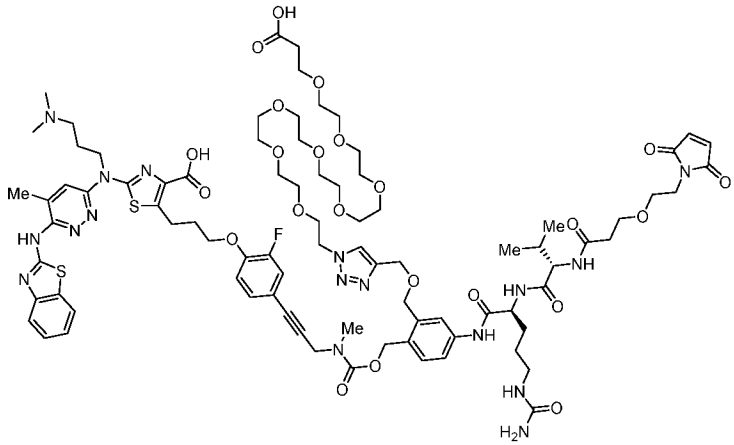
[1030] To a solution of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (30.0 mg, 0.039 mmol) and tert-butyl ((R)-3-methyl-1-(((R)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (36.5 mg, 0.051 mmol, 1.3 equiv.) in DMF (1.0 mL) was added DIPEA (0.034 mL, 0.197 mmol, 5.0 equiv.). The mixture was stirred for 2 hours at RT. DMSO (1.0 mL) was added and the solution was purified by RP-HPLC ISCO gold chromatography (0-100% MeCN/H2O, 0.1% TFA modifier). Upon lyophilisation, 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1262.5100; Rt=2.47 min (5 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((R)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid



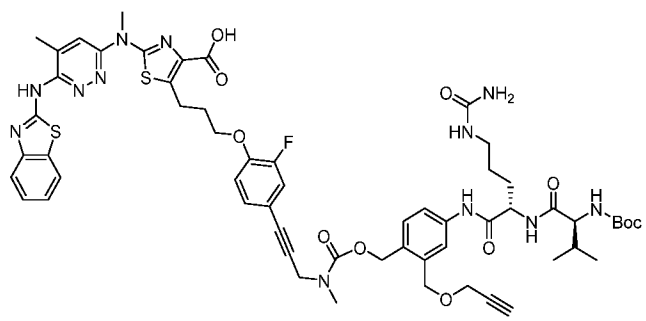
[1031] Following **GENERAL PROCEDURE 8** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (40.0 mg, 0.032 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (11.8 mg, 0.038 mmol, 1.2 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((R)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1357.5262; Rt=1.16 min (2 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (L7C-P6)



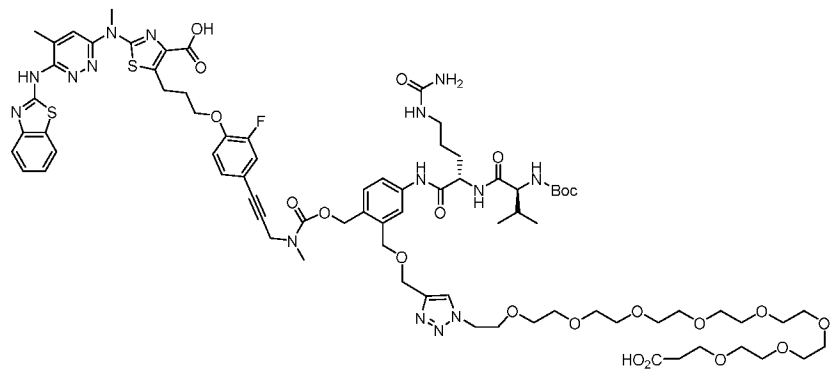
[1032] Following **GENERAL PROCEDURE 7** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((4-((S)-2-((R)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (10.0 mg, 0.008 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (6.9 mg, 0.015 mmol, 2.0 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(3-(dimethylamino)propyl)amino)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1824.7700; Rt=2.19 min (5 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid



[1033] Following **GENERAL PROCEDURE 10** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (50.0 mg, 0.081 mmol) and tert-butyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)-3-((prop-2-yn-1-yloxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (57.7 mg, 0.081 mmol, 1.0 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1192.2; Rt=0.88 min (2 min basic method).

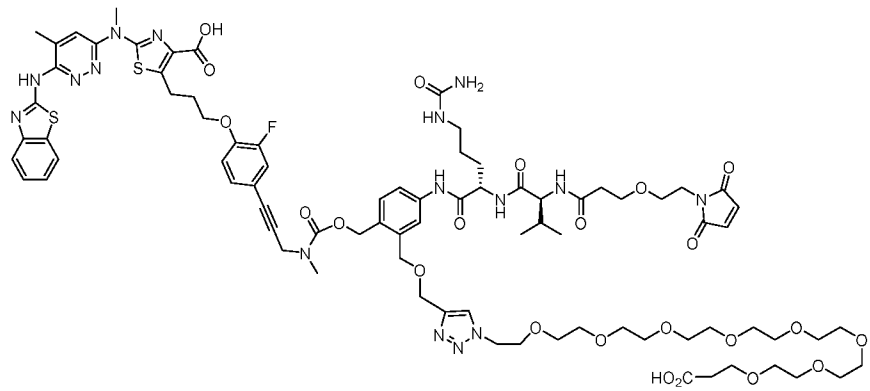
Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid



[1034] Following **GENERAL PROCEDURE 7** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (38.0 mg, 0.032 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (23.9 mg, 0.051 mmol, 1.6 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-

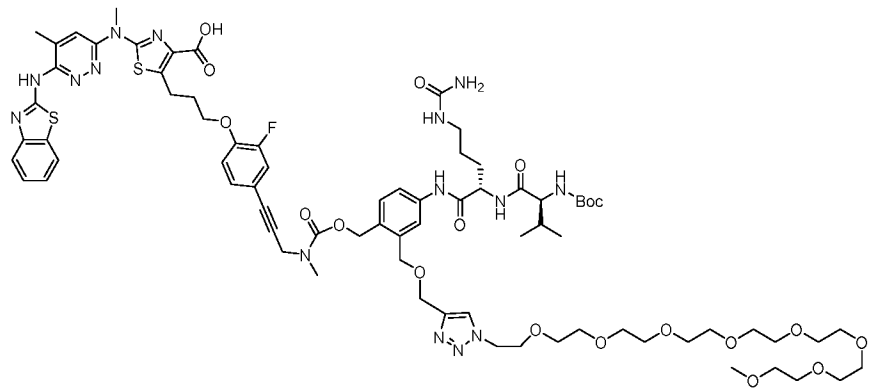
fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 830.6; Rt=0.73 min (2 min basic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (L7C-P7)



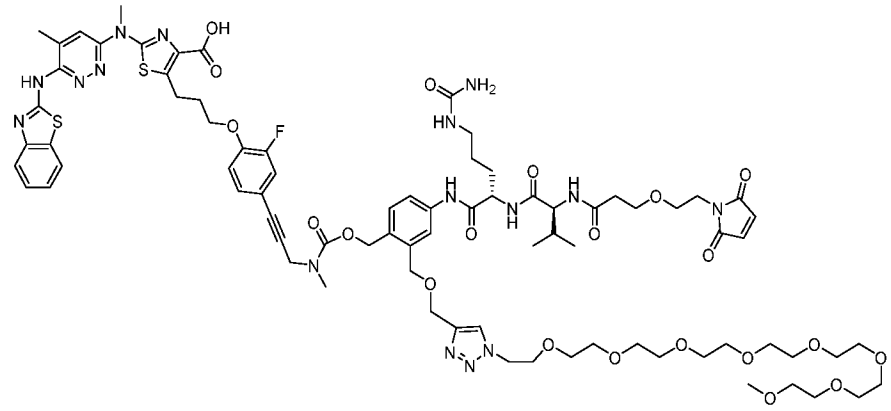
[1035] Following **GENERAL PROCEDURE 8** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (25.0 mg, 0.015 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (7.0 mg, 0.023 mmol, 1.5 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((2-(((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 877.9; Rt=1.07 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)thiazole-4-carboxylic acid



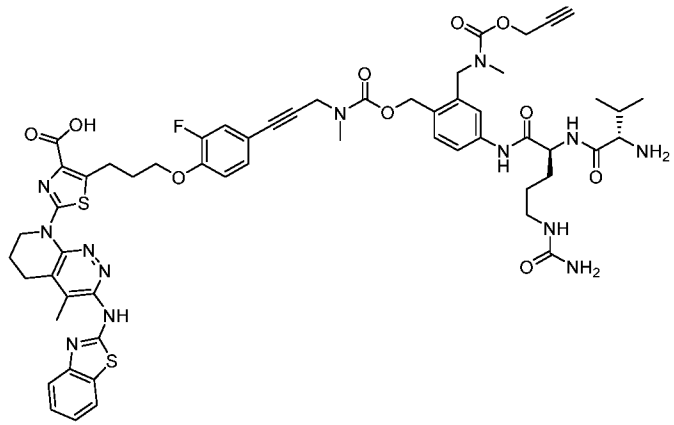
[1036] Following **GENERAL PROCEDURE 7** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yloxy)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (45.0 mg, 0.038 mmol) and 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (21.7 mg, 0.053 mmol, 1.4 equiv.), 5-(3-(4-(3-((((2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 800.9; Rt=1.14 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)thiazole-4-carboxylic acid (L8C-P7)



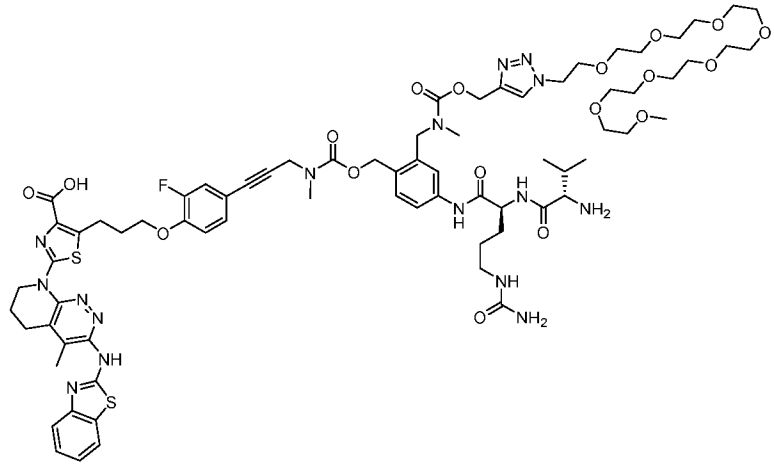
[1037] Following **GENERAL PROCEDURE 8** with 5-(3-(4-(3-((((2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)thiazole-4-carboxylic acid (49.0 mg, 0.031 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (14.3 mg, 0.046 mmol, 1.5 equiv.), 5-(3-(4-(3-((((2-(((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(methyl)amino)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 848.6; Rt=1.08 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



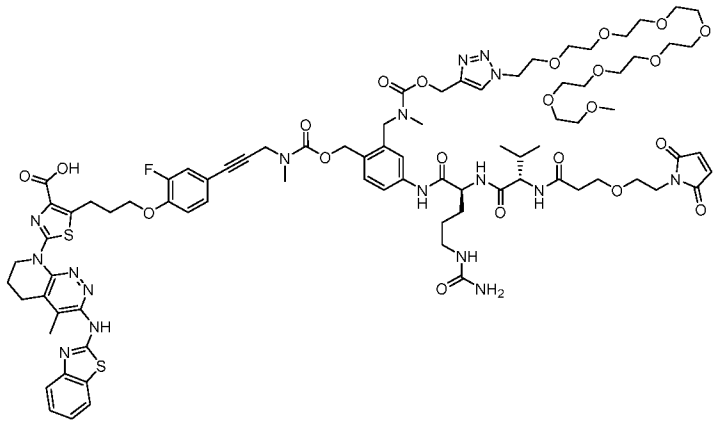
[1038] Following **GENERAL PROCEDURE 9** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(methyl)carbamate (72.0 mg, 0.081 mmol) and 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (52.0 mg, 0.081 mmol, 1.0 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1174.3; Rt=1.12 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1039] Following **GENERAL PROCEDURE 7** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (22.0 mg, 0.019 mmol) and 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (23.0 mg, 0.056 mmol, 3.0 equiv.), 5-(3-(4-(3-((((2-((((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1583.8199; Rt=2.30 min (5 min acidic method).

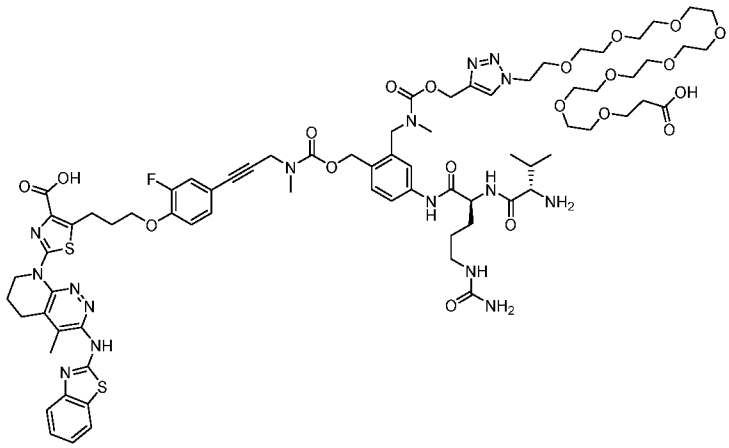
Synthesis of 5-(3-(4-(3-((((2-((((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (L8C-P3)



[1040] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((2-((((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (12.3 mg, 0.008 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (4.8 mg, 0.016 mmol, 2.0 equiv.), 5-(3-(4-(3-((((2-((((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-

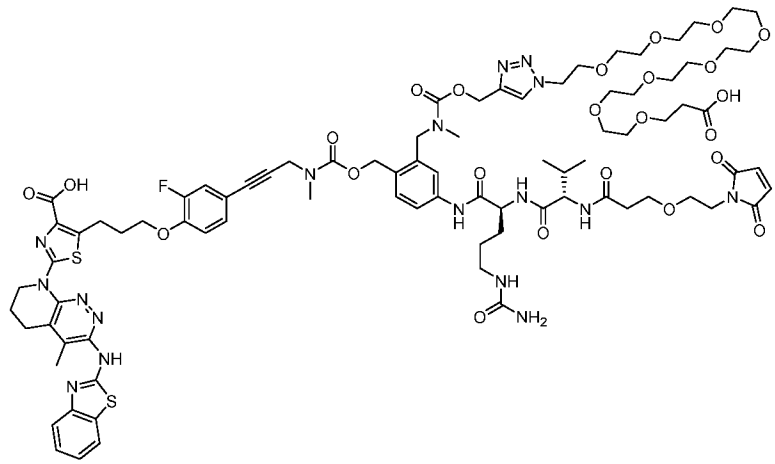
ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1778.6500; Rt=2.67 min (5 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



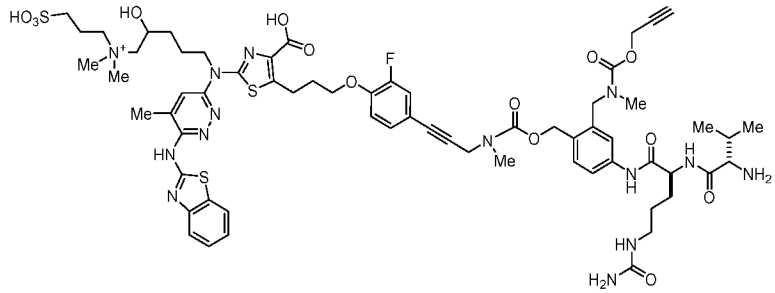
[1041] Following **GENERAL PROCEDURE 7** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (20.0 mg, 0.017 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (23.9 mg, 0.051 mmol, 3.0 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1641.8900; Rt=2.24 min (5 min acidic method).

Synthesis of 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (L3C-P3)



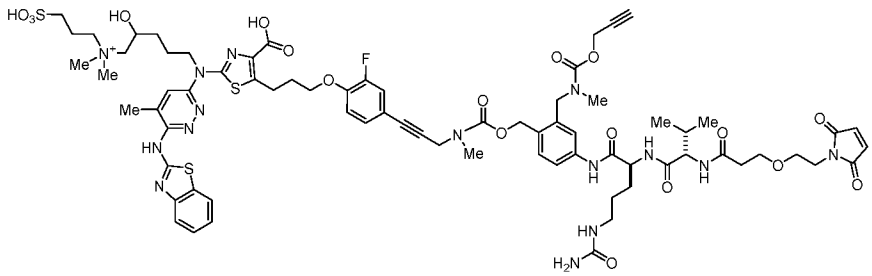
[1042] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (5.5 mg, 0.003 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (2.1 mg, 0.007 mmol, 2.0 equiv.), 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 1837.6300; Rt=2.61 min (5 min acidic method).

Synthesis of 5-((5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-4-carboxythiazol-2-yl)(6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium



[1043] Following **GENERAL PROCEDURE 9** with prop-2-yn-1-yl (5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)carbamate (30.0 mg, 0.034 mmol) and 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (28.8 mg, 0.034 mmol, 1.0 equiv.), 5-((5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-4-carboxythiazol-2-yl)(6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium was obtained. LCMS: M+H = 1387.1; Rt=0.98 min (2 min acidic method).

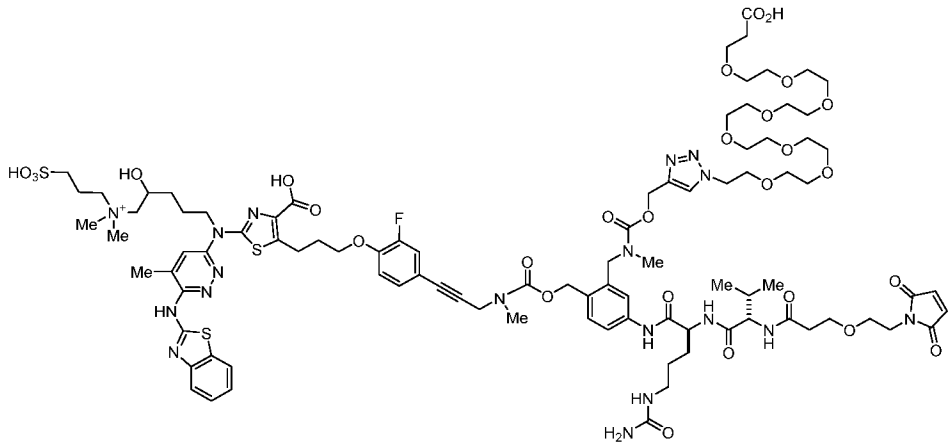
Synthesis of 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium



[1044] Following **GENERAL PROCEDURE 5** with 5-((5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-4-carboxythiazol-2-yl)(6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (35.6 mg, 0.026 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

yl)ethoxy)propanoate (12.0 mg, 0.039 mmol, 1.5 equiv.), 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium was obtained. LCMS: M/2+H = 791.2; Rt=1.01 min (2 min acidic method).

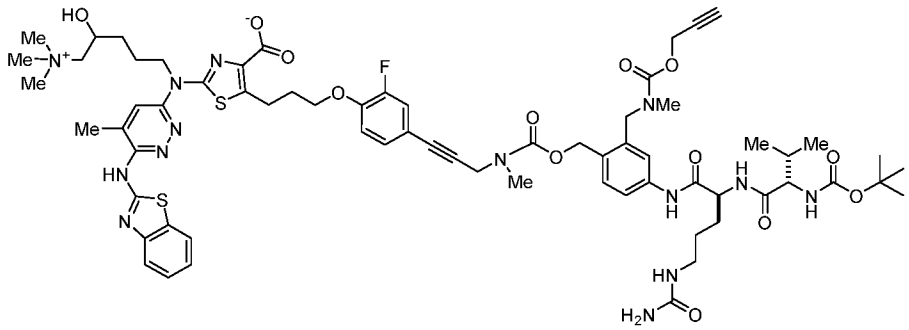
Synthesis of 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (L3C-P4)



[1045] Following **GENERAL PROCEDURE 7** with 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (23.0 mg, 0.015 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (20.4 mg, 0.044 mmol, 3.0 equiv.), 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-

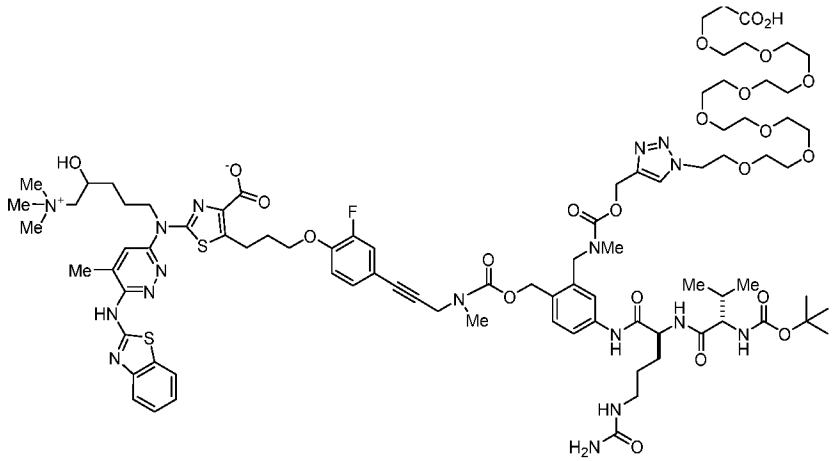
yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium was obtained. HRMS: M+H = 2047.8101; Rt=2.24 min (5 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate



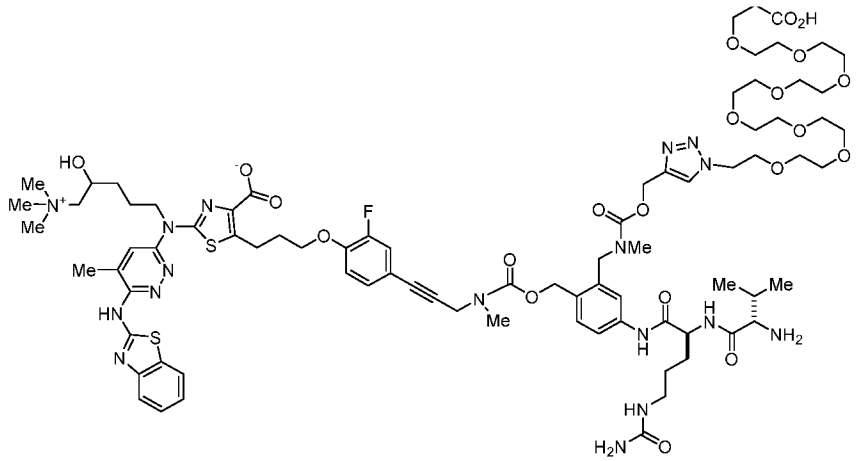
[1046] Following **GENERAL PROCEDURE 10** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylate (40.0 mg, 0.054 mmol) and prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(methyl)carbamate (41.2 mg, 0.054 mmol, 1.0 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate was obtained. LCMS: M+H = 1378.1; Rt=1.11 min (2 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate



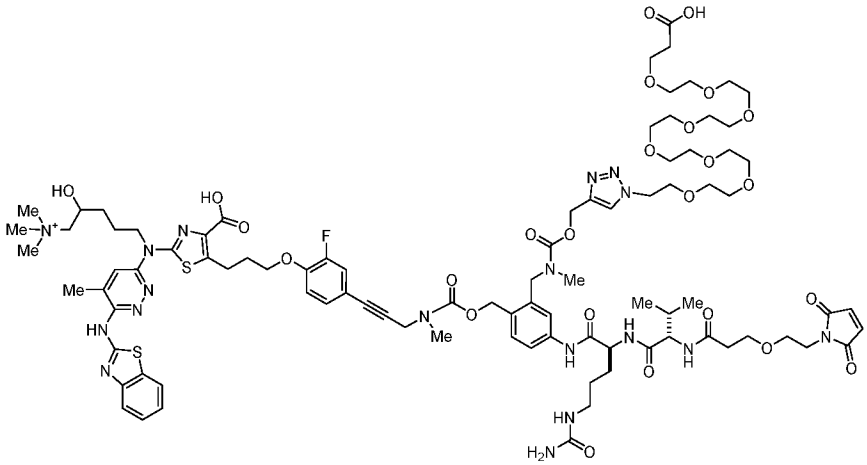
[1047] Following **GENERAL PROCEDURE 7** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methyl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate (67.0 mg, 0.049 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (34.1 mg, 0.073 mmol, 1.5 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate was obtained. LCMS: M/2+H = 923.6; Rt=1.06 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate



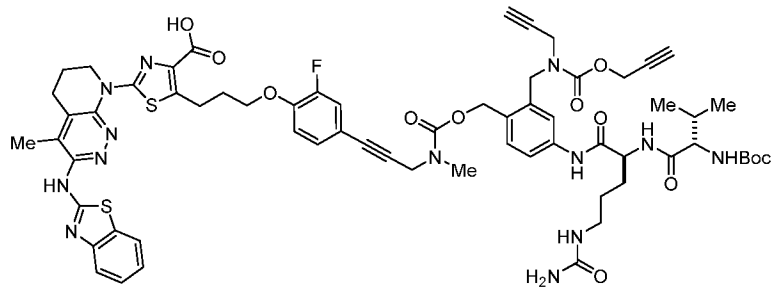
[1048] Following **GENERAL PROCEDURE 4** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate (53.7 mg, 0.029 mmol), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate was obtained. LCMS: M/2+H = 873.5; Rt=1.03 min (2 min acidic method).

Synthesis of 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N,N-trimethylpentan-1-aminium (L3C-P5)



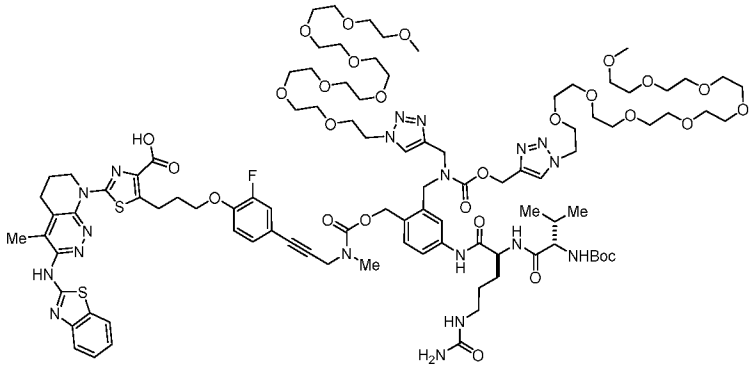
[1049] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate (50.8 mg, 0.029 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (13.6 mg, 0.044 mmol, 1.5 equiv.), 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)(methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N,N-trimethylpentan-1-aminium was obtained. HRMS: M+H = 1939.8199; Rt=2.17 min (5 min acidic method).

Synthesis of 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid



[1050] Following **GENERAL PROCEDURE 10** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate (49.3 mg, 0.062 mmol) and 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic (40.0 mg, 0.062 mmol, 1.0 equiv.), 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1297.0; Rt=1.28 min (2 min acidic method).

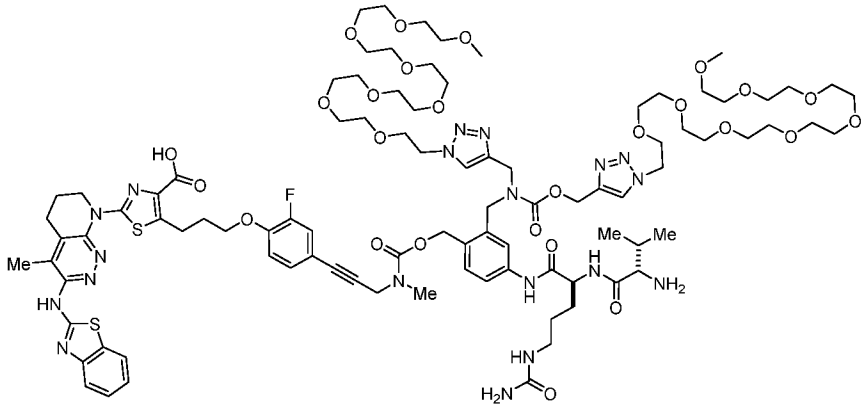
Synthesis of 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1051] Following **GENERAL PROCEDURE 7** with 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (49.0 mg, 0.038 mmol) and 25-azido-2,5,8,11,14,17,20,23-octaoxapentacosane (34.0 mg, 0.083 mmol, 2.2 equiv.), 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-

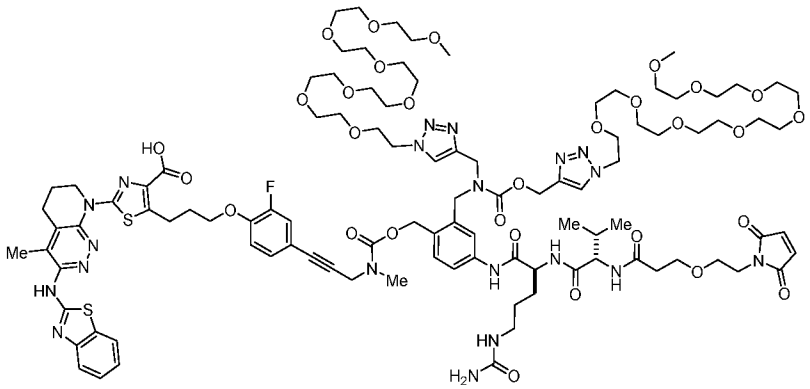
c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 1059.4; Rt=1.16 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



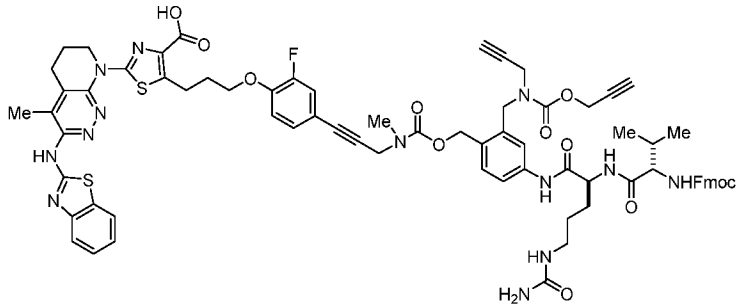
[1052] Following **GENERAL PROCEDURE 4** with 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (80.0 mg, 0.038 mmol), 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 1009.2; Rt=1.14 min (2 min acidic method).

Synthesis of 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (L1C-P3)



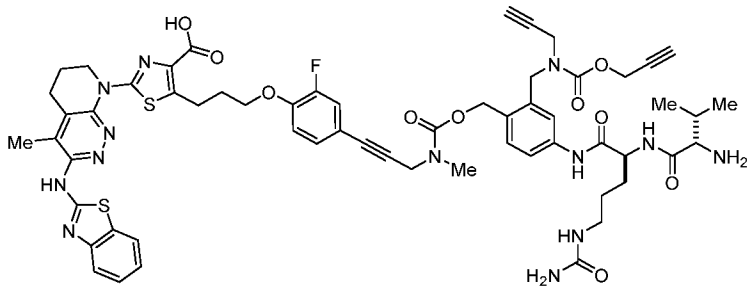
[1053] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (76.0 mg, 0.038 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (23.4 mg, 0.075 mmol, 2.0 equiv.), 5-(3-(4-(3-((((2-(((((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 2211.9700; Rt=2.56 min (5 min acidic method).

Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1054] Following **GENERAL PROCEDURE 10** with prop-2-yn-1-yl 5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl(prop-2-yn-1-yl)carbamate (56.9 mg, 0.062 mmol) and 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (40.0 mg, 0.062 mmol, 1.0 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1422.6; Rt=1.33 min (2 min acidic method).

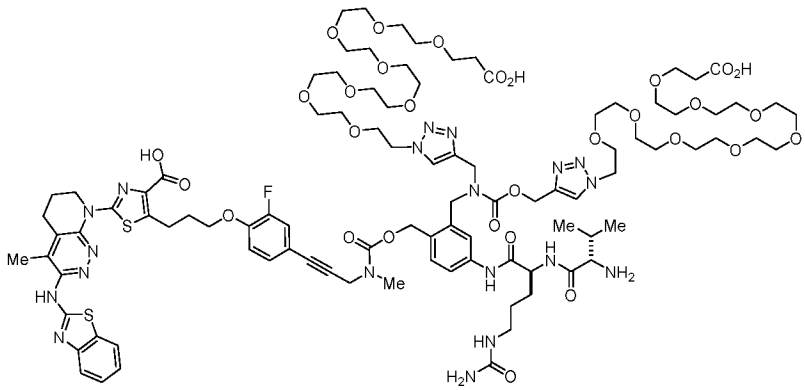
Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1055] A solution of 5-(3-(4-(3-((((4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-

yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (88.0 mg, 0.062 mmol) in 2.0 M dimethylamine in THF (3.1 mL, 6.20 mmol, 100.0 equiv.) was stirred for 80 min. at RT. The solvents were removed in vacuo, diluted residue in DMSO (1.0 mL) and purified by RP-HPLC ISCO gold chromatography (0-100% MeCN/H2O, 0.05% TFA modifier). Upon lyophilisation, 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M+H = 1199.2; Rt=1.06 min (2 min acidic method).

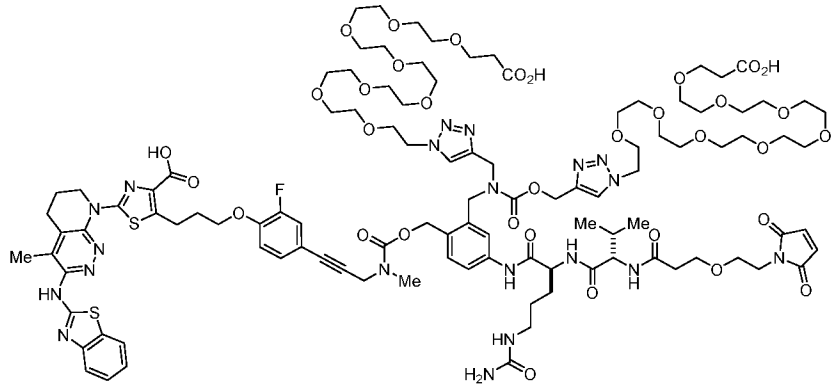
Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid



[1056] Following **GENERAL PROCEDURE 7** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-((prop-2-yn-1-yl((prop-2-yn-1-yloxy)carbonyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (20.8 mg, 0.017 mmol) and 1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid (17.9 mg, 0.038 mmol, 2.2 equiv.), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-

(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid was obtained. LCMS: M/2+H = 1068.2; Rt=0.99 min (2 min acidic method).

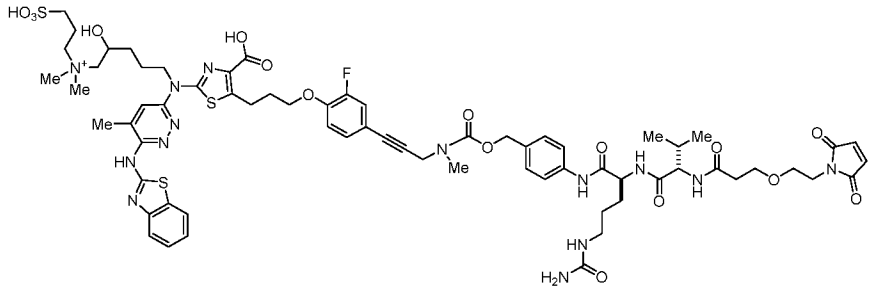
Synthesis of 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (L10C-P3)



[1057] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)thiazole-4-carboxylic acid (36.3 mg, 0.017 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (5.3 mg, 0.017 mmol, 1.0 equiv.), 2-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-5-(3-(4-(3-((((2-(((((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methoxy)carbonyl)((1-(26-carboxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-

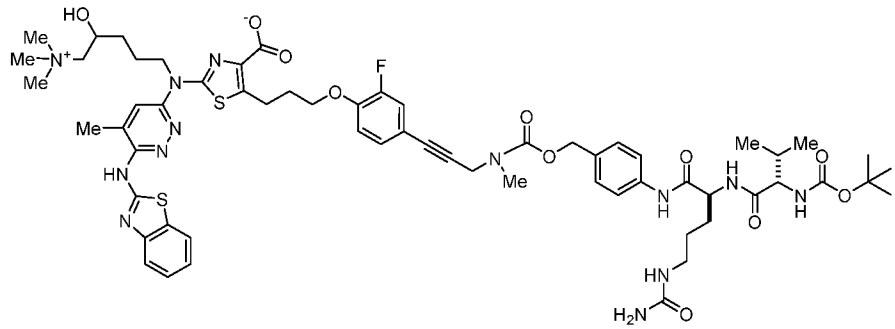
yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid was obtained. HRMS: M+H = 2327.9800; Rt=2.45 min (5 min acidic method).

Synthesis of 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (L9C-P4)



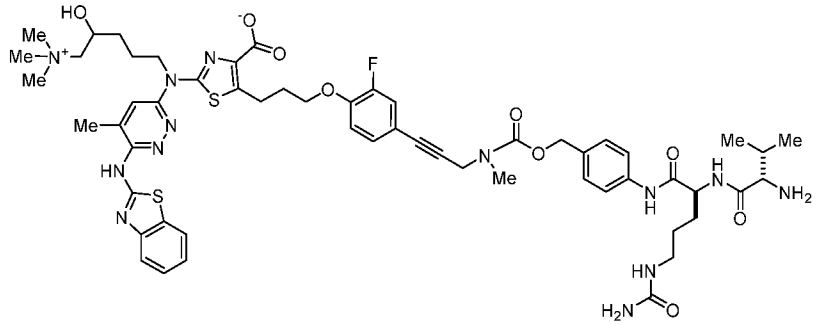
[1058] Following **GENERAL PROCEDURE 10** with 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate (20.0 mg, 0.027 mmol) and 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium (23.1 mg, 0.027 mmol, 1.0 equiv.), 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N-dimethyl-N-(3-sulfopropyl)pentan-1-aminium was obtained. HRMS: M+H = 1455.5300; Rt=2.31 min (5 min acidic method).

Synthesis of 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate



[1059] Following **GENERAL PROCEDURE 10** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(2-fluoro-4-(3-(methylamino)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylate (40.0 mg, 0.054 mmol) and tert-butyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (34.5 mg, 0.054 mmol, 1.0 equiv.), 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate was obtained. LCMS: M+H = 1253.8; Rt=1.11 min (2 min acidic method).

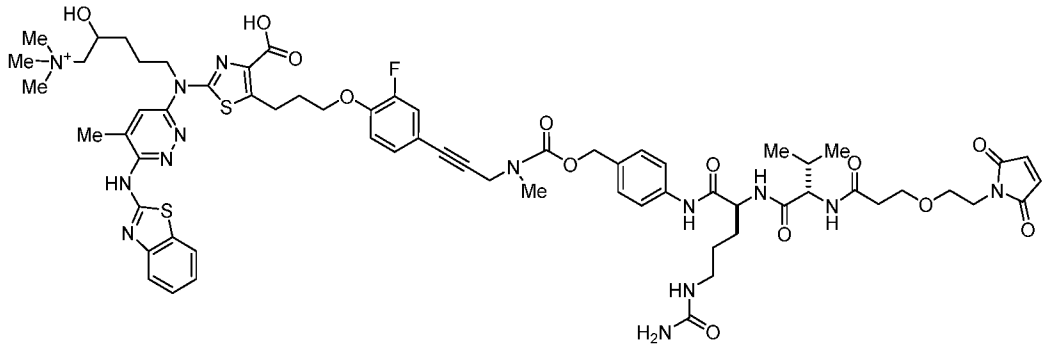
Synthesis of 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate



[1060] Following **GENERAL PROCEDURE 4** with 2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)-5-(3-(4-(3-((((4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazole-4-carboxylate (64.8 mg, 0.052 mmol), 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-

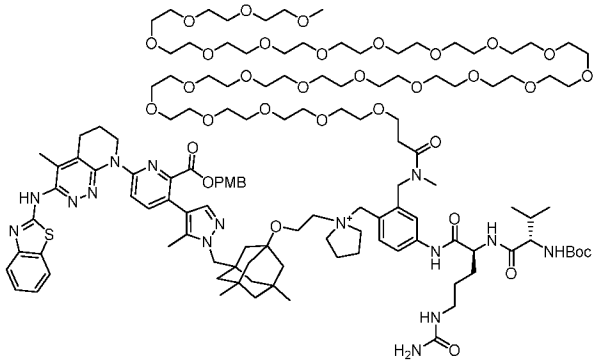
fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate was obtained. LCMS: M/2+H = 576.6; Rt=0.99 min (2 min acidic method).

Synthesis of 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N,N-trimethylpentan-1-aminium (L9C-P5)



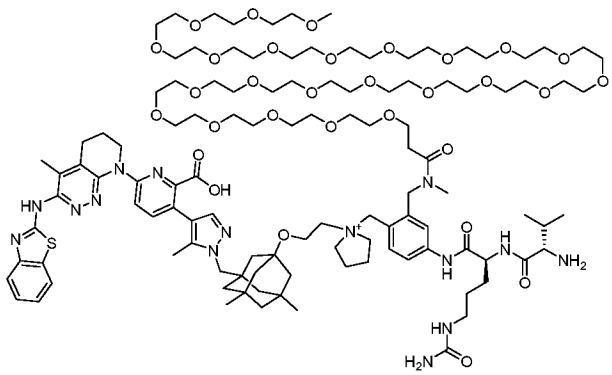
[1061] Following **GENERAL PROCEDURE 5** with 5-(3-(4-(3-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-hydroxy-5-(trimethylammonio)pentyl)amino)thiazole-4-carboxylate (59.0 mg, 0.051 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (19.1 mg, 0.061 mmol, 1.2 equiv.), 5-((6-(benzo[d]thiazol-2-ylamino)-5-methylpyridazin-3-yl)(4-carboxy-5-(3-(4-(3-((((4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)thiazol-2-yl)amino)-2-hydroxy-N,N,N-trimethylpentan-1-aminium was obtained. HRMS: M+H = 1347.5300; Rt=2.23 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)pyrrolidin-1-ium



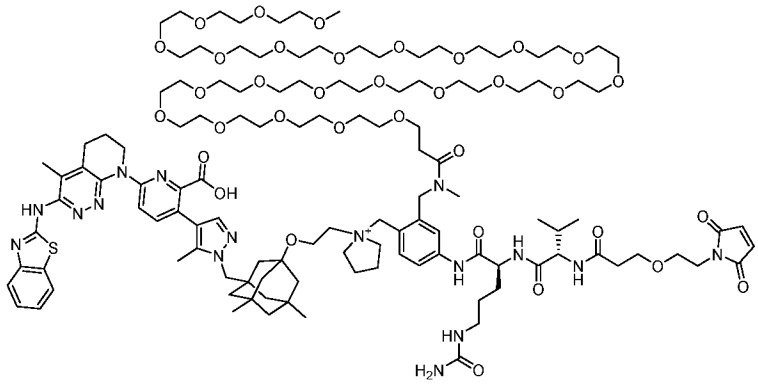
[1062] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (40 mg, 0.028 mmol) and 2,5-dioxopyrrolidin-1-yl 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxatetraheptacontan-74-oate (51.5 mg, 0.042 mmol, 1.5 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2511.4099; Rt=2.44 min (5 min acidic method).

Synthesis of 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium



[1063] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)pyrrolidin-1-ium (50 mg, 0.0199 mmol), 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2291.3101; Rt=1.93 min (5 min acidic method).

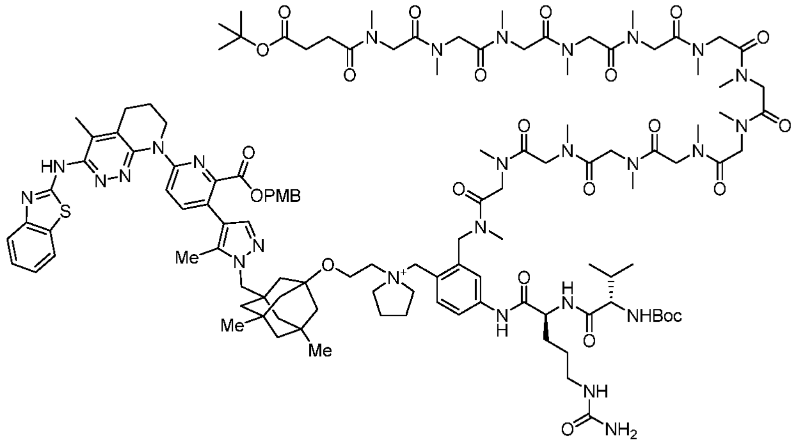
Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)pyrrolidin-1-ium (L30A-P21)



[1064] Following **GENERAL PROCEDURE 5** with 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium (37 mg, 0.015 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (11.4 mg, 0.0367 mmol, 2.5 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-

(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-(75-methyl-74-oxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetracosaoxa-75-azahexaheptacontan-76-yl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2486.3301; Rt=2.14 min (5 min acidic method).

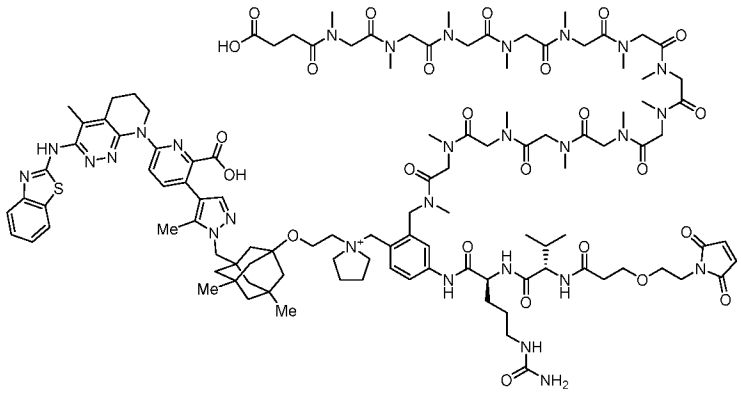
Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,44,44-pentadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42-tetradecaoxo-43-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazapentatetracontyl)benzyl)pyrrolidin-1-ium



[1065] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (35 mg, 0.021 mmol) and 3,6,9,12,15,18,21,24,27,30,33,36,42,42-tetradecamethyl-4,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxo-41-oxa-3,6,9,12,15,18,21,24,27,30,33,36-dodecaazatritetracontanoic acid (21.9 mg, 0.021 mmol, 1.0 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,44,44-pentadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42-tetradecaoxo-43-oxa-

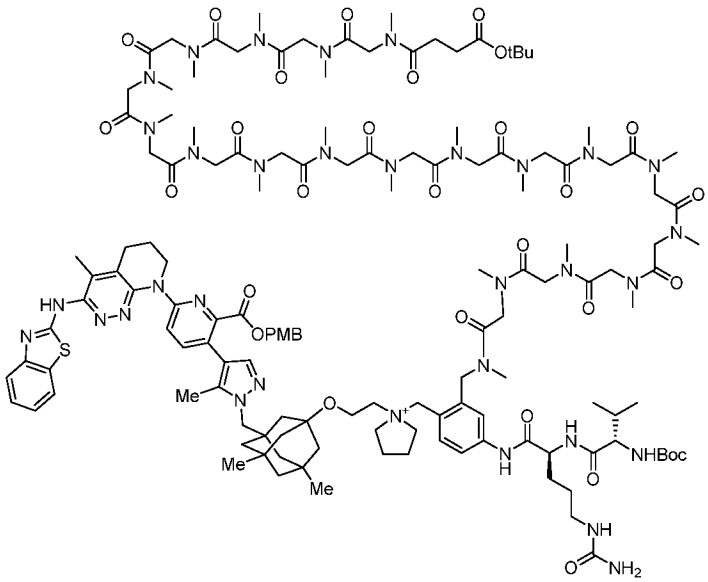
2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazapentatetracontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: $[(M+)+H+]^{+2}/2$=1211.6500; Rt=2.31 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(41-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazahentetracontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium (L35A-P21)



[1066] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,44,44-pentadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42-tetradecaoxo-43-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazapentatetracontyl)benzyl)pyrrolidin-1-ium (24 mg, 0.0095 mmol) and then taking the crude product on and following **GENERAL PROCEDURE 5** with 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (5.9 mg, 0.019 mmol, 2 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(41-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazahentetracontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2340.1699; Rt=1.87 min (5 min acidic method).

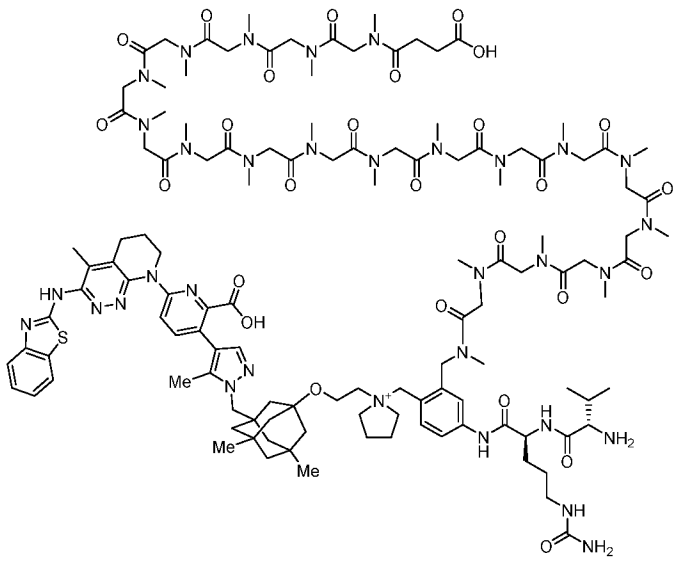
Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,62,62-henicosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60-icosaoxo-61-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazatrihexacontyl)benzyl)pyrrolidin-1-ium



[1067] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (47 mg, 0.0286 mmol) and 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,60,60-icosamethyl-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58-nonadecaoxo-59-oxa-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54-octadecaazahenhexacontanoic acid (41.6 mg, 0.0286 mmol, 1.0 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,62,62-henicosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60-icosaoxo-61-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-

nonadecaazatrihexacontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2487.5400; Rt=2.26 min (5 min acidic method).

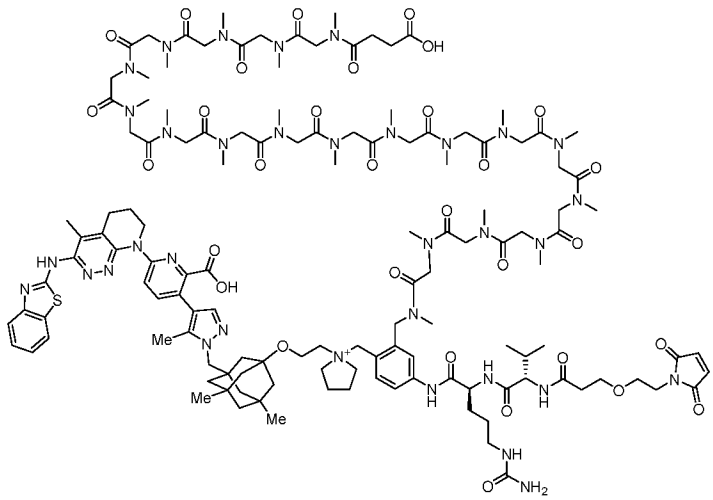
Synthesis of 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(59-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazanonapentacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium



[1068] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,62,62-henicosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60-icosaoxo-61-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazatrihexacontyl)benzyl)pyrrolidin-1-ium (46 mg, 0.0155 mmol), 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(59-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazanonapentacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-

methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2571.3401; Rt=1.60 min (5 min acidic method).

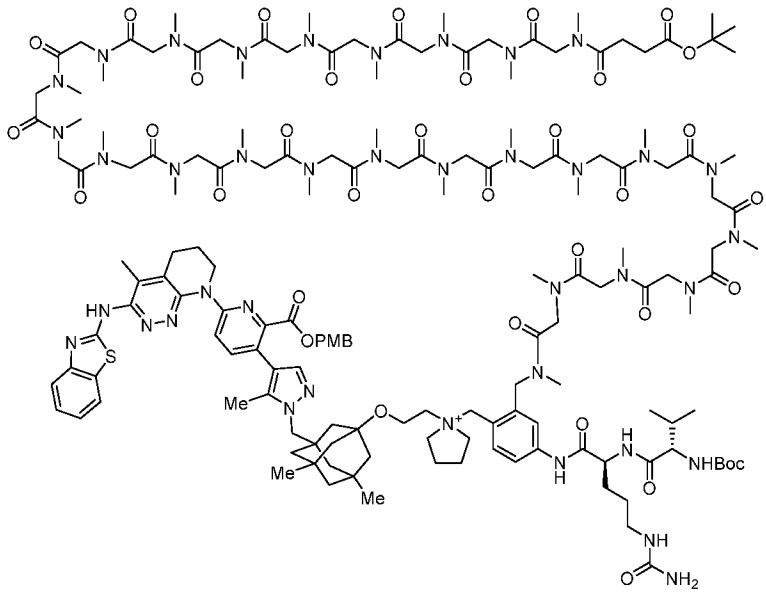
Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(59-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazanonapentacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium (L36A-P21)



[1069] Following **GENERAL PROCEDURE 5** with 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(59-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazanonapentacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium (17.0 mg, 0.0055 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (2.4 mg, 0.0076 mmol, 1.4 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(59-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57-nonadecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56-nonadecaazanonapentacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-

methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2766.3899; Rt=1.82 min (5 min acidic method).

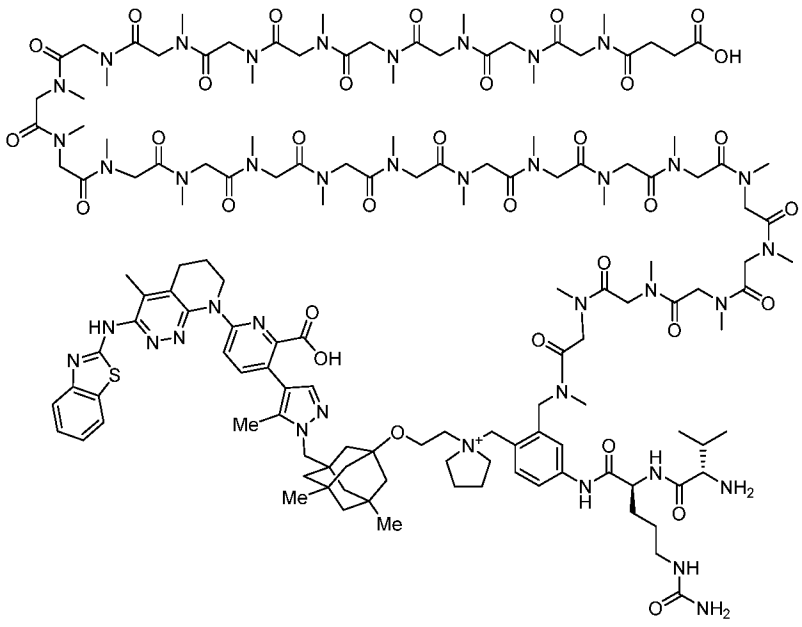
Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,80,80-heptacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-hexacosaoxo-79-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazahenoctacontyl)benzyl)pyrrolidin-1-ium



[1070] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (40 mg, 0.028 mmol) and 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,78,78-hexacosamethyl-4,7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-pentacosaoxo-77-oxa-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaazanonaheptacontanoic acid (58.5 mg, 0.031 mmol, 1.1 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-

c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,80,80-heptacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-hexacosaoxo-79-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazahenoctacontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 3273.7500; Rt=2.24 min (5 min acidic method).

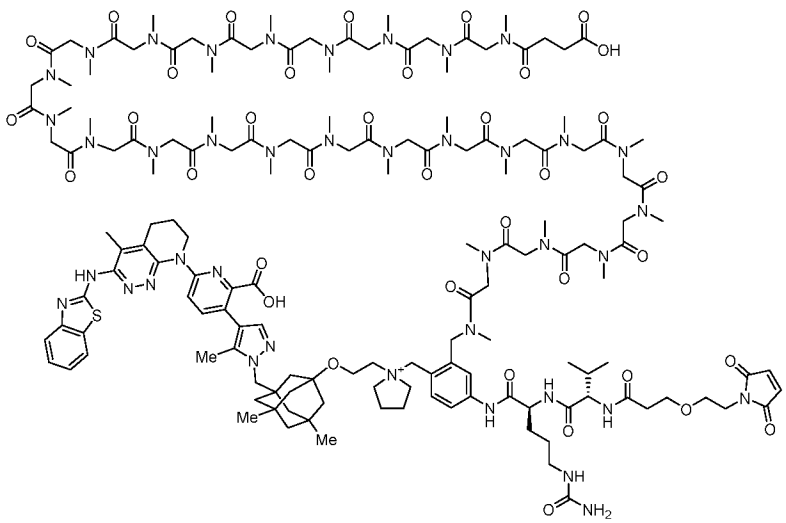
Synthesis of 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(77-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75-pentacosaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazaheptaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium



[1071] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-

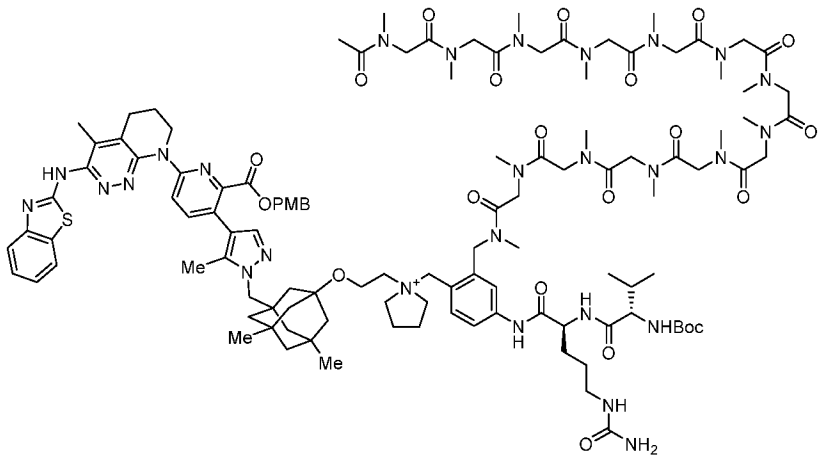
(2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,80,80-heptacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78-hexacosaoxo-79-oxa-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazahenoctacontyl)benzyl)pyrrolidin-1-ium (20 mg, 0.0063 mmol), 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(77-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75-pentacosaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazaheptaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2997.6001; Rt=1.65 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(77-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75-pentacosaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazaheptaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium (L37A-P21)



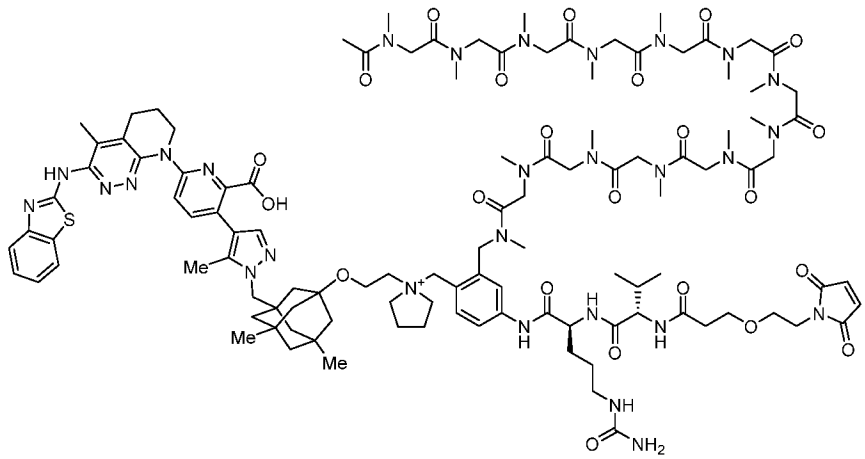
[1072] Following **GENERAL PROCEDURE 5** with 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(77-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75-pentacosaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazaheptaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium (35 mg, 0.011 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (4.7 mg, 0.015 mmol, 1.4 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(77-carboxy-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75-pentacosaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74-pentacosaazaheptaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 3192.6399; Rt=1.86 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazatetracontyl)benzyl)pyrrolidin-1-ium



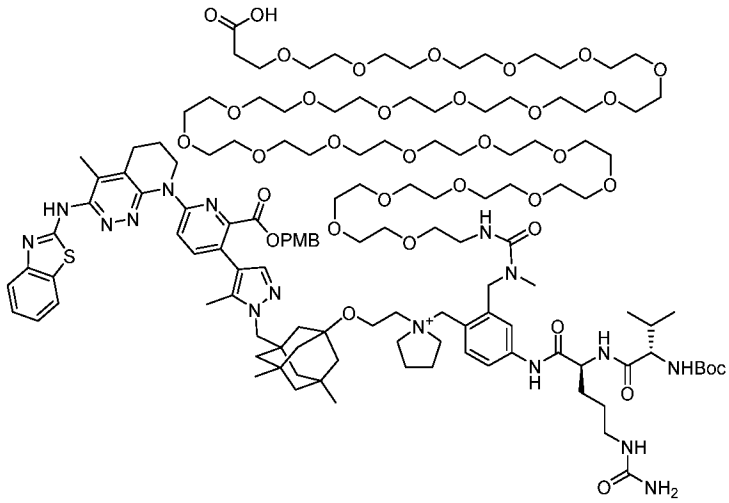
[1073] Following **GENERAL PROCEDURE 3** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (70 mg, 0.043 mmol) and 3,6,9,12,15,18,21,24,27,30,33,36-dodecamethyl-4,7,10,13,16,19,22,25,28,31,34,37-dodecaoxo-3,6,9,12,15,18,21,24,27,30,33,36-dodecaazaoctatriacontanoic acid (38.9 mg, 0.043 mmol, 1.0 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazatetracontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2307.2300; Rt=2.20 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazatetracontyl)benzyl)pyrrolidin-1-ium (L38A-P21)



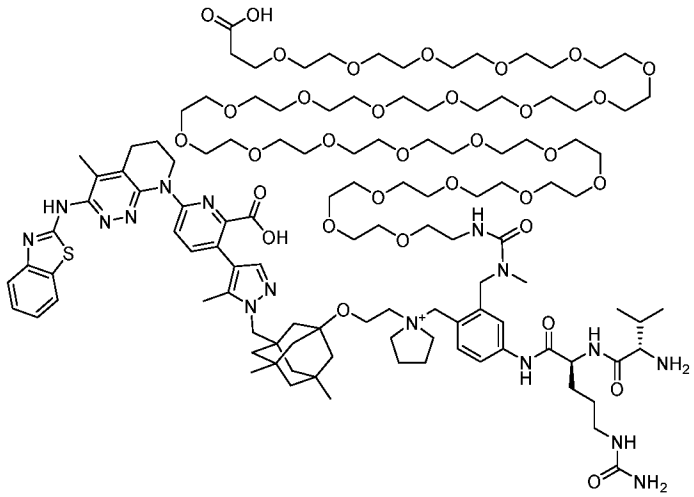
[1074] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazatetracontyl)benzyl)pyrrolidin-1-ium (67 mg, 0.029 mmol) and then taking the crude reaction product and following **GENERAL PROCEDURE 5** with 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (13.5 mg, 0.044 mmol, 1.5 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)-2-(2,5,8,11,14,17,20,23,26,29,32,35,38-tridecamethyl-3,6,9,12,15,18,21,24,27,30,33,36,39-tridecaoxo-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaazatetracontyl)benzyl)pyrrolidin-1-iumwas obtained. HRMS: M+= 2282.2500; Rt=1.89 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)pyrrolidin-1-ium



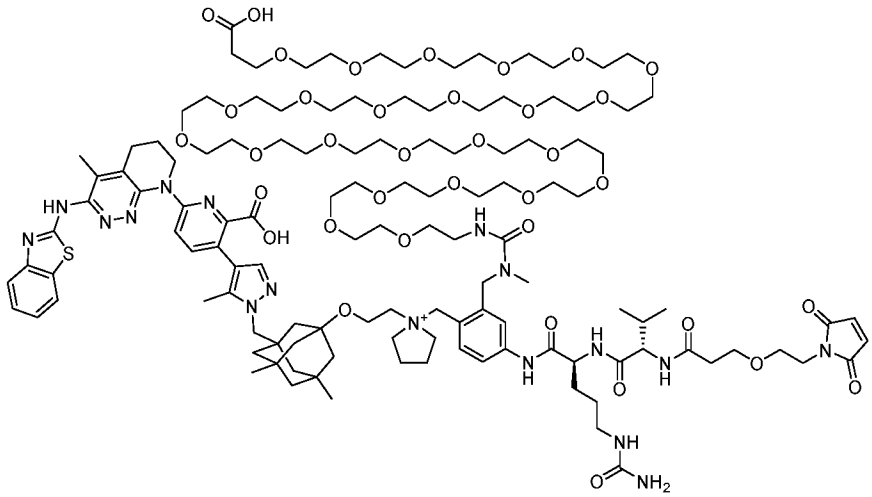
[1075] A mixture of 1-amino-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxapentaheptacontan-75-oic acid (67 mg, 0.059 mmol, 1.28 equiv.), bis(4-nitrophenyl) carbonate (17 mg, 0.057 mmol, 1.25 equiv.), and DIPEA (48 µL, 0.28 mmol, 6.0 equiv.)) in DMF (1 mL) was stirred at RT for 1 h at which time 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (65 mg, 0.046 mmol, 1.0 equiv.) and additional DIEA (80 uL, 0.46 mmol, 10 equiv.) were added. After stirring for 1 hour the solution was diluted with DMSO (2.5 mL) and purified by RP-HPLC. After lyophilization, 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2584.4399; Rt=2.39 min (5 min acidic method).

Synthesis of 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium



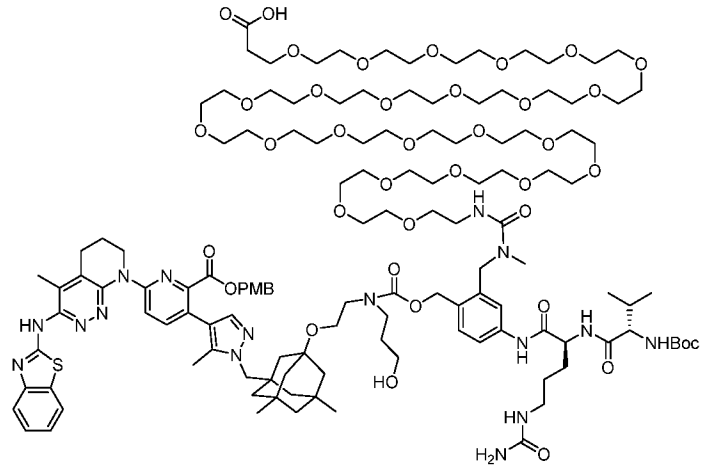
[1076] Following **GENERAL PROCEDURE 4** with 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)pyrrolidin-1-ium (58 mg, 0.021 mmol), 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium was obtained. HRMS: $[(M+)+H+)]^{+2}/2$= 1183.1700; Rt=1.88 min (5 min acidic method).

Synthesis of 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium (L42A-P21)



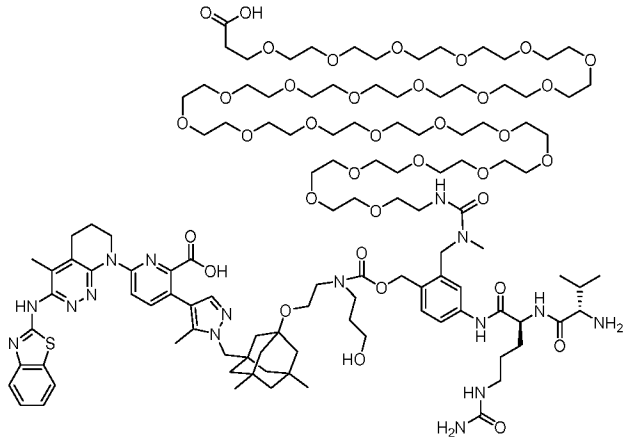
[1077] Following **GENERAL PROCEDURE 5** with 1-(4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)-1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)pyrrolidin-1-ium (61 mg, 0.024 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (10.2 mg, 0.033 mmol, 1.4 equiv.), 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)pyrrolidin-1-ium was obtained. HRMS: M+= 2559.3701; Rt=2.07 min (5 min acidic method).

Synthesis of 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazanonaheptacontan-79-oic acid



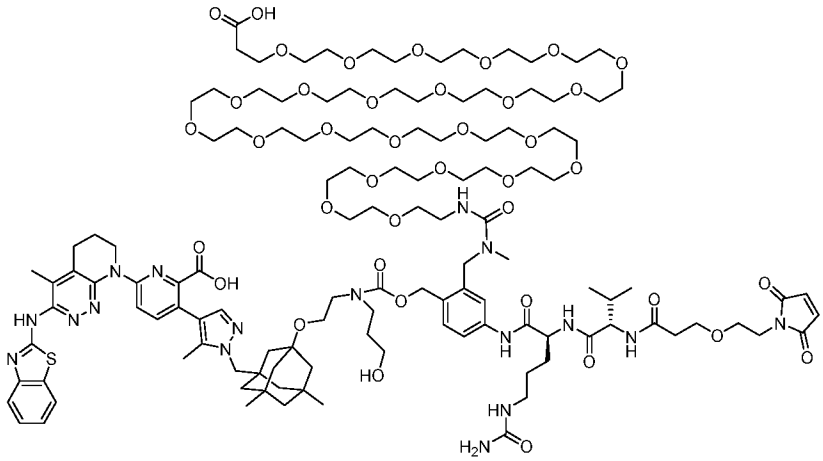
[1078] A mixture of 1-amino-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxapentaheptacontan-75-oic acid (45.9 mg, 0.040 mmol, 1.3 equiv.), bis(4-nitrophenyl) carbonate (12 mg, 0.0394 mmol, 1.28 equiv.), and DIPEA (32 µL, 0.184 mmol, 6.0 equiv.)) in DMF (1 mL) was stirred at RT for 1 h at which time 1-(2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)-1-(4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)-2-((methylamino)methyl)benzyl)pyrrolidin-1-ium (50 mg, 0.0308 mmol, 1.0 equiv.) and additional DIEA (53.7 uL, 0.308 mmol, 10 equiv.) were added. After stirring for 1 hour the solution was diluted with DMSO (2.5 mL) and purified by RP-HPLC. After lyophilization, 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazanonaheptacontan-79-oic acid was obtained. HRMS: $(M+2H+)^{+2}/2$= 1316.7200; Rt=2.64 min (5 min acidic method).

Synthesis of 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid



[1079] Following **GENERAL PROCEDURE 4** with 1-(2-((((2-(((1s,3r,5R,7S)-3-((4-(6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-2-(((4-methoxybenzyl)oxy)carbonyl)pyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(3-hydroxypropyl)carbamoyl)oxy)methyl)-5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenyl)-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazanonaheptacontan-79-oic acid (39 mg, 0.014 mmol), 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid was obtained. General Procedure 4 was modified to clip small amount of TFA ester which formed on the primary hydroxyl. Upon concentration of TFA/CH2Cl2 the residue was dissolved in DMSO (1 mL), DIEA (125 uL, 50 equiv) was added followed by MeOH (1 mL). After standing 1 hour the ester was clipped and the solution was purified. HRMS: MH+= 2412.3101; Rt=2.03 min (5 min acidic method).

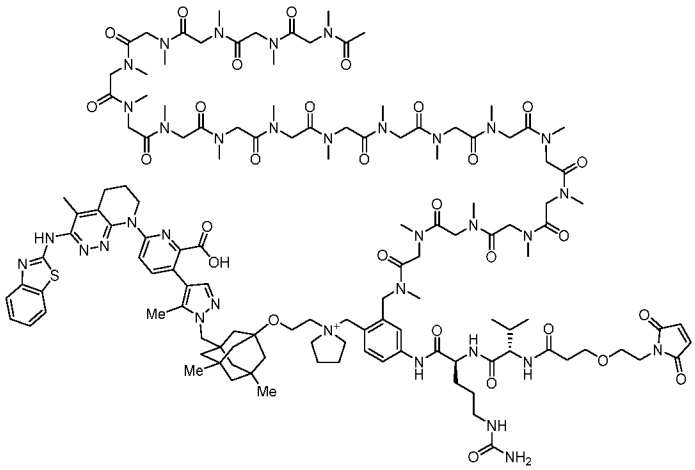
Synthesis of 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (L42C-P25)



[1080] Following **GENERAL PROCEDURE 5** with 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)-2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)picolinic acid (26 mg, 0.010 mmol) and 2,5-dioxopyrrolidin-1-yl 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoate (4.0 mg, 0.013 mmol, 1.25 equiv.), 6-(3-(benzo[d]thiazol-2-ylamino)-4-methyl-6,7-dihydropyrido[2,3-c]pyridazin-8(5H)-yl)-3-(1-(((1r,3s,5R,7S)-3-(2-((((2-(78-carboxy-2-methyl-3-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43,46,49,52,55,58,61,64,67,70,73,76-tetracosaoxa-2,4-diazaoctaheptacontyl)-4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(3-hydroxypropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid was obtained. HRMS: MH+= 2607.3601; Rt=2.27 min (5 min acidic method).

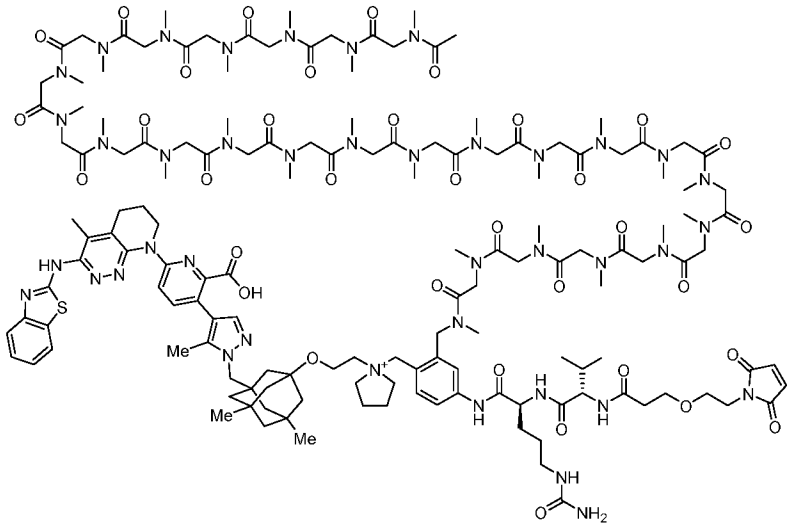
[1081] The following compounds were prepared using procedures similar to those described for L38A-P21:

L39A-P21



HRMS: M+= 2708.3999; Rt=1.85 min (5 min acidic method).

L40A-P21



HRMS: M+= 3134.6201; Rt=1.81 min (5 min acidic method).

[1082] The following compounds could be prepared using procedures similar to those described above:

L11A-P1, L11A-P21, L11A-P27, L11C-P19, L11C-P25, L30A-P1, L30C-P19, L30A-P21, L30C-P25, L30A-P27, L35A-P1, L35C-P19, L35A-P21, L35C-P25, L35A-P27, L36A-P1, L36C-P19, L36A-P21, L36C-P25, L36A-P27, L37A-P1, L37C-P19, L37A-P21, L37C-P25, L37A-P27, L38A-P1, L38C-P19, L38A-P21, L38C-P25, L38A-P27, L39A-P1, L39C-P19, L39A-P21, L39C-P25, L39A-P27, L40A-P1, L40C-P19, L40A-P21, L40C-P25, L40A-P27, L42A-P1, L42C-P19, L42A-P21, L42C-P25, L42A-P27, L67A-P1, L67C-P19, L67A-P21, L67C-P25, L67A-P27, L100A-P1, L100C-P19, L100A-P21, L100C-P25, L100A-P27, L103A-P1, L103C-P19, L103A-P21, L103C-P25, L103A-P27, L111A-P1, L111C-P19, L111A-P21, L111C-P25, and L111A-P2. The structures of the compounds are shown in Table B.

Example 5. Synthesis and Characterization of Bcl-xL Inhibitor ADCs

[1083] Exemplary antibody-drug conjugates (ADCs) were synthesized using the exemplary methods described below.

Abbreviations:

Ab antibody
ADC antibody-drug conjugate
BCN (*N*-[(1*R*,8*S*,9*S*)-bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane)
BTG bacterial transglutaminase
CV column volume
DAR drug-to-antibody ratio
DBCO dibenzo cyclooctyne
DFA difluoroacetic acid
DMA dimethylacetamide
DMF dimethylformamide
DMSO dimethyl sulfoxide
DTT dithiothreitol
FA formic acid
HIC hydrophobic interaction chromatography
LC-MS liquid chromatography mass spectrometry
L/P linker-payload
mAb monoclonal antibody
PBS phosphate buffer saline
PES polyether sulfone
PG propylene glycol
PLRP-s polymeric reverse phase column
rmp reduction modifiable protein
SEC size exclusion chromatography
TFA trifluoroacetic acid
Tris tris(hydroxymethyl)aminomethane

Materials and methods: Conjugation and analytical characterization of ADCs BCL-xL Antibodies specifications

[1084] Exemplary antibody-drug conjugates (ADCs) were synthesized using the exemplary methods described below. Antibodies Cetuximab (anti-EGFR), anti-CD7, anti-CD7 DANAPA, anti-chicken lysozyme DANAPA, Milatuzumab (anti-CD74), anti-CD38, anti-CD48 DANAPA

and Trastuzumab (anti-Her2) used for the preparation of the exemplary ADCs were defined respectively by the abbreviation Ab C, Ab D, Ab E, Ab F, Ab G, Ab H, Ab I and Ab T (Table 12). Antibody sequences in Table 12 are disclosed on the internet at go.drugbank.com/drugs/DB00002, in international application publication WO2018/098306, and in U.S. Patent No. US6870034B2, which are incorporated by reference in their entireties.

Table 12: Antibodies used for the synthesis of the exemplified ADC

Antibody abbreviation	Antibody	Mutation	Sequence (VL/VH)	Allotype
Ab C	Cetuximab Anti-EGFR	E163C S386C (E152C S375C)	C225	G1m3-1
Ab D	anti-CD7	E152C S375C	TH69	G1m3-1
Ab E (Ab D DANAPA)	Anti-CD7 DANAPA	E152C S375C	TH69	G1m3-1
Ab F	anti-chicken lysozyme DANAPA	E163C S386C (or E152C S375C)	3207	G1m3-1
Ab G	Milatuzumab Anti-CD74	E152C S375C		
Ab H	Anti-CD38	E152C S375C		
Ab I	Anti-CD48 DANAPA	E152C S375C	SGN-CD48A	
Ab T	Trastuzumab	N297Q	SEQ 1 / 2	G1m3-1

[1085] Two site-specific bioconjugations were exploited for the synthesis of the exemplified ADCs. The antibodies C, D, E, F, G, H and I were endowed with cysteine mutations incorporated inside the heavy chain and used to conjugate linker-payloads via maleimide group by method M1, M2, M3 and M4 (FIG.1).

[1086] Antibody T endowed with a bacterial transglutaminase (BTG) - reactive glutamines, was specifically functionalized with amine containing cyclooctyne BCN as described by Innate Pharma 2013 (presentation at ADC Summit, San Francisco, California, Oct. 15, 2013.), WO2017059160A1 and WO2016144608A1. These modifications allowed the conjugation of the described azide containing precursors using following method M5 (FIG. 2).

Conjugation

General antibody preparation for site-specific cysteine conjugation:

[1087] The conjugations were performed in a range of 5 mg antibody. The mAb was bound on rmp Protein A resin (GE Healthcare) at a ratio of 10 mg Ab to 1 ml resin in PBS by mixing in Biorad sized disposable column for 30 minutes. To deblock the reactive cysteines, cysteine hydrochloride monohydrate was added to a final concentration of 20 mM. The mixture was agitated at room temperature for 30 minutes followed by the washing of the resin with 5x50 CV of PBS on a vacuum manifold. The resin was then resuspended in an equal volume of PBS containing 250 nM of $CuCl_2$ and incubated for 1.5 h. Then conjugation methods M1, 2, 3 and 4 were used for the attachment of the linker-payload.

Conjugation method M1:

[1088] The re-oxidized antibody attached to protein A, was washed 5x50 CV of PBS on a vacuum manifold and resuspended in an equal resin volume of PBS. To the mixture were added 10-fold molar excess of a 20 mM solution of linker-payload and equal volume of DMSO. The reaction was incubated at room temperature for 2 h. To monitor the conjugation 20 µl of resin slurry were removed, centrifuged, and after the supernatant was removed, the resin was eluted with 40 µl of antibody elution buffer (Thermo Fisher Scientific) and analysed by PRLP-s. After elimination of the excess of linker-payload by washing the resin 5x50 CV of PBS on a vacuum manifold, the ADC was eluted from protein A with antibody elution buffer and neutralized with 0.1 CV of 1 M Tris buffer solution at pH 9.0. Exemplified ADC by method M1 were purified by SEC column HiLoad® 26/600 Superdex® 200 prep grade with 20% DMA in PBS.

Conjugation method M2:

[1089] The re-oxidized antibody attached to protein A, was washed 5x50 CV of PBS on a vacuum manifold and resuspended in an equal resin volume of PBS. To the mixture were added 10-fold molar excess of 20 mM solution of the linker-payload and equal volume of DMSO. The reaction was incubated at room temperature for 2 h. To monitor the conjugation 20 µl of resin slurry were removed, centrifuged and after the supernatant was removed, the resin was eluted with 40 µl of the antibody elution buffer (Thermo Fisher Scientific) and analysed by PRLP-s. After elimination the excess of linker-payload by washing the resin 5x50 CV of PBS on a vacuum manifold, the ADC was eluted from protein A with antibody elution buffer and neutralized with 0.1 CV of 1 M Tris buffer solution at pH 9.0.

Conjugation method M3:

[1090] The re-oxidized antibody was washed 5x50 CV of PBS and was eluted from protein A with 4 CV of antibody elution buffer (Thermo Fisher Scientific). After buffer exchange in

PBS using Vivaspin 20, 50KD, PES (Sartorius Stedim, VS2031), DMF and 10-fold molar excess of 20 mM linker-payload solution were added to the mAb leading to final solvent percentage in the medium of 20%. The reaction was agitated at room temperature for 18 h. The mixture was centrifuged (14000 G at +4 °C) for 20 minutes and purified by SEC column HiLoad® 26/600 Superdex® 200 prep grade with 20% DMA in PBS.

Conjugation method M4:

[1091] The re-oxidized antibody was washed 5x50 CV of PBS and was eluted from protein A with 4 CV of antibody elution buffer (Thermo Fisher Scientific). After buffer exchange in PBS using Vivaspin 20, 50KD, PES (Sartorius Stedim, VS2031), DMF and 10-fold molar excess of 20 mM linker-payload solution were added to the mAb leading to final solvent percentage in the medium of 20%. The reaction was agitated at room temperature for 18 h. To remove the excess of L/P, the conjugate was bound to rmp protein A resin (GE Healthcare) at a ratio of 5 mg Ab to 500µl resin in PBS with no more than 5% final solvent percentage in the slurry. After washing step with 5% DMF in PBS solution (5x50 CV) followed by second washing step in PBS (5x50CV), the conjugate was eluted from protein A with the antibody elution buffer and neutralized with 0.1 CV of 1 M Tris buffer solution at pH 9.0.

[1092] All exemplified ADCs synthesized with method **M1**, **M2**, **M3**, and **M4** were buffer exchanged by dialysis (Thermo Fisher, 88254) in PBS 1X pH 7.4 (Sigma Life Science, P3813, 10PAK), concentrated using Vivaspin 20, 50KD, PES (Sartorius Stedim, VS2031), filtered sterilely through 0.2µm sterile PES Filter, 25mm (Whatmann, G896-2502) and stored at 4 °C. They were characterized by analytical size exclusion chromatography Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) to determine monomer percentage and LC-MS for drug-to-antibody ratio (**DAR**) determination. To monitor the conjugation, reverse phase chromatography using an Agilent PLRP-S column 4000A 5 um, 4.6 x 50 mm column (Buffer A water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80 °C, Flowrate 1.5 ml/min) was used.

General procedure for site-specific transglutaminase conjugation:

[1093] The site-specific transglutaminase conjugations were performed on antibody Trastuzumab where glutamines present in the Fc region of the antibody were functionalized by bacterial transglutaminase with 4 BCN linkers as described above.

Conjugation method M5:

[1094] The conjugations were performed in a range of 5 mg antibody. To the Ab solution was added DMA followed by 10-fold molar excess of the linker-warhead payload solution (5 mM in PG/DMA) leading to final solvent percentage in the medium of 20%. The reaction was stirred at 64 rpm at room temperature for 18 h. Then, this solution was incubated with 10-fold molar excess of DBCO-containing Tentagel resin (0.1-0.2 mmol/g, Iris Biotech, CS-0477.0500) for 6 h in order to remove the excess of the linker-payload. The solution was centrifuged (14000 G at 4 °C) for 20 minutes and the supernatant was loaded onto HiLoad 26/600 Superdex 200 pg (GE Healthcare, 28989336) SEC chromatography column. The ADC was purified with 20% DMA in PBS (Sigma Life Science, P3813, 10PAK) followed by 2 cycle's dialysis (16 and 4 h) in PBS 1X pH 7.4 (Sigma Life Science, P3813, 10PAK). The conjugate was concentrated using Vivaspin 20, 50KD, PES (Sartorius Stedim, VS2031), filtered sterilely through 0.2µm sterile PES Filter, 25mm (Whatmann, G896-2502) and stored at 4 °C.

[1095] All exemplified ADCs synthesized with method **M5** were characterized by analytical size exclusion chromatography Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) to determine monomer percentage and LC-MS for DAR determination.

[1096] To monitor the conjugation, HIC chromatography with TOSOH Tskgel Butyl-NPR column 2,5µm 4,6 x 35 mm (Buffer A 1.5 M $(NH_4)_2SO_4^{2-}$/ 25 mM KH_2PO_4 pH7.0; Buffer B 25 mM KH_2PO_4 /20% iPrOH pH to 7.0, column held at 21 °C, Flowrate 0.6 ml/min) was used.

Characterization

LC-MS General Methodology

[1097] Drug-to-antibody ratio (**DAR**) of exemplary ADCs was determined by liquid chromatography hyphenated with mass spectrometry (LC-MS) with one of the following methods (i.e., **LC-I**, **LC-II**, **LC-III, LC-IV and LC-V**). For **LC-I, LC-II, LC-III and LC-IV** methods, mobile phase A was purified MS grade water (Biosolve, Dieuze, France, 00232141B1BS), mobile phase B was MS grade acetonitrile (Biosolve, Dieuze, France, 0001204101BS) and mobile phase D purified MS grade water supplemented with 1% of FA (Honeywell/Fluka, Bucharest, Romania, 56302). Mobile phase D was fixed at 10% in order to maintain a 0.1% FA mobile phase composition. Alternatively, for **LC-IV** method, mobile phase A was ultrapure water obtained with Milli-Q® system and mobile phase B was MS grade acetonitrile (Biosolve, Dieuze, France, 0001204101BS) supplemented with 0.1% of FA (Fisher Chemical: A117-50-50ML). For **LC-V** method, mobile phase A was ultrapure water obtained with Milli-Q® system and mobile phase B was MS grade acetonitrile (Biosolve, Dieuze, France, 0001204101BS) supplemented with 0.1% of DFA (Waters, 186009201).

Column temperature was set at 80 °C. A general MS method was optimized for all synthesized ADCs in order to determine average **DAR** (Table 13).

[1098] LC-I*:* ADC was loaded onto a MassPREP Micro desalting column (2.1 x 5.0 mm, Waters, Saint- Quentin-en-Yvelines, France, 186004032). For intact mass analysis, a desalting step was performed for 0.5 min at 5% mobile phase B with a flow rate of 0.5 mL/min. Elution step was performed with a gradient from 0.5 min at 5% B to 2.0 min at 85 % B with a flow rate of 0.2 mL/min. Two wash steps were set from 2.1 min to 2.7 min and from 2.8 min to 3.4 min at 5% B to 85% B with a flow rate of 0.5 mL/min. Finally, a conditioning step was used at 3.5 min for 0.5 min at 5 % B (0.5 mL/min). For ADC analysis in reduced condition, a desalting step was performed for 0.5 min at 5% B with a flow rate of 0.2 mL/min. Then, the elution step started with a gradient from 0.51 min at 10% B to 7.61 min at 50 % B with a flow rate of 0.2 mL/min. At 8.0 min, Phase B was at 90% with a flow rate of 0.5mL/min. Two washing steps were set 8.1 min to 8.6 min and from 8.7 min to 9.2 min from 5% B to 90% B (0.5 mL/min). Finally, a conditioning step was performed at 9.3 min for 0.5 min at 5 % B with a flow rate of 0.5 mL/min.

[1099] LC-II: ADC was loaded onto a MabPac RP column (2.1 x 100mm, 4µm, Thermo Scientific, Rockford, IL, 088647). For analysis in both intact and reduced conditions, a desalting step was performed for 1.4 min at 20% of B with a flow rate of 0.4 mL/min. Then, the elution step was performed with a gradient from 1.5 min at 20% B to 11.5 min at 70 % B with a flow rate of 0.3 mL/min. A wash step was set from 11.75 min to 13.75 min at 90% B with a flow rate of 0.5 mL/min. Finally, a conditioning step was used at 14.0 min for 1.0 min at 20 % B with a flow rate of 0.4 mL/min.

[1100] LC-III: ADC was loaded onto a Bioresolve RP mAb Polyphenyl,column 450A, 2.7µm, 2.1*150mm (Waters, Saint- Quentin-en-Yvelines, France, 186008946). For analysis in both intact and reduced conditions, a desalting step was performed for 1.5 min at 20% of B with a flow rate of 0.6 mL/min. Elution step was performed with a gradient from 1.5 min at 20% B to 16.5 min at 50 % B with a flow rate of 0.6 mL/min. A wash step was set from 16.8 min to 18.8 min at 90% B with a flow rate of 0.6 mL/min. Finally, a conditioning step was used at 19.1 min for 1.9 min at 20 % B with a flow rate of 0.6 mL/min (Total run time=21min).

[1101] LC-IV (80% Phase A (Water/0.1% AF), 20% Phase B (Acetonitrile/0.1%AF))**:** ADC was loaded onto a Bioresolve RP mAb Polyphenyl,column 450A, 2.7µm, 2.1*150mm (Waters, Saint- Quentin-en-Yvelines, France, 186008946). For analysis in both intact and

reduced conditions, a desalting step was performed for 1.5 min at 20% of B with a flow rate of 0.6 mL/min. Elution step was performed with a gradient from 1.5 min at 20% B to 16.5 min at 50 % B with a flow rate of 0.6 mL/min. A wash step was set from 16.8 min to 18.8 min at 100% B with a flow rate of 0.6 mL/min. Finally, a conditioning step was used at 19.2 min for 1.8 min at 20 % B with a flow rate of 0.6 mL/min (Total run time=21min).

[1102] LC-V (80% Phase A (Water/0.1% DFA), 20% Phase B (Acetonitrile/0.1%DFA))**:** ADC was loaded onto a Bioresolve RP mAb Polyphenyl,column 450A, 2.7µm, 2.1*150mm (Waters, Saint- Quentin-en-Yvelines, France, 186008946). For analysis in both intact and reduced conditions, a desalting step was performed for 1.5 min at 20% of B with a flow rate of 0.6 mL/min. Elution step was performed with a gradient from 1.5 min at 20% B to 16.5 min at 50 % B with a flow rate of 0.6 mL/min. A wash step was set from 16.8 min to 18.8 min at 100% B with a flow rate of 0.6 mL/min. Finally, a conditioning step was used at 19.2 min for 1.8 min at 20 % B with a flow rate of 0.6 mL/min (Total run time=21min).

[1103] LC-MS analysis was performed using a Waters UPLC H-Class Bio chromatography system hyphenated with a Xevo G2 XS Q-TOF ESI mass spectrometer (Waters, Manchester, UK). The ADC was either analysed in intact condition (no preliminary treatment), or with a deglycosylation step using PNGase F enzyme (New England Biolabs®, P0705L) or following reduction with 5 mM (final concentration) of dithiothreitol DTT (Thermo Scientific, Rockford, IL, 20291). Subsequently, treated ADC was analysed using one of the above-mentioned **LC-I**, **LC-II, LC-III, LC-IV** or **LC-V** (Table 13). Electrospray-ionization time-of-flight mass spectra of the analytes were acquired using MassLynx™ acquisition software (Waters, Manchester, UK). Then, the extracted intensity vs. m/z spectrum was deconvoluted using Maximum Entropy (MaxEnt1) method of MassLynx™ software in order to determine the mass of each intact antibody species or each reduced antibody fragment depending on the treatment. Finally, **DAR** was determined from the deconvoluted spectra or UV chromatogram by summing the integrated MS (total ion current) or UV (280 nm) peak area of unconjugated and conjugated given species (mAb or associated fragment). For the **DAR** determination by UV chromatogram, relative area percentage of each specie was multiplied by the number of drugs attached. The summed, weighted areas of every species were divided by the sum of total relative area percentage and the results produced an estimation of the final average **DAR** value for the full ADC. For the **DAR** determination by deconvoluted spectra, the percentage of each specie identified was calculated by intensity peak value from deconvoluted spectra. The percentage obtained, was multiplied by the number of drugs attached. The summed results produced an estimation of the final average **DAR** value for the full ADC.

[1104] Size Exclusion Chromatography: Size exclusion chromatography (SEC) was performed for quality control of each ADCs by measuring monomer percentage of the conjugate. The analysis was performed on analytical column Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) in isocratic conditions 100% PBS pH7.4 (Sigma Life Science, P3813, 10PAK), flow 0.45 ml/min for 12 minutes. The % aggregate fraction of the conjugate sample was quantified based on the peak area absorbance at 280 nm. Its calculation was based on the ratio between the high molecular weight eluent at 280 nm divided by the sum of peak area absorbance at the same wavelength of the high molecular weight and monomeric eluents multiplied by 100.

Results

[1105] Characterization of the exemplary ADCs is summarized in Table 13 (coupling, LC-MS method, DAR, aggregation status, ADC stability and yield). The average DAR values were determined using the above LC-MS methods and the percentage of aggregates was measured by size exclusion chromatography (SEC) during the quality control of the ADC and after the stability study (incubation at 37 °C for 168 h).

Table 13: ADC analytical characterization and coupling methodology

ADC	Coupling Method	LC-MS Method	DAR (by LC-MS)	% Agg (by SEC)	Stab w1 +37°C % Agg (by SEC)	% Yield
Ab C - L9A-P8	M3	LC-III	3.8	7	7	23
Ab C - L9A-P9	M3	LC-III	3.8	3.3	ND	23
Ab C - L9A-P10	M3	LC-III	2.7	4.2	ND	13
Ab C - L9A-P11	M3	LC-III	4.2	2.3	2.4	67
Ab C - L9C-P12	M3	LC-III	3.3	2.5	4	23
Ab C - L9A-P13	M3	LC-III	3.4	2.5	0.2	21
Ab C - L9A-P14	M3	LC-III	3.5	2	7	15
Ab C - L9A-P15	M3	LC-III	3.2	2.9	3	19
Ab C - L9C-P16	M3	LC-III	3.8	1.3	2.4	47
Ab C - L9A-P1	M3	LC-III	4.1	3.4	6	28
Ab C - L9C-P17	M3	LC-III	3.1	22	22	15
Ab C - L9A-P18	M3	LC-III	3.4	2	3	23
Ab C - L9C-P19	M3	LC-III	3.6	2	2	85

Ab C - L9A-P20	M3	LC-III	3.8	2	4	46
Ab C - L9A-P21	M3	LC-III	3.6	2	4	50
Ab C - L9C-P22	M3	LC-III	4.1	2	2	59
Ab C - L9C-P23	M3	LC-III	3.3	3	5	27
Ab C - L9C-P24	M3	LC-III	3.2	2	2	61
Ab C - L9A-P2	M1	LC-III	3.5	3.5	ND	10
Ab C - L9C-P25	M2	LC-III	3	4	4	78
Ab C - L9C-P26	M2	LC-III	3.2	2	3	50
Ab C - L9A-P27	M3	LC-IV	4	16	16	32
Ab C - L9A-P28	M3	LC-IV	4.9	11	15	11
Ab C - L9C-P29	M3	LC-IV	3.5	3	16	18
Ab C - L9A-P30	M3	LC-IV	3.5	1	5	55
Ab C - L9C-P31	M3	LC-IV	3.9	4	6	32
Ab C - L9A-P32	M3	LC-IV	2.8	2	2	57
Ab C - L9A-P33	M3	LC-IV	3.9	4	4	52
Ab C - L9A-P34	M4	LC-IV	3.9	6	6	31
Ab C - L9A-P35	M4	LC-IV	3.8	4	6	38
Ab C - L9A-P36	M4	LC-IV	4.2	10	7	35
Ab C - L9A-P37	M4	LC-IV	4	9	9	53
Ab C - L9A-P38	M4	LC-IV	4	4	6	38
Ab C - L9A-P39	M4	LC-IV	3.8	6	ND	32
Ab C - L9C-P40	M4	LC-IV	5.2	30	27	45
Ab C - L9A-P41	M2	LC-IV	2.3	7	9	80
Ab C - L9A-P42	M4	LC-IV	4.1	9	9	47
Ab C - L9A-P43	M4	LC-IV	3.8	4	6	44
Ab C - L21A-P2	M1	LC-II	3.6	4	4	73
Ab C - L27C-P3	M1	LC-III	3.7	6	17	23
Ab C -L106A-P2	M1	LC-III	3.3	7	ND	10
Ab C -L106C-P7	M1	LC-II	3	10	29	60
Ab C -L107C-P7	M1	LC-II	2.9	8	9	56

Ab C -L107A-P2	M1	LC-II	3.7	3	5	60
Ab C -L108A-P2	M1	LC-II	4.1	4	5	70
Ab D - L27A-P1	M3	LC-III	3.9	4	9	45
Ab D - L9A-P1	M3	LC-III	4.4	8	8	44
Ab D - L9A-P9	M3	LC-III	3.9	8	10	33
Ab D - L9A-P13	M3	LC-III	4.7	10	10	44
Ab D - L9A-P8	M3	LC-III	3.4	12	12	54
Ab D - L9A-P10	M3	LC-III	3.2	9	12	54
Ab D - L9C-P44	M3	LC-III	3.4	6	4	70
Ab D - L9C-P45	M3	LC-III	4.3	5	2	38
Ab D - L9C-P46	M3	LC-III	4.2	4	6	66
Ab D - L9A-P20	M3	LC-III	3.9	3	4	46
Ab D - L9C-P19	M3	LC-III	3.6	3	3	26
Ab D - L9A-P21	M3	LC-III	3.5	4	4	66
Ab D - L9C-P22	M3	LC-III	3.8	2	2	72
Ab D - L9C-P17	M3	LC-III	2	4	6	88
Ab T - L13A-P2	M5	LC-I	4	2	3	62
Ab T - L19C-P7	M5	LC-I	4	4	7	72
Ab T - L23C-P7	M5	LC-II	4	4	4	51
Ab T - L110C-P7	M5	LC-II	4	3	4	48
Ab C - L9C-P3	M4	LC-V	0.5	4	6	72
Ab C - L9A-P61	M4	LC-IV	4.8	5	7	46
Ab C - L9C-P60	M4	LC-IV	2.3	3	4	75
Ab C - L9A-P64	M4	LC-IV	4.2	10	7	73
Ab C - L9A-P67	M4	LC-IV	3.3	2	2	67
Ab C - L9A-P65	M4	LC-IV	4.0	10	6	51
Ab C - L9A-P63	M4	LC-IV	4.1	5	6	54
Ab C - L9A-P66	M4	LC-IV	3.6	5	7	50
Ab C - L9A-P68	M4	LC-IV	4.1	10	10	46
Ab C - L9A-P62	M4	LC-IV	4.0	6	6	49

Ab C - L9C-P69	M3	LC-IV	4.3	4	6	58
Ab C - L9A-P70	M4	LC-IV	3.6	1	2	49
Ab C - L9A-P48	M4	LC-IV	3.4	2	2	54
Ab C - L112A-P1	M4	LC-IV	4.0	5	7	99
Ab C - L9C-P71	M4	LC-IV	1.7	6	8	53
Ab C - L9C-P72	M4	LC-IV	3.8	12	5	51
Ab C - L9A-P50	M4	LC-IV	3.5	10	10	57
Ab C - L9A-P52	M4	LC-IV	3.5	12	27	45
Ab C - L9C-P53	M4	LC-IV	3.7	4	3	21
Ab C - L9C-P51	M4	LC-IV	4.3	29	ND	50
Ab C - L9A-P49	M4	LC-IV	3.8	2	3	39
Ab C - L9A-P55	M4	LC-IV	3.8	4	3	40
Ab C - L9C-P54	M4	LC-IV	3.6	4	2	20
Ab C - L9C-P47	M4	LC-IV	3.5	2	2	34
Ab C - L9A-P56	M4	LC-IV	3.4	3	3	53
Ab C - L9A-P57	M4	LC-IV	3.6	2	2	44
Ab C - L9A-P58	M4	LC-IV	3.4	2	2	44
Ab C - L9A-P74	M3	LC-IV	3.8	11	24	27
Ab C - L9A-P75	M3	LC-IV	3.7	10	16	29
Ab C - L9A-P76	M3	LC-IV	4.0	13	15	19
Ab C - L9A-P73	M3	LC-IV	3.7	8	18	21
Ab G - L9C-P25	M2	LC-IV	3.2	5	14	99
Ab G - L11C-P25	M1	LC-IV	3.3	3	ND	60
Ab H - L9C-P25	M1	LC-IV	3.2	4	ND	77
Ab H - L11C-P25	M1	LC-IV	3.2	4	ND	74
Ab I - L9C-P25	M1	LC-IV	3.2	2	3	40
Ab I - L11C-P25	M2	LC-IV	3.1	11	8	66
Ab D - L9C-P25	M2	LC-IV	3.3	4	4	73
Ab D - L11C-P25	M2	LC-IV	3.2	3	4	75
Ab F - L9A-P21	M3	LC-IV	3.0	2	8	36

Ab E - L9A-P21	M3	LC-IV	2.7	2	10	25
Ab E - L9C-P25	M4	LC-IV	3.8	5	10	47
Ab E - L9A-P1	M3	LC-IV	4.0	1	1	27
Ab E - L9C-P40	M3	LC-IV	4.0	9	16	26
Ab E - L9A-P33	M4	LC-IV	4.0	3	3	55
Ab F - L9C-P25	M1	LC-IV	3.5	4	4	38

Example 6. Synthesis and Characterization of EGFR1 CysMab ADCs

Drug Substance Intermediate (DSi) Preparation (Re-ox material)

[1106] 200 mg of EGFR1 CysMab (1.36 µM) at 10 mg/ml was incubated with 20ml of settled RMP Protein A resin (GE Lifesciences, 17513803) and agitated for 15 minutes. Cysteine HCl monohydrate was added to a final concentration of 20 mM and incubated with agitation for 30 min at room temperature to allow the reactive cysteines to be deblocked. The resin was washed quickly with 50 column volumes PBS on a vacuum manifold. The resin was then resuspended in an equal volume PBS containing 250 nM $CuCl_2$. Reformation of antibody interchain disulfides was monitored by taking time points. At each time point, 25 µL of resin slurry was removed, 1 µL of 20 mM MC-valcit-MMAE was added, and the tube flicked several times. The resin was spun down, supernatant removed, and then eluted with 50 µL Antibody elution buffer (Thermo). The resin was pelleted and the supernatant analyzed by reverse phase chromatography using an Agilent PLRP-S 4000A 5um, 4.6x50mm column (Buffer A is water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80 C, Flowrate 1.5 ml/min; Gradient 0 minutes – 30%B, 5 minutes – 45%B, 6.5 min – 100%B, 8 minutes – 100%B, 10 minutes – 30%). At 60 minutes after addition of $CuCl_2$, $CuCl_2$ was removed by washing with 50 column volumes of PBS on a vacuum manifold and then20 ml of PBS was added to resuspend and drained by gravity. The antibody was eluted with 100 ml antibody elution buffer (Thermo Scientific, 21004) and then buffer exchanged into 1X PBS pH 7.2. The material was then concentrated using a centrifugal concentrator using an Amicon Ultra-15, 50KDa, regenerated cellulose (Millipore, UFC0905024), to 6.6 mg/ml aliquoted into 5 mg aliquots and flash frozen in liquid nitrogen and stored at -80 C until used.

Conjugation method using Drug Substance Intermediate (DSi) (Re-ox material)

Preparation of EGFR-L11C-P19

[1107] To a solution of EGFR (also labeled as EGFR1 CysMab) DSi antibody (2.0 mg, 274 µl of a 7.3 mg/ml solution in 1X PBS buffer solution, 0.013 µmoles, 1.0 equiv.) was added DMSO (20 uL) and **L11C-P19** (5.28 µl of a 20 mM solution in DMSO, 0.106 µmoles, 8.0 equiv.). The total DMSO amount was </= 10%. The resulting mixture was shaken at 400 rpm at ambient temperature for 2 hours, at which time the mixture was purified by ultracentrifugation (4 ml Amicon 30 kD cutoff membrane filter, diluting sample to 4 ml total volume with 1X PBS buffer followed by centrifugation for 10 minutes at 7500 x g, repeated 6 times). After dilution with 1X PBS buffer to 5.0 mg/ml, **EGFR- L11C-P19** was obtained (1.9 mg, 0.012 µmoles, 90%). The following analyses were performed: analytical size-exclusion chromatography (SEC) to determine percent monomer, mass spectroscopy of reduced aliquot (MS) to determine DAR, and protein concentration determined by A280 utilizing extinction coefficient and molecular weight of antibody. HRMS data (protein method) indicated a mass of 58050 for HC+2 linker payloads attached, with a DAR of 4.0. SEC indicated 1.0% aggregation, as determined by comparison of the area of the high-molecular-weight peak absorbance at 280 nm with the area of the peak absorbance for monomeric ADC. Note: in some cases, conjugation reactions were purified via Protein A method followed by 1X PBS buffer swap using ultacentrifugation.

[1108] Following the conjugation method using DSi (Re-ox material) described above, the following conjugates were prepared:

ADC	Yield	DAR4 M+ or (HC+2LP)+	DAR	%Agg
EGFR-L1A-P1	95%	157294 (degly)	3.8	1.6
EGFR-L1A-P2	95%	160074	4.0	2.1
EGFR-L1C-P3	89%	160307	4.0	2.0
EGFR-L3A-P1	42%	158641l HC+2LP=55916	3.9	5.0
EGFR-L3C-P3	82%	159415	3.7	12.0
EGFR-L3C-P4	94%	159649	3.6	1.0
EGFR-L3C-P5	95%	159200	3.6	2.0
EGFR-L4A-P1	95%	158443	4.0	1.6
EGFR-L7A-P1	95%	158448	4.0	2.0
EGFR-L7A-P2	43%	158342	4.2	2.5
EGFR-L7C-P3	50%	158567	3.9	9.0
EGFR-L7C-P6	47%	158728	3.7	3.0
EGFR-L7C-P7	76%	158782	3.8	8.0
EGFR-L8A-P1	95%	158218	3.9	2.0
EGFR-L8C-P7	39%	158250	3.6	2.0

EGFR-L9A-P1	49%	54731 (HC+2LP)	4.0	1.0
EGFR-L9A-P2	53%	54678 (HC+2LP)	4.0	4.1
EGFR-L9C-P4	40%	157276	3.6	4.5
EGFR-L9C-P5	95%	156834	3.6	1.0
EGFR-L109A-P1	93%	161262	3.5	4.5
EGFR-L10A-P1	95%	160640	4.0	1.0
EGFR-L10A-P2	95%	160548	4.0	2.0
EGFR-L10C-P3	95%	160763	3.8	1.0
EGFR-L11A-P21	87%	57891 (HC+2LP)	3.8	3.0
EGFR-L11A-P27	87%	57839 (HC+2LP)	3.8	3.0
EGFR-L11C-P19	90%	58050 (HC+2L)	4.0	1.0
EGFR-L11C-P25	84%	57988 (HC+2LP)	3.8	1.0
EGFR-L30A-P21	66%	57688 (HC+2LP)	4.0	2.0
EGFR-L35A-P21	64%	57360 (HC+2LP)	4.0	2.0
EGFR-L36A-P21	63%	58248 (HC+2LP)	4.0	2.0
EGFR-L37A-P21	67%	59102 (HC+2LP)	4.0	2.0
EGFR-L38A-P21	60%	57280 (HC+2LP)	4.0	2.0
EGFR-L39A-P21	69%	58132 (HC+2LP)	4.0	2.0
EGFR-L40A-P21	70%	58948 (HC+2LP)	4.0	2.0
EGFR-L42A-P21	54%	57836 (HC+2LP)	4.0	1.0
EGFR-L42C-P25	57%	57930 (HC+2LP)	4.0	1.0

Example 7. Synthesis of BCL-xL Inhibitor ADCs for Multi-Target *In Vitro* Assays

Expression and purification of antibodies

[1109] Table 14 lists the antibodies that were used to synthesize antibody drug conjugates disclosed herein. Antibody heavy chain sequences were modified to include cysteine mutations at E152 and S375 positions (according to EU numbering) to facilitate conjugation to linker-payloads disclosed herein. Certain exemplary antibody sequences in Table 14 are disclosed in international application publications such as WO2016/179257, WO2011/097627, WO2017/214282, WO2017/214301, WO2017/214233, WO2013/126810, WO2008/056833, WO2020/236817, and WO2017/214335, which are incorporated by reference in their entireties.

Table 14: Antibody Sequences

Antibody Target/ Code	Antibody Name	Heavy Chain (CysMab) Sequence	Light Chain (CysMab) Sequence

EGFR1	Cetuximab	QVQLKQSGPGLVQPSQS LSITCTVSGFSLTNYGV HWVRQSPGKGLEWLGVI WSGGNTDYNTPFTSRLS INKDNSKSQVFFKMNSL QSNDTAIYYCARALTYY DYEFAYWGQGTLVTVSA ASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFP CPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISK AKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGF YPCDIAVEWESNGQPEN NYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO:92)	DILLTQSPVILSVS PGERVSFSCRASQS IGTNIHWYQQRTNG SPRLLIKYASESIS GIPSRFSGSGSGTD FTLSINSVESEDIA DYYCQQNNNWPTTF GAGTKLELKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:93)
TFRC	CD71 (CX-2029)	QVQLVQSGAEVKKPGAS VKMSCKASGYTFTSYWM HWVRQAPGQGLEWIGAI YPGNSETGYAQKFQGRA TLTADTSTSTAYMELSS LRSEDTAVYYCTRENWD PGFAFWGQGTLITVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL	DIVLTQSPLSLAVS LGQPAIISCKASQS VSFAGTSLMHWYHQ KPGQQPRLLIYRAS NLEAGVPDRFSGSG SKTDFTLTISPVEA EDAATYYCQQSREY PYTFGGGTKLEIKR TVAAPSVFIFPPSD EQLKSGTASVVCLL NNFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSST LTLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO:95)

		SLSPGK (SEQ ID NO:94)	
EPCAM	Oportuzumab* *Variable heavy chain and variable light chain sequence of oportuzumab monatox were combined with human heavy chain constant region and light chain constant region to produce full IgG antibody sequence	EVQLVQSGPGLVQPGGS VRISCAASGYTFTNYGM NWVKQAPGKGLEWMGWI NTYTGESTYADSFKGRF TFSLDTSASAAYLQINS LRAEDTAVYYCARFAIK GDYWGQGTLLTVSSAST KGPSVFPLAPSSKSTSG GTAALGCLVKDYFPCPV TVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPS VFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKG QPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSL SPGK (SEQ ID NO:96)	DIQMTQSPSSLSAS VGDRVTITCRSTKS LLHSNGITYLYWYQ QKPGKAPKLLIYQM SNLASGVPSRFSSS GSGTDFTLTISSLQ PEDFATYYCAQNLE IPRTFGQGTKVELK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO:97)
FOLR1	Mirvetuximab	QVQLVQSGAEVVKPGAS VKISCKASGYTFTGYFM NWVKQSPGQSLEWIGRI HPYDGDTFYNQKFQGKA TLTVDKSSNTAHMELLS LTSEDFAVYYCTRYDGS RAMDYWGQGTTVTVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY	DIVLTQSPLSLAVS LGQPAIISCKASQS VSFAGTSLMHWYHQ KPGQQPRLLIYRAS NLEAGVPDRFSGSG SKTDFTLTISPVEA EDAATYYCQQSREY PYTFGGGTKLEIKR TVAAPSVFIFPPSD EQLKSGTASVVCLL NNFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSST LTLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO:99)

		SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO:98)	
ENPP3	ENPP3 (AGS16-7.8)	QVQLQESGPGLVKPSQT LSLTCTVSGGSISSGGY YWSWIRQHPGKGLEWIG IIYYSGSTYYNPSLKSR VTISVDTSKNQFSLKLN SVTAADTAVFYCARVAI VTTIPGGMDVWGQGTTV TVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVK DYFPCPVTVSWNSGALT SGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEK TISKAKGQPREPQVYTL PPSREEMTKNQVSLTCL VKGFYPCDIAVEWESNG QPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHY TQKSLSLSPGK (SEQ ID NO:100)	EIVLTQSPDFQSVT PKEKVTITCRASQS IGISLHWYQQKPDQ SPKLLIKYASQSFS GVPSRFSGSGSGTD FTLTINSLEAEDAA TYYCHQSRSFPWTF GQGTKVEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:101)

MET	Telisotuzumab	QVQLVQSGAEVKKPGAS VKVSCKASGYIFTAYTM HWVRQAPGQGLEWMGWI KPNNGLANYAQKFQGRV TMTRDTSISTAYMELSR LRSDDTAVYYCARSEIT TEFDYWGQGTLVTVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO:102)	DIVMTQSPDSLAVS LGERATINCKSSES VDSYANSFLHWYQQ KPGQPPKLLIYRAS TRESGVPDRFSGSG SGTDFTLTISSLQA EDVAVYYCQQSKED PLTFGGGTKVEIKR TVAAPSVFIFPPSD EQLKSGTASVVCLL NNFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSST LTLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO:103)
AXL	Enapotamab	EVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAM NWVRQAPGKGLEWVSTT SGSGASTYYADSVKGRF TISRDNSKNTLYLQMNS LRAEDTAVYYCAKIWIA FDIWGQGTMVTVSSAST KGPSVFPLAPSSKSTSG GTAALGCLVKDYFPCPV TVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPS VFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKG QPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSL	EIVLTQSPGTLSLS PGERATLSCRASQS VSSSYLAWYQQKPG QAPRLLIYGASSRA TGIPDRFSGSGSGT DFTLTISRLEPEDF AVYYCQQYGSSPYT FGQGTKLEIKRTVA APSVFIFPPSDEQL KSGTASVVCLLNNF YPREAKVQWKVDNA LQSGNSQESVTEQD SKDSTYSLSSTLTL SKADYEKHKVYACE VTHQGLSSPVTKSF NRGEC (SEQ ID NO:105)

		SPGK (SEQ ID NO:104)	
SLC34A2	Lifastuzumab	EVQLVESGGGLVQPGGS LRLSCAASGFSFSDFAM SWVRQAPGKGLEWVATI GRVAFHTYYPDSMKGRF TISRDNSKNTLYLQMNS LRAEDTAVYYCARHRGF DVGHFDFWGQGTLVTVS SASTKGPSVFPLAPSSK STSGGTAALGCLVKDYF PCPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPE NNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:106)	DIQMTQSPSSLSAS VGDRVTITCRSSET LVHSSGNTYLEWYQ QKPGKAPKLLIYRV SNRFSGVPSRFSGS GSGTDFTLTISSLQ PEDFATYYCFQGSF NPLTFGQGTKVEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO:107)

NECTIN4	Enfortumab	EVQLVESGGGLVQPGGS LRLSCAASGFTFSSYNM NWVRQAPGKGLEWVSYI SSSSSTIYYADSVKGRF TISRDNAKNSLSLQMNS LRDEDTAVYYCARAYYY GMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAK GQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLS LSPGK (SEQ ID NO:108)	DIQMTQSPSSVSAS VGDRVTITCRASQG ISGWLAWYQQKPGK APKFLIYAASTLQS GVPSRFSGSGSGTD FTLTISSLQPEDFA TYYCQQANSFPPTF GGGTKVEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:109)
TACSTD2	Sacituzumab	QVQLQQSGSELKKPGAS VKVSCKASGYTFTNYGM NWVKQAPGQGLKWMGWI NTYTGEPTYTDDFKGRF AFSLDTSVSTAYLQISS LKADDTAVYFCARGGFG SSYWYFDVWGQGSLVTV SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDY FPCPVTVSWNSGALTSG VHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVK GFYPCDIAVEWESNGQP ENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQ	DIQLTQSPSSLSAS VGDRVSITCKASQD VSIAVAWYQQKPGK APKLLIYSASYRYT GVPDRFSGSGSGTD FTLTISSLQPEDFA VYYCQQHYITPLTF GAGTKVEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:111)

		KSLSLSPGK (SEQ ID NO:110)	
SLC39A6	Ladiratuzumab	QVQLVQSGAEVKKPGAS VKVSCKASGLTIEDYYM HWVRQAPGQGLEWMGWI DPENGDTEYGPKFQGRV TMTRDTSINTAYMELSR LRSDDTAVYYCAVHNAH YGTWFAYWGQGTLVTVS SASTKGPSVFPLAPSSK STSGGTAALGCLVKDYF PCPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPE NNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:112)	DVVMTQSPLSLPVT LGQPASISCRSSQS LLHSSGNTYLEWYQ QRPGQSPRPLIYKI STRFSGVPDRFSGS GSGTDFTLKISRVE AEDVGVYYCFQGSH VPYTFGGGTKVEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO:113)

GPNMB	Glembatumumab	QVQLQESGPGLVKPSQT LSLTCTVSGGSISSFNY YWSWIRHHPGKGLEWIG YIYYSGSTYSNPSLKSR VTISVDTSKNQFSLTLS SVTAADTAVYYCARGYN WNYFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFP CPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISK AKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGF YPCDIAVEWESNGQPEN NYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO:114)	EIVMTQSPATLSVS PGERATLSCRASQS VDNNLVWYQQKPGQ APRLLIYGASTRAT GIPARFSGSGSGTE FTLTISSLQSEDFA VYYCQQYNNWPPWT FGQGTKVEIKRTVA APSVFIFPPSDEQL KSGTASVVCLLNNF YPREAKVQWKVDNA LQSGNSQESVTEQD SKDSTYSLSSTLTL SKADYEKHKVYACE VTHQGLSSPVTKSF NRGEC (SEQ ID NO:115)
MSLN	Anetumab	QVELVQSGAEVKKPGES LKISCKGSGYSFTSYWI GWVRQAPGKGLEWMGII DPGDSRTRYSPSFQGQV TISADKSISTAYLQWSS LKASDTAMYYCARGQLY GGTYMDGWGQGTLVTVS SASTKGPSVFPLAPSSK STSGGTAALGCLVKDYF PCPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPE NNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQK	DIALTQPASVSGSP GQSITISCTGTSSD IGGYNSVSWYQQHP GKAPKLMIYGVNNR PSGVSNRFSGSKSG NTASLTISGLQAED EADYYCSSYDIESA TPVFGGGTKLTVLG QPKAAPSVTLFPPS SEELQANKATLVCL ISDFYPGAVTVAWK ADSSPVKAGVETTT PSKQSNNKYAASSY LSLTPEQWKSHRSY SCQVTHEGSTVEKT VAPTECS (SEQ ID NO:117)

		SLSLSPGK (SEQ ID NO:116)	
CD74	Milatuzumab	QVQLQQSGSELKKPGAS VKVSCKASGYTFTNYGV NWIKQAPGQGLQWMGWI NPNTGEPTFDDDFKGRF AFSLDTSVSTAYLQISS LKADDTAVYFCSRSRGK NEAWFAYWGQGTLVTVS SASTKGPSVFPLAPSSK STSGGTAALGCLVKDYF PCPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPE NNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:118)	DIQLTQSPLSLPVT LGQPASISCRSSQS LVHRNGNTYLHWFQ QRPGQSPRLLIYTV SNRFSGVPDRFSGS GSGTDFTLKISRVE AEDVGVYFCSQSSH VPPTFGAGTRLEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO:119)
F3	Tisotumab	EVQLLESGGGLVQPGGS LRLSCAASGFTFSNYAM SWVRQAPGKGLEWVSSI SGSGDYTYYTDSVKGRF TISRDNSKNTLYLQMNS LRAEDTAVYYCARSPWG YYLDSWGQGTLVTVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE	DIQMTQSPPSLSAS AGDRVTITCRASQG ISSRLAWYQQKPEK APKSLIYAASSLQS GVPSRFSGSGSGTD FTLTISSLQPEDFA TYYCQQYNSYPYTF GQGTKLEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:121)

		EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO:120)	
MUC16	Sofituzumab	EVQLVESGGGLVQPGGS LRLSCAASGYSITNDYA WNWVRQAPGKGLEWVGY ISYSGYTTYNPSLKSRF TISRDTSKNTLYLQMNS LRAEDTAVYYCARWTSG LDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSG GTAALGCLVKDYFPCPV TVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPS VFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKG QPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSL SPGK (SEQ ID NO:122)	DIQMTQSPSSLSAS VGDRVTITCKASDL IHNWLAWYQQKPGK APKLLIYGATSLET GVPSRFSGSGSGTD FTLTISSLQPEDFA TYYCQQYWTTPFTF GQGTKVEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:123)

EGFR2	Aba	EVQLQESGPGLVKPSQT LSLTCTVSGYSISRDFA WNWIRQPPGKGLEWMGY ISYNGNTRYQPSLKSRI TISRDTSKNQFFLKLNS VTAADTATYYCVTASRG FPYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSG GTAALGCLVKDYFPCPV TVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPS VFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKG QPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSL SPGK (SEQ ID NO:124)	DIQMTQSPSSMSVS VGDRVTITCHSSQD INSNIGWLQQKPGK SFKGLIYHGTNLDD GVPSRFSGSGSGTD YTLTISSLQPEDFA TYYCVQYAQFPWTF GGGTKLEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:125)
CD7	Ab D	EVQLVESGGGLVKPGGS LKLSCAASGLTFSSYAM SWVRQTPEKRLEWVASI SSGGFTYYPDSVKGRFT ISRDNARNILYLQMSSL RSEDTAMYYCARDEVRG YLDVWGAGTTVTVSSAS TKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAK GQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLS	DIQMTQTTSSLSAS LGDRVTISCSASQG ISNYLNWYQQKPDG TVKLLIYYTSSLHS GVPSRFSGSGSGTD YSLTISNLEPEDIA TYYCQQYSKLPYTF GGGTKLEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO:144)

		LSPGK (SEQ ID NO:143)	
SEZ6	SC17.46	QVQLVQSGAEVKKPGAS VKVSCKASGYTFTSYWI NWVRQAPGQGLEWIGNI FPDTTTTNYNEKFKGRV TLTRDTSISTAYMELSR LRSDDTAVYYCAREYYD GTYDAMDYWGQGTLVTV SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDY FPCPVTVSWNSGALTSG VHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEP KSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVK GFYPCDIAVEWESNGQP ENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 217)	AIQMTQSPSSLSAS VGDRVTITCKASQS VNNDVAWYQQKPGK APKLLIYYASNRYT GVPSRFSGSGSGTD FTLTISSLQPEDFA TYFCQQDYSSPRTF GQGTKLEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO: 218)
CD56	Lorovtuzumab	QVQLVESGGGVVQPGRS LRLSCAASGFTFSSFGM HWVRQAPGKGLEWVAYI SSGSFTIYYADSVKGRF TISRDNSKNTLYLQMNS LRAEDTAVYYCARMRKG YAMDYWGQGTLVTVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK	DVVMTQSPLSLPVT LGQPASISCRSSQI IIHSDGNTYLEWFQ QRPGQSPRRLIYKV SNRFSGVPDRFSGS GSGTDFTLKISRVE AEDVGVYYCFQGSH VPHTFGQGTKVEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO: 220)

		TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 219)	
DLL3	Rovalpituzumab	QVQLVQSGAEVKKPGAS VKVSCKASGYTFTNYGM NWVRQAPGQGLEWMGWI NTYTGEPTYADDFKGRV TMTTDTSTSTAYMELRS LRSDDTAVYYCARIGDS SPSDYWGQGTLVTVSSA STKGPSVFPLAPSSKST SGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKA KGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENN YKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 221)	EIVMTQSPATLSVS PGERATLSCKASQS VSNDVVWYQQKPGQ APRLLIYYASNRYT GIPARFSGSGSGTE FTLTISSLQSEDFA VYYCQQDYTSPWTF GQGTKLEIKRTVAA PSVFIFPPSDEQLK SGTASVVCLLNNFY PREAKVQWKVDNAL QSGNSQESVTEQDS KDSTYSLSSTLTLS KADYEKHKVYACEV THQGLSSPVTKSFN RGEC (SEQ ID NO: 222)
DLK1	DI-2-14	EVQLQQSGAELVKPGAS VKLSCTASGFNIRDTYI HWVKQRPEQGLEWIGRI DPPNGNLKYDPKFQGKA TITADTSSNTAYLQFSS LTSEDTAVYYCARSDGY SFAYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPE	DIVMTQAAPSVPVT PGESVSISCRSSKS LLHSNGNTYLYWFL QRPGQSPQLLIYRM SNLASGVPDRFSGS GSGTAFTLRISRVE AEDVGVYYCMQHVE YPFTFGSGTKLEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV

		VKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAK GQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 223)	TKSFNRGEC (SEQ ID NO: 224)
B7-H3	ABBV-155	EVQLVQSGAEVKKPGSS VKVSCKASGYTFSSYWM HWVRQAPGQGLEWIGLI HPESGSTNYNEMFKNRA TLTVDRSTSTAYMELSS LRSEDTAVYYCAGGGRL YFDYWGQGTTVTVSSAS TKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAK GQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 225)	DIVMTQSPLSLPVT PGEPASISCRSSQS LVHSNRDTYLRWYL QKPGQSPQLLIYKV SNRFSGVPDRFSGS GSGTDFTLKISRVE AEDVGVYYCSQSTH VPYTFGGGTKVEIK RTVAAPSVFIFPPS DEQLKSGTASVVCL LNNFYPREAKVQWK VDNALQSGNSQESV TEQDSKDSTYSLSS TLTLSKADYEKHKV YACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO: 226)

[1110] Expression vectors coding for heavy chains and light chains of the antibodies listed in Table 14 were transfected into suspension HEK293 cells using polyethylenimine and typically cultured for 5 days. Culture supernatants were harvested by centrifugation, filtered, and antibodies purified by Protein A affinity chromatography. If needed, aggregates were removed by size exclusion chromatography. Antibody purity after affinity chromatography was determined by analytical size exclusion chromatography and were >98% monomer. Antibodies were buffered in phosphate buffered saline pH 7.2.

[1111] Conjugate Production (L109A-P1): 12.5 mg of each antibody (0.085 µmoles, 1.0 equiv.) was incubated with 1.25 ml of settled RMP Protein A resin (GE Lifesciences,

17513803) and agitated for 15 minutes. Cysteine HCl monohydrate was added to a final concentration of 20 mM and incubated with agitation for 30 min at room temperature to allow the reactive cysteines to be deblocked. The resin was washed rapidly with 50 column volumes PBS on a vacuum manifold. The resin was then resuspended in an equal volume PBS containing 250 nM $CuCl_2$. Reformation of antibody interchain disulfides was monitored by taking time points. At each time point, 25 µL of resin slurry was removed, 1 µL of 20 mM MC-valcit-PAB-MMAE was added, and the tube flicked several times. The resin was spun down, supernatant removed, and then eluted with 50 µL Antibody elution buffer (Thermo Scientific, 21004). The resin was pelleted and the supernatant analyzed by reverse phase chromatography using an Agilent PLRP-S 4000A 5um, 4.6x50mm column (Buffer A is water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80 C, Flowrate 1.5 ml/min; Gradient 0 minutes – 30%B, 5 minutes – 45%B, 6.5 min – 100%B, 8 minutes – 100%B, 10 minutes – 30%). At 90 minutes after addition of $CuCl_2$, $CuCl_2$ was removed by washing with 50 column volumes of PBS on a vacuum manifold and then 1.25 ml of PBS was added to resuspend. To this slurry of resin and antibody. Respective Linker-Payload (42 µl of a 20 mM solution in DMSO, 1.63 µmoles, 10 equiv.) was added. The resulting mixture was then incubated at ambient temperature for 3 hours. The resin was then washed with 50 column volumes PBS. The ADC was eluted from the resin with Antibody elution buffer (Thermo Scientific, 21004). The ADC was then buffer exchanged into 1X PBS (20X PBS, TeknovaP0191) by dialysis in Dulbecco's PBS pH 7.2 (Hyclone SH30028.03). The material was then concentrated using a centrifugal concentrator using an Amicon Ultra-15, 50KDa, regenerated cellulose (Millipore, UFC0905024), to >3 mg/ml and filtered sterilely through 0.22 µm sterile PVDF Filter, 25mm (Millapore, SLGV013SL) and stored at 4°C. The following analyses were performed: analytical size-exclusion chromatography (SEC) to determine percent monomer, mass spectroscopy (MS) to determine DAR, LAL test to determine endotoxin load and protein concentration determined by A280 utilizing extinction coefficient and molecular weight of antibody. HRMS data (protein method) indicated a dominant mass of the heavy chain+2 species, giving a DAR of ~4.0 was calculated by comparing MS intensities of peaks for DAR1 DAR2 and DAR3 species. SEC indicated 1.8% aggregation, as determined by comparison of the area of the high-molecular-weight peak absorbance at 210 and 280 nm with the area of the peak absorbance for monomeric ADC.

[1112] General Methodology: Drug-to-antibody ratio (DAR) of exemplary ADCs was determined by liquid chromatography-mass spectrometry (LC/MS) according to the following method. For all LC methods, mobile phase A was purified MS grade water (Honeywell, LC015-1), mobile phase B was MS grade 80% Isopropanol (Honeywell LC323-1): 20% acetonitrile (Honeywell, LC015-1), LC323-1), supplemented with 1 % of formic acid (FA) (Thermo Scientific, 85178). The column temperature was set at 80°C. A general MS

method was optimized for all ADCs synthesized. The column used for analysis was an Agilent PLRP-S 4000 A; 2.1x150mm, 8um (Agilent, PL1912-3803). Flowrate used was 0.3 ml/min. The gradient used was 0-0.75 minute 95%A, 0.76 -1.9 minute 75%A, 1.91-11.0 minute 50%A, 11.01-11.50 10%A, 11.51-13.50 minute 95%A,13.51-18 minute 95%A on an Acuity Bio H-Class Quaternary UPLC (Waters). MS system was Xevo G2-XS QToF ESI mass spectrometer (Waters) and data acquired from 1.5-11 minutes and masses were analyzed between 15000-80000 daltons. DAR was determined from the deconvoluted spectra or UV chromatogram by summing the integrated MS (total ion current) or UV (280 nm) peak area of unconjugated and conjugated given species (mAb or associated fragment), weighted by multiplying each area by the number of drug attached. The summed, weighted areas were divided by the sum of total area and the results produced a final average DAR value for the full ADC.

[1113] Size exclusion chromatography (SEC) was performed to determine the quality of the ADCs and aggregation percentage (%) after purification. The analysis was performed on analytical column Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) in isocratic conditions 100% PBS pH 7.2 ((Hyclone SH30028.03)), flow 0.45 ml/min for 8 minutes. The % aggregate fraction of the ADC sample was quantified based on the peak area absorbance at 280 nm. Calculation was based on the ratio between the high molecular weight eluent at 280 nm divided by the sum of peak area absorbance at the same wavelength of the high molecular weight and monomeric eluents multiplied by 100%. Data was aquired on an Agilent Bio-Inert 1260 HPLC outfitted with a Wyatt miniDAWN light scattering and Treos refractive index detectors (Wyatt Technologies, Santa Barbara, CA).

[1114] All exemplified ADCs were characterized by analytical size exclusion chromatography Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) to determine monomer percentage and LC-MS for DAR determination. The average DAR values were determined using the above LC/MS methods and percentage aggregation was determined using the above SEC methods (Table 15).

Table 15: Drug-Antibody Ratios of ADCs Used in *In Vitro* Screening

ADC	DAR
EGFR1 CysMab-L109-P1	3.8
TFRC CysMab-L109-P1	3.8
EPCAM CysMab-L109-P1	4.3
FOLR1 CysMab-L109-P1	3.5
ENPP3 CysMab-L109-P1	3.4
MET CysMab-L109-P1	4
AXL CysMab-L109-P1	3.8
SLC34A2 CysMab-L109-P1	4
NECTIN4 CysMab-L109-P1	4
TACSTD2 CysMab-L109-P1	4
SLC39A6 CysMab-L109-P1	3.6

GPNMB CysMab-L109-P1	4.1
MSLN CysMab-L109-P1	3.8
CD74 CysMab-L109-P1	4
F3 CysMab-L109-P1	3.6
MUC16 CysMab-L109-P1	4
IgG CysMab-L109-P1	4
EGFR2 CysMab-L109-P1	4

Preparation of anti-CD74 BCLxL L11C-P25 conjugates

[1115] To prepare anti-CD74 BCLxL L11C-P25 conjugates, 5 mg of anti-CD74 antibody VHmil x VK1aNQ (34 nmol) at 10 mg/ml was incubated with 0.5 ml of settled RMP Protein A resin (GE Lifesciences, 17513803) and agitated for 15 minutes. Cysteine HCl monohydrate was added to a final concentration of 20 mM and incubated with agitation for 30 min at room temperature to allow the reactive cysteines to be deblocked. The resin was quickly washed with 20 column volumes PBS on a vacuum manifold. The resin was then resuspeneded in an equal volume of PBS containing 250 nM $CuCl_2$. Reformation of antibody interchain disulfides was monitored by taking time points. At each time point, 25 µL of resin slurry was removed, 1 µL of 20 mM MC-valcit-MMAE was added, and the tube flicked several times. The resin was spun down, supernatant removed, and then eluted with 50 µL Antibody elution buffer (ThermoFisher Scientific 21004). The resin was pelleted and the supernatant was analyzed by reverse phase chromatography using an Agilent PLRP-S 4000A 5um, 4.6x50mm column (Buffer A is water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80°C, Flowrate 1.5 ml/min; Gradient 0 minutes – 30%B, 5 minutes – 45%B, 6.5 min – 100%B, 8 minutes – 100%B, 10 minutes – 30%). At 65 minutes after addition of $CuCl_2$ to the Ab/resin slurry, it was removed by washing with 20 column volumes of PBS on a vacuum manifold and then 1 ml of PBS was added to resuspend. DMSO was added to a final concentration of 10% (v/v) and then 10 equivalents of L11C-P25 (20 mM in DMSO) was added. DMSO was added to a final concentration of 10% (v/v). The linker-payload was incubated at room temperature for at least 90 minutes. The excess linker-payload was washed away by washing the resin with 20 column volumes of PBS pH 7.2. The antibody was eluted with 5 ml antibody elution buffer and then buffer exchanged into 1X PBS pH 7.2 by dialysis. The material was then concentrated to 1 ml using a centrifugal concentrator using an Amicon Ultra-15, 50KDa, regenerated cellulose (Millipore, UFC0905024), to 3.8 mg/ml and flash frozen in liquid nitrogen and stored at -80°C until used.

[1116] The following analyses were performed: analytical size-exclusion chromatography (SEC) to determine percent monomer, mass spectroscopy (MS) to determine DAR, LAL test to determine endotoxin load and protein concentration determined by A280 utilizing extinction coefficient and molecular weight of antibody. HRMS data (protein method) indicated a dominant mass of the heavy chain was 55898 da, giving a DAR of 4.0 as

calculated by comparing MS intensities of peaks for DAR1 DAR2 and DAR3 species. SEC indicated </= 2% aggregation, as determined by comparison of the area of the high-molecular-weight peak absorbance at 210 and 280 nm with the area of the peak absorbance for monomeric ADC.

Preparation of anti-CD74 BCLxL L11A-P21 conjugates.

[1117] To prepare anti-CD74 BCLxL L11A-P21 conjugates, 5 mg of anti-CD74 antibody VHmil x VK1aNQ (34 nmol) at 10 mg/ml was incubated with 0.5 ml of settled RMP Protein A resin (GE Lifesciences, 17513803) and agitated for 15 minutes. Cysteine HCl monohydrate was added to a final concentration of 20 mM and incubated with agitation for 30 min at room temperature to allow the reactive cysteines to be deblocked. The resin was quickly washed with 20 column volumes PBS on a vacuum manifold. The resin was then resuspeneded in an equal volume PBS containing 250 nM $CuCl_2$. Reformation of antibody interchain disulfides was monitored by taking time points. At each time point, 25 µL of resin slurry was removed, 1 µL of 20 mM MC-valcit-MMAE was added, and the tube flicked several times. The resin was spun down, supernatant removed, and then eluted with 50 µL Antibody elution buffer (ThermoFisher Scientific 21004). The resin was pelleted and the supernatant analyzed by reverse phase chromatography using an Agilent PLRP-S 4000A 5um, 4.6x50mm column (Buffer A is water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80°C, Flowrate 1.5 ml/min; Gradient 0 minutes – 30%B, 5 minutes – 45%B, 6.5 min – 100%B, 8 minutes – 100%B, 10 minutes – 30%). At 65 minutes after addition of $CuCl_2$ to the Ab/resin slurry, it was removed by washing with 20 column volumes of PBS on a vacuum manifold and then 1 ml of PBS was added to resuspend. DMSO was added to a final concentration of 10% (v/v) and then 10 equivalents of L11A-P21 (20 mM in DMSO) was added. DMSO was added to a final concentration of 10% (v/v). The linker-payload was incubated at room temperature for at least 90 minutes. The excess linker-payload was washed away by washing the resin with 20 column volumes of PBS pH 7.2. The antibody was eluted with 5 ml antibody elution buffer and then buffer exchanged into 1X PBS pH 7.2 by dialysis. The material was then concentrated to 1 ml using a centrifugal concentrator using an Amicon Ultra-15, 50KDa, regenerated cellulose (Millipore, UFC0905024), to 3.5 mg/ml and flash frozen in liquid nitrogen and stored at -80°C until used.

[1118] The following analyses were performed: analytical size-exclusion chromatography (SEC) to determine percent monomer, mass spectroscopy (MS) to determine DAR, LAL test to determine endotoxin load and protein concentration determined by A280 utilizing extinction coefficient and molecular weight of antibody. HRMS data (protein method) indicated a dominant mass of the heavy chain was 55802 da, giving a DAR of 4.0 as calculated by comparing MS intensities of peaks for DAR1 DAR2 and DAR3 species. SEC

indicated </= 2.9% aggregation, as determined by comparison of the area of the high-molecular-weight peak absorbance at 210 and 280 nm with the area of the peak absorbance for monomeric ADC.

Example 8. *In Vitro* Assessment of anti-CD7-, anti-EGFR- and anti-HER2- BCL-xLi ADCs

In vitro activity of anti-CD7-BCL-xLi ADCs and payloads in ALL-SIL cell line (CTG 72h):

[1119] As shown in FIG. 3 and Table 16, the payloads and the anti-CD7-BCL-xLi ADCs induced a dose dependent decrease in the viability of ALL-SIL cells in CTG assay.

In vitro activity of anti-CD7-BCLxLi ADCs and payloads in DND-41 cell line (CTG 72h):

[1120] ALL-SIL cells were cultivated in RPMI supplemented with 20% heat inactivated fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO2. Cells were seeded in 96 well clear bottom plates (96 well clear-bottom, white, Corning reference 3903) and exposed to the payloads or ADCs for 72h (serially diluted; 9 concentrations each, triplicates). Effects of payloads or ADCs on cell viability were assessed after 3 days of incubation at 37°C/5% CO2 by quantification of cellular ATP levels using CellTiterGlo at 75µL reagent/well. All the conditions were tested in triplicates. Luminescence was quantified on a multipurpose plate reader. IC50s were calculated using standard four-parametric curve fitting. IC50 is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the control. Each experiment was performed at least twice, with results being reproducible.

[1121] DND-41 cells were cultivated in RPMI supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96 well clear bottom plates (96 well clear-bottom, white, Corning reference 3903) and exposed to the payloads or ADCs for 72h (serially diluted; 9 concentrations each, triplicates). Effects of payloads or ADCs on cell viability were assessed after 3 days of incubation at 37°C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo at 75µL reagent/well. All the conditions were tested in triplicates. Luminescence was quantified on a multipurpose plate reader. IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the control. Each experiment was performed at least twice, with results being reproducible.

[1122] As shown in FIG. 4 and Table 16, the payloads and the anti-CD7-BCLxLi ADCs induced a dose dependent decrease in the viability of DND-41 cells in CTG assay.

Table 16

	ALLSIL CTG 72h IC50 nM n1	**ALLSIL CTG 72h IC50 nM n2**	**ALLSIL CTG 72h IC50 nM Mean of n1 and n2**	**DND41 CTG 72h IC50 nM n1**	**DND41 CTG 72h IC50 nM n2**	**DND41 CTG 72h IC50 nM Mean of n1 and n2**
Ab D – L27A - P1	0.028	0.018	**0.023**	0.21	0.28	**0.245**
Ab D – L9A-P1	0,025	0,037	**0,031**	0,160	0,140	**0,150**
Ab D – L9A-P9	0,036	0,044	**0,040**	0,190	0,160	**0,175**
Ab D – L9A-P13	0,041	0,051	**0,046**	0,210	0,130	**0,170**
Ab D – L9A-P8	0,020	0,014	**0,017**	0,090	0,066	**0,078**
Ab D – L9A-P10	0,018	0,018	**0,018**	0,089	0,065	**0,077**
Ab D – L9C-P44	0,039	0,027	**0,033**	0,740	0,660	**0,700**
Ab D - L9C-P45	0,360	0,380	**0,370**	>100	>100	**>100**
Ab D – L9C-P46	0,180	0,200	**0,190**	>100	>100	**>100**
Ab D – L9A-P20	0,003	0,009	**0,006**	0,070	0,070	**0,070**
Ab D – L9C-P19	0,003	0,008	**0,006**	0,060	0,050	**0,055**
Ab D - L9A-P21	0,005	0,012	**0,009**	0,100	0,060	**0,080**
Ab D – L9C-P22	0,005	0,004	**0,005**	0,080	0,070	**0,075**
Ab D – L9C-P17	0.006	0.010	**0.008**	2.100	0.380	**1.240**
naked anti-CD7 antibody	>30	>30	**>30**	>100	>100	**>100**
P1	0.38	0.14	**0.26**	1.3	0.26	**0.78**
P9	3,31	1,10	**2,21**	21,80	19,30	**20,55**
P13	2,60	1,44	**2,02**	11,80	12,10	**11,95**
P8	0,02	0,03	**0,02**	0,69	0,41	**0,55**
P10	<Cmin	0,01	**0,01**	0,20	0,29	**0,25**
P44	4,64	3,14	**3,89**	36,00	33,60	**34,80**
P45	3,11	4,01	**3,56**	17,00	16,80	**16,90**
P46	62,20	50,00	**56,10**	211,70	220,00	**215,85**
P20	0,02	0,08	**0,05**	0,38	0,24	**0,31**
P19	4,02	3,50	**3,76**	10,80	7,20	**9,00**

P21	0,02	0,05	**0,04**	0,31	0,20	**0,26**
P22	0,26	0,50	**0,38**	0,45	0,26	**0,36**
P17	0,13	0,26	**0,20**	0,40	0,70	**0,55**

In vitro activity of anti-EGFR-BCL-xLi ADCs and payloads in H1650 cell line (3D, CTG 120h):

[1123] H1650 cells were cultivated in RPMI supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96 microwell round bottom plates (96 microwell Low attachment plates , Costar reference 7007) and exposed to the payloads or ADCs for 120h (serially diluted; 9 concentrations each, duplicates). Effects of payloads or ADCs on cell viability were assessed after 5 days of incubation at 37°C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo at 75µL reagent/well. All the conditions were tested in duplicates. Luminescence was quantified on a multipurpose plate reader. IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the control. Each experiment was performed at least twice, with results being reproducible.

[1124] As shown in FIGs. 5A and 5B and Table 17, the payloads and the anti-EGFR-BCLxLi ADCs induced a dose dependent decrease in the viability of H1650 cells in CTG assay.

Table 17

	H1650 3D CTG IC50 nM (120h)		
	n1	**n2**	**Mean of n1 and n2**
Ab C – L9A-P8	0,14	0,30	**0,22**
Ab C – L9A-P9	0,22	0,30	**0,26**
Ab C – L9A-P10	0,30	0,50	**0,40**
Ab C – L9A-P11	0,50	0,50	**0,50**
Ab C - L9C-P12	0,60	0,90	**0,75**
Ab C – L9A-P13	0,90	0,70	**0,80**
Ab C – L106A-P2	1,00	0,70	**0,85**
Ab C – L9A-P14	1,30	0,40	**0,85**
Ab C – L21A-P2	1,60	1,10	**1,35**
Ab C – L107C-P7	1,60	1,60	**1,60**
Ab C – L106C-P7	1,80	1,50	**1,65**
Ab C – L9A-P15	1,50	1,80	**1,65**
Ab C – L9C-P16	3,90	4,20	**4,05**
Ab C – L107A-P2	3,50	2,40	**2,95**
Ab C – L108A-P2	2,10	0,97	**1,54**
Ab C – L27C-P3	1,40	2,60	**2,00**

Ab C – L9A-P1	0,49	0,33	**0,41**
Ab C – L9C-P17	2,85	2,51	**2,68**
Ab C – L9A-P18	1,20	1,25	**1,23**
Ab C – L9C-P19	0,11	0,13	**0,12**
Ab C – L9A-P20	0,03	0,03	**0,03**
Ab C - L9A-P21	0,09	0,09	**0,09**
Ab C – L9C-P22	0,04	0,05	**0,05**
Ab C – L9C-P23	0,23	0,17	**0,20**
Ab C – L9C-P24	0,17	0,16	**0,17**
Ab C – L9A-P2	4,20	7,00	**5,60**
Ab C - L9C-P25	0,08	0,07	**0,08**
Ab C – L9C-P26	0,12	0,11	**0,12**
Ab C – L9A-P27	0,30	0,22	**0,26**
Ab C – L9A-P28	0,21	0,20	**0,21**
Ab C – L9C-P29	0,13	0,09	**0,11**
Ab C - L9A-P30	0,80	0,52	**0,66**
Ab C – L9C-P31	0,07	0,10	**0,09**
Ab C – L9A-P32	0,04	0,06	**0,05**
Ab C – L9A-P33	0,02	0,03	**0,03**
Ab C – L9A-P34	0,37	0,75	**0,56**
Ab C - L9A-P35	0,02	0,04	**0,03**
Ab C - L9A-P36	0,03	0,03	**0,03**
Ab C - L9A-P37	0,04	0,05	**0,05**
Ab C - L9A-P38	0,03	0,03	**0,03**
Ab C - L9A-P39	0,04	0,05	**0,05**
Ab C - L9C-P40	0,04	0,06	**0,05**
Ab C - L9A-P41	0,81	0,74	**0,78**
Ab C - L9A-P42	0,08	0,11	**0,09**
Ab C - L9A-P43	0,08	0,12	**0,10**
Ab C - L112A-P1	0.3500	0.4200	0.3850
Ab C - L9A-P48	0.2100	0.1900	0.2000
Ab C - L9A-P49	0.0880	0.0810	0.0845
Ab C - L9A-P50	0.0760	0.0900	0.0830
Ab C - L9A-P52	0.0580	0.0850	0.0715
Ab C - L9A-P55	0.2500	0.2900	0.2700
Ab C - L9A-P56	0.1100	0.1600	0.1350
Ab C - L9A-P57	0.0970	0.3500	0.2235
Ab C - L9A-P58	0.2400	0.4900	0.3650
Ab C - L9A-P62	0.0660	0.0600	0.0630
Ab C - L9A-P63	0.1500	0.0700	0.1100
Ab C - L9A-P64	0.5100	0.8700	0.6900
Ab C - L9A-P65	0.1700	0.2200	0.1950
Ab C - L9A-P66	0.5200	0.2400	0.3800
Ab C - L9A-P68	0.0480	0.0750	0.0615

Ab C - L9A-P70	0.1600	0.1800	0.1700
Ab C - L9A-P73	6.4800	5.4800	5.9800
Ab C - L9A-P74	0.3400	0.5600	0.4500
Ab C - L9A-P75	0.4900	0.3500	0.4200
Ab C - L9A-P76	1.0700	1.4800	1.2750
Ab C - L9C-P3	4.4000	11.3000	7.8500
Ab C - L9C-P47	0.2200	0.2100	0.2150
Ab C - L9C-P51	0.0590	0.0460	0.0525
Ab C - L9C-P53	0.3800	0.1600	0.2700
Ab C - L9C-P54	0.3700	0.4100	0.3900
Ab C - L9C-P69	0.0790	0.1800	0.1295
Ab C - L9C-P71	0.9300	0.7700	0.8500
Ab C - L9C-P72	0.0430	0.0920	0.0675
anti-EGFR naked mAb	>100	>100	**>100**
P2	1,06	1,90	**1,48**
P11	0,70	1,20	**0,95**
P1	0,51	1,28	**0,90**
P8	0,70	1,80	**1,25**
P9	0,70	1,40	**1,05**
P18	1,80	3,50	**2,65**
P10	0,30	0,80	**0,55**
P15	0,98	1,20	**1,09**
P12	0,95	2,00	**1,48**
P17	3,15	5,30	**4,23**
P14	1,90	5,40	**3,65**
P13	1,80	3,00	**2,40**
P27	0,22	0,30	**0,26**
P21	0,31	0,37	**0,34**
P30	ND	ND	**ND**
P20	0,36	0,50	**0,43**
P19	2,89	1,43	**2,16**
P22	0,77	0,79	**0,78**
P23	4,00	2,80	**3,40**
P24	2,10	1,80	**1,95**
P25	2,80	3,00	**2,90**
P26	0,68	0,69	**0,69**
P28	0,39	0,18	**0,29**
P29	0,57	0,47	**0,52**
P39	0,21	0,34	**0,28**
P38	0,19	0,33	**0,26**
P31	0,57	1,40	**0,99**
P33	0,89	0,57	**0,73**
P32	0,24	0,22	**0,23**
P34	0,65	0,87	**0,76**

P35	0,23	0,27	**0,25**
P36	0,26	0,31	**0,29**
P37	0,40	1,20	**0,80**
P41	3,30	2,10	**2,70**
P42	9,40	12,90	**11,15**
P43	0,53	0,50	**0,52**
P40	ND	ND	**ND**
P7	7,50	4,70	**6,10**
P3	4,00	28,00	**16,00**
P16	6,10	6,20	**6,15**
P47	6.540	7.350	6.945
P48	2.270	2.790	2.530
P49	1.300	1.120	1.210
P50	0.550	0.210	0.380
P51	1.970	2.830	2.400
P52	0.730	0.380	0.555
P53	37.700	35.000	36.350
P54	10.700	11.800	11.250
P55	6.150	6.880	6.515
P56	13.900	2.950	8.425
P57	6.750	14.300	10.525
P58	5.730	9.080	7.405
P62	0.520	ND	0.520
P63	3.730	2.030	2.880
P64	1.600	2.900	2.250
P65	1.000	1.200	1.100
P66	0.490	0.220	0.355
P68	0.840	0.680	0.760
P69	3.780	5.560	4.670
P70	9.650	9.490	9.570
P71	16.300	13.400	14.850
P72	5.070	5.570	5.320
P73	8.600	9.410	9.005
P74	2.000	1.860	1.930
P75	2.300	3.030	2.665
P76	2.000	3.870	2.935

ND = not tested

Effect of anti-HER2-Bclxli ADCs at single agent and in combination with Paclitaxel in HCC1569 cell viability using CTG assay

[1125] HCC1569 cells were cultivated in RPMI supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2. HCC1569

cells were seeded in 96 microwell (clear-bottom, white, Corning reference 3903) and exposed to the ADCs or the corresponding payloads for 120h (5 fold serially diluted; 9 concentrations each, triplicates) in the absence or in the presence of 10nM of Paclitaxel. Effects of ADCs on cell viability were assessed after 5 days of incubation at 37°C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo at 75µL reagent/well. All the conditions were tested in triplicates. Luminescence was quantified on a multipurpose plate reader. IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the control. Each experiment was performed at least twice, with results being reproducible.

[1126] As shown in FIG. 6 and Table 18, all the payloads and anti-HER2-Bclxli ADCs induced a dose dependent decrease in the viability of HCC1569 cells in CTG assay. Interestingly, the activity of the payloads and ADCs was significantly improved when in combination with 10 nM of Paclitaxel, while no significant effect was observed after treatment of these cells with the corresponding naked antibody at single agent or in combination with Paclitaxel.

Table 18

	HCC1569 CTG 120h IC50 nM N1	**HCC1569 CTG 120h IC50 nM N2**	**HCC1569 CTG 120h IC50 nM Mean N1 and N2**	**HCC1569 CTG 120h IC50 nM + Paclitaxel 10nM N1**	**HCC1569 CTG 120h IC50 nM + Paclitaxel 10nM N2**	**HCC1569 CTG 120h IC50 nM + Paclitaxel 10nM Mean N1 and N2**
Ab T–L13A-P2	>300	>300	**>300**	0.239	0.084	**0.162**
Ab T–L19C-P7	>300	>300	**>300**	0.208	0.124	**0.166**
Ab T–L23C-P7	>300	>300	**>300**	0.287	0.139	**0.213**
Ab T–L110C-P7	>300	>300	**>300**	0.197	0.164	**0.181**
HER2 naked antibody	>300	>300	**>300**	>300	>300	**>300**
Payload P2	13.10	11.70	**12.40**	0.126	0.096	**0.111**
Payload P7	40.30	25.60	**32.95**	0.443	0.429	**0.436**

Example 9. *In Vitro* Assessment of Bcl-xLi payloads and anti-CD7-, anti-CD74-, anti-CD38 and anti-CD48- Bcl-xLi ADCs in haematological malignancies cell lines

[1127] Cell lines were cultured in the media described above at 37°C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96 well clear bottom plates (96 well clear-bottom, white, Corning reference 3903) and exposed to the payloads or ADCs at single agents or in 1/1 combinations with vincristine, ABT-199 or compound A2 for 72h (serially diluted; 9 concentrations each, triplicates). Effects of payloads or ADCs on cell viability were assessed after 3 days of incubation at 37°C/5% CO_2 by quantification of cellular ATP levels using CellTiter- Glo reagent (Promega Ref : G7571) at 75µL reagent/well. All the conditions

were tested in triplicates. Luminescence was quantified on a multipurpose plate reader. IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the control. Each experiment was performed at least twice, with results being reproducible.

Culture media:

- MM1S and LOUCY : RPMI 1640 + Glutamax Medium (Gibco #61870), 10% FBS (Dutscher # 500105Y1 batch S18367S1810), 1% Penicilline-Streptomycine (Gibco #15140), 1% Hepes (Gibco # 15630)
- HPB-ALL and ALL-SIL : RPMI 1640 + Glutamax Medium (Gibco #61870), 20% FBS (Dutscher # 500105Y1 batch S18367S1810) 1% Penicilline-Streptomycine (Gibco #15140), 1% Hepes (Gibco # 15630)
- SUDHL8: RPMI 1640 + Glutamax Medium (Gibco #61870), 20% FBS (Pan Biotech #P30-1302), 1% Penicilline-Streptomycine (Gibco #15140), 1% Hepes (Gibco # 15630)

Plating conditions:

- SUDHL8 : 75 µL/well of 375000 cells/mL for 72h in 96-well plate
- MM1S : 75 µL/well of 300000 cells/mL for 72h in 96-well plate
- ALL-SILL : 75 µL/well of 375000 cells/mL for 72h in 96-well plate
- LOUCY: 75 µL/well of 375000 cells/mL for 72h in 96-well plate
- HPB-ALL: 75 µL/well of 300000 cells/mL for 72h in 96-well plate

[1128] As shown in FIGs. 7A, 7B, 7C and 7D and Tables 19 and 20, the payloads and ADCs induced a dose dependent decrease in the viability of the cell lines tested. Interestingly, the activity of the payloads or ADCs was frequently improved when in combination with vincristine, ABT-199 or Compound A2.

Table19

Compounds	SUDHL8 (DLBCL) IC50 (nM)	MM1S (MM) IC50 (nM)	HPB-ALL (T-ALL) IC50 (nM)	ALL-SILL (T-ALL) IC50 (nM)	LOUCY (T-ALL) IC50 (nM)
P25 n1	5.920	56.700	4.200	2.100	0.800
P25 n2	9.640	92.900	5.000	1.390	1.260
P25 mean	**7.780**	**74.800**	**4.600**	**1.745**	**1.030**
P21 n1	0.340	10.400	1.330	0.400	0.730
P21 n2	0.140	24.300	0.690	0.860	ND
P21 mean	**0.240**	**17.350**	**1.010**	**0.630**	**0.730**
ABT-199 n1	4820.000	4470.000	8920.000	234.000	19.000
ABT-199 n2	7460.000	4600.000	9210.000	213.000	30.000

ABT-199 mean	**6140.000**	**4535.000**	**9065.000**	**223.500**	**24.500**
Compound A2 n1	6670.000	7360.000	5850.000	128.000	30.000
Compound A2 n2	4820.000	7360.000	6300.000	121.000	41.000
Compound A2 mean	**5745.000**	**7360.000**	**6075.000**	**124.500**	**35.500**
P25 + ABT-199 n1	4.690	16.600	2.230	0.140	0.460
P25 + ABT-199 n2	9.960	16.500	2.330	1.240	0.350
P25 + ABT-199 mean	**7.325**	**16.550**	**2.280**	**0.690**	**0.405**
P25 + Compound A2 n1	6.050	14.300	2.690	0.180	0.350
P25 + Compound A2 n2	8.550	11.000	2.180	1.160	0.260
P25 + Compound A2 mean	**7.300**	**12.650**	**2.435**	**0.670**	**0.305**
P21 + ABT-199 n1	0.400	6.440	0.870	0.140	0.250
P21 + ABT-199 n2	0.170	6.260	0.670	0.320	ND
P21 + ABT-199 mean	**0.285**	**6.350**	**0.770**	**0.230**	**0.250**
P21 + Compound A2 n1	0.370	6.090	0.320	0.300	0.360
P21 + Compound A2 n2	0.150	6.370	0.590	0.450	ND
P21 + Compound A2 mean	**0.260**	**6.230**	**0.455**	**0.375**	**0.360**
Vincristine n1	ND	ND	0.680	0.094	0.960
Vincristine n2	ND	ND	0.730	0.083	0.500
Vincristine mean	ND	ND	**0.705**	**0.089**	**0.730**
P25 + Vincristine n1	ND	ND	0.330	0.097	0.230
P25 + Vincristine n2	ND	ND	0.320	0.074	0.150
P25 + Vincristine mean	ND	ND	**0.325**	**0.086**	**0.190**
P21 + Vincristine n1	ND	ND	0.320	0.081	0.130
P21 + Vincristine n2	ND	ND	0.170	0.033	ND
P21 + Vincristine mean	ND	ND	**0.245**	**0.057**	**0.130**
Ab F - L9A-P21 n1	ND	ND	ND	23.900	ND
Ab F - L9A-P21 n2	ND	ND	ND	32.000	ND
Ab F - L9A-P21 mean	ND	ND	ND	**27.950**	ND
Ab E - L9A-P21 n1	ND	ND	ND	0.017	ND
Ab E - L9A-P21 n2	ND	ND	ND	0.022	ND
Ab E - L9A-P21 mean	ND	ND	ND	**0.020**	ND
Ab E - L9C-P25 n1	ND	ND	ND	0.006	ND
Ab E - L9C-P25 n2	ND	ND	ND	0.009	ND
Ab E - L9C-P25 mean	ND	ND	ND	**0.007**	ND
Ab E - L9A-P1 n1	ND	ND	ND	0.027	ND
Ab E - L9A-P1 n2	ND	ND	ND	0.036	ND
Ab E - L9A-P1 mean	ND	ND	ND	**0.032**	ND
Ab E - L9C-P40 n1	ND	ND	ND	0.008	ND
Ab E - L9C-P40 n2	ND	ND	ND	0.012	ND
Ab E - L9C-P40 mean	ND	ND	ND	**0.010**	ND
Ab E - L9A-P33 n1	ND	ND	ND	0.011	ND
Ab E - L9A-P33 n2	ND	ND	ND	0.018	ND

Ab E - L9A-P33 mean	ND	ND	ND	**0.015**	ND
P40 n1	ND	ND	ND	1.060	ND
P40 n2	ND	ND	ND	1.260	ND
P40 mean	ND	ND	ND	**1.160**	ND
P33 n1	ND	ND	ND	0.100	ND
P33 n2	ND	ND	ND	0.190	ND
P33 mean	ND	ND	ND	**0.145**	ND
Ab H - L9C-P25 n1	0.910	> 300	> 300	127.000	110.000
Ab H - L9C-P25 n2	6.180	> 300	>300	73.700	113.000
Ab H - L9C-P25 Mean	**3.545**	**> 300**	**>300**	**100.350**	**111.500**
Ab H - L9C-P25 + ABT-199 n1	0.750	> 300	110.000	13.500	0.930
Ab H - L9C-P25 + ABT-199 n2	4.910	199.000	110.000	11.400	1.170
Ab H - L9C-P25 + ABT-199 Mean	**2.830**	**199.000**	**110.000**	**12.450**	**1.050**
Ab H - L9C-P25 + Compound A2 n1	1.150	> 300	61.800	15.300	0.960
Ab H - L9C-P25 + Compound A2 n2	4.840	> 300	109.000	7.580	1.490
Ab H - L9C-P25 + Compound A2 Mean	**2.995**	> 300	**85.400**	**11.440**	**1.225**
Ab H - L9C-P25 + Vincristine n1	ND	ND	0.350	0.069	0.130
Ab H - L9C-P25 + Vincristine n2	ND	ND	0.390	0.040	0.220
Ab H - L9C-P25 + Vincristine Mean	ND	ND	**0.370**	**0.055**	**0.175**
Ab H - L11C-P25 n1	0.900	> 300	159.000	50.000	37.900
Ab H - L11C-P25 n2	2.450	> 300	174.000	31.900	64.500
Ab H - L11C-P25 Mean	**1.675**	**> 300**	**166.500**	**40.950**	**51.200**
Ab H - L11C-P25 + ABT-199 n1	0.480	257.000	27.700	9.830	0.970
Ab H - L11C-P25 + ABT-199 n2	2.590	229.000	32.200	5.900	1.240
Ab H - L11C-P25 + ABT-199 Mean	**1.535**	**243.000**	**29.950**	**7.865**	**1.105**
Ab H - L11C-P25 + Compound A2 n1	0.370	221.000	31.000	10.700	1.310
Ab H - L11C-P25 + Compound A2 n2	2.860	211.000	39.200	7.320	2.300
Ab H - L11C-P25 + Compound A2 Mean	**1.615**	**216.000**	**35.100**	**9.010**	**1.805**
Ab H - L11C-P25 + Vincristine n1	ND	ND	0.280	0.057	0.120
Ab H - L11C-P25 + Vincristine n2	ND	ND	0.180	0.027	0.190
Ab H - L11C-P25 + Vincristine Mean	ND	ND	**0.230**	**0.042**	**0.155**
Ab I - L9C-P25 n1	> 300	25.40	99.80	72.60	68.40
Ab I - L9C-P25 n2	241.00	>300	ND	52.60	ND
Ab I - L9C-P25 Mean	**241.00**	**25.40**	**99.80**	**62.60**	**68.40**
Ab I - L9C-P25 + ABT-199 n1	202.00	3.83	17.50	28.70	4.57
Ab I - L9C-P25 + ABT-199 n2	148.00	5.57	ND	15.80	ND
Ab I - L9C-P25 + ABT-199 Mean	**175.00**	**4.70**	**17.50**	**22.25**	4.57
Ab I - L9C-P25 + Compound A2 n1	267.00	4.68	21.20	24.50	7.34
Ab I - L9C-P25 + Compound A2 n2	155.00	17.50	ND	15.90	ND
Ab I - L9C-P25 + Compound A2 Mean	**211.00**	**11.09**	**21.20**	**20.20**	**7.34**
Ab I - L9C-P25 + Vincristine n1	ND	ND	0.49	0.08	0.19
Ab I - L9C-P25 + Vincristine n2	ND	ND	ND	0.06	ND

Ab I - L9C-P25 + Vincristine Mean	ND	ND	**0.49**	**0.07**	**0.19**
Ab I - L11C-P25 n1	54.10	11.00	32.80	73.90	74.60
Ab I - L11C-P25 n2	39.10	>300	18.90	44.70	89.40
Ab I - L11C-P25 Mean	**46.60**	**11.00**	**25.85**	**59.30**	**82.00**
Ab I - L11C-P25 + ABT-199 n1	36.50	3.50	3.74	18.80	4.19
Ab I - L11C-P25 + ABT-199 n2	19.20	6.50	3.06	12.00	4.17
Ab I - L11C-P25 + ABT-199 Mean	**27.85**	**5.00**	**3.40**	**15.40**	**4.18**
Ab I - L11C-P25 + Compound A2 n1	62.10	3.03	3.96	17.80	6.79
Ab I - L11C-P25 + Compound A2 n2	25.60	5.40	3.45	12.00	6.36
Ab I - L11C-P25 + Compound A2 Mean	**43.85**	**4.22**	**3.71**	**14.90**	**6.58**
Ab I - L11C-P25+ Vincristine n1	ND	ND	0.25	0.07	0.31
Ab I - L11C-P25+ Vincristine n2	ND	ND	0.19	0.03	0.36
Ab I - L11C-P25 + Vincristine Mean	ND	ND	**0.22**	**0.05**	**0.34**
Ab F - L9C-P25 n1	296.00	>300	186.00	171.00	122.00
Ab F - L9C-P25 n2	>300	>300	>300	175.00	118.00
Ab F - L9C-P25 Mean	**>300**	**>300**	**>300**	**173.00**	**120.00**
Ab F - L9C-P25 + ABT-199 n1	226.00	>300	143.00	53.70	9.20
Ab F - L9C-P25 + ABT-199 n2	244.00	>300	166.00	55.80	13.10
Ab F - L9C-P25 + ABT-199 Mean	**235.00**	**>300**	**154.50**	**54.75**	**11.15**
Ab F - L9C-P25+ Compound A2 n1	234.00	>300	137.00	56.40	13.40
Ab F - L9C-P25 + Compound A2 n2	117.00	>300	157.00	60.40	15.40
Ab F - L9C-P25 + Compound A2 Mean	**175.50**	**>300**	**147.00**	**58.40**	**14.40**
Ab F - L9C-P25 + Vincristine n1	ND	ND	1.00	0.08	0.37
Ab F - L9C-P25 + Vincristine n2	ND	ND	0.12	0.07	0.51
Ab F - L9C-P25 + Vincristine Mean	ND	ND	**0.56**	**0.08**	**0.44**

Table 20

Compounds	HPB-ALL (T-ALL) IC50 (nM)	ALL-SILL (T-ALL) IC50 (nM)	LOUCY (T-ALL) IC50 (nM)
Ab D - L9C-P25 n1	0.03000	0.01200	0.01700
Ab D - L9C-P25 n1	0.04400	0.01600	0.01700
Ab D - L9C-P25 Mean	**0.03700**	**0.01400**	**0.01700**
Ab D - L9C-P25 + ABT-199 n1	0.02400	0.01100	0.01400
Ab D - L9C-P25 + ABT-199 n2	0.04400	0.01400	0.01800
Ab D - L9C-P25 + ABT-199 Mean	**0.03400**	**0.01250**	**0.01600**
Ab D - L9C-P25 + Compound A2 n1	0.03200	0.01200	0.01500
Ab D - L9C-P25+ Compound A2 n2	0.05400	0.00920	0.01800
Ab D - L9C-P25 + Compound A2 Mean	**0.04300**	**0.01060**	**0.01650**
Ab D - L9C-P25 + Vincristine n1	0.04500	0.01200	0.01600
Ab D - L9C-P25 + Vincristine n2	0.05400	0.01000	0.01200

Ab D - L9C-P25 + Vincristine Mean	**0.04950**	**0.01100**	**0.01400**
Ab D - L11C-P25 n1	0.02600	0.01200	0.01000
Ab D - L11C-P25 n2	0.03000	0.00790	0.01000
Ab D - L11C-P25 Mean	**0.02800**	**0.00995**	**0.01000**
Ab D - L11C-P25 + ABT-199 n1	0.02600	0.00700	0.01300
Ab D - L11C-P25 + ABT-199 n2	0.04600	0.00910	0.01400
Ab D - L11C-P25 + ABT-199 Mean	**0.03600**	**0.00805**	**0.01350**
Ab D - L11C-P25 + Compound A2 n1	0.02800	0.01100	0.01000
Ab D - L11C-P25 + Compound A2 n2	0.04100	0.00980	0.01000
Ab D - L11C-P25 + Compound A2 Mean	**0.03450**	**0.01040**	**0.01000**
Ab D - L11C-P25 + Vincristine n1	0.02200	0.01000	0.01200
Ab D - L11C-P25 + Vincristine n2	0.02100	0.00610	0.00970
Ab D - L11C-P25 + Vincristine Mean	**0.02150**	**0.00805**	**0.01085**
Compounds	**SUDHL8 (DLBCL) IC50 (nM)**	**MM1S (MM) IC50 (nM)**	**ALL-SILL (T-ALL) IC50 (nM)**
VHmil x VK1aNQ-L11C-P25 n1	0.05	7.84	109.00
VHmil x VK1aNQ-L11C-P25 n2	0.06	31.00	97.80
VHmil x VK1aNQ-L11C-P25 Mean	**0.06**	**19.42**	**103.40**
VHmil x VK1aNQ-L11C-P25 + ABT-199 n1	0.05	2.56	ND
VHmil x VK1aNQ-L11C-P25 + ABT-199 n2	0.06	3.15	ND
VHmil x VK1aNQ-L11C-P25 + ABT-199 Mean	**0.06**	2.86	ND
VHmil x VK1aNQ-L11C-P25 + Compound A2 n1	0.06	3.11	ND
VHmil x VK1aNQ-L11C-P25 + Compound A2 n2	0.08	3.96	ND
VHmil x VK1aNQ-L11C-P25 + Compound A2 Mean	**0.07**	3.54	ND
VHmil x VK1aNQ-L11C-P21 n1	0.14	24.90	162.00
VHmil x VK1aNQ-L11C-P21 n2	0.19	>300	132.00
VHmil x VK1aNQ-L11C-P21 Mean	**0.17**	**24.90**	**147.00**
VHmil x VK1aNQ-L11C-P21 + ABT-199 n1	0.16	3.69	ND
VHmil x VK1aNQ-L11C-P21 + ABT-199 n2	0.15	5.19	ND
VHmil x VK1aNQ-L11C-P21 + ABT-199 Mean	**0.16**	**4.44**	ND
VHmil x VK1aNQ-L11C-P21 + Compound A2 n1	0.12	5.02	ND
VHmil x VK1aNQ-L11C-P21 + Compound A2 n2	0.14	5.75	ND

VHmil x VK1aNQ-L11C-P21 + Compound A2 Mean	**0.13**	**5.39**	ND
Ab G - L9C-P25 n1	0.06	4.36	175.00
Ab G - L9C-P25 n2	0.08	3.99	133.00
Ab G - L9C-P25 Mean	**0.07**	**4.18**	**154.00**
Ab G - L11C-P25 n1	0.10	7.00	147.00
Ab G - L11C-P25 n2	0.13	9.81	111.00
Ab G - L11C-P25 Mean	**0.12**	**8.41**	**129.00**

Example 10. *In Vitro* Assessment of BCL-xL antibody drug conjugates in NCI-H1650 Cell Lines

[1129] The BCL-xL antibody drug conjugates were tested against an endogenous cancer cell line in NCI-H1650: (ATCC No. CRL-5883 cultured in RPMI-1640 + 10% FBS). One target was assessed: EGFR.

Inhibition of cell proliferation and survival

[1130] The ability of the BCL-xL antibody drug conjugates to inhibit cell proliferation and survival was assessed using the Promega CellTiter-Glo® proliferation assay.

[1131] Cell lines were cultured in media that is optimal for their growth at 5% CO_2, 37°C in a tissue culture incubator. Prior to seeding for the proliferation assay, the cells were split at least 2 days before the assay to ensure optimal growth density. On the day of seeding, adherent cells were lifted off tissue culture flasks using 0.25% trypsin. Cell viability and cell density were determined using a cell counter (Vi-Cell XR Cell Viability Analyzer, Beckman Coulter). Cells with higher than 85% viability were seeded for the assay.

[1132] The NCI-H1650 cell line was seeded in black, clear round bottom 384-well ultra-low attachment spheroid microplates (Corning cat. # 3830). Cells were seeded at a density of 3,000 cells per well in 45 uL of standard growth media. Plates were spun in a centrifuge for 5 minutes and 1,000 RPM. Plates were incubated at 5% CO_2, 37°C for 72 hours in a tissue culture incubator. On the day of dosing, EGFR targeting BCL-xL ADCs were prepared at 10X in standard growth media. The prepared drug treatments were then added to the cells resulting in final concentrations of 0.0005 – 500 nM and a final volume of 50 µL per well. Each drug concentration was tested in quadruplets. Plates were incubated at 5% CO_2, 37°C for 5 days in a tissue culture incubator.

[1133] Cell viability was assessed through the addition of 40 µL of CellTiter Glo® 3D Cell Viability Assay substrate (Promega, cat# G9681), a reagent which lyses cells and measures total adenosine triphosphate (ATP) content. Wells were mixed thoroughly and plates were incubated at room temperature for 30 minutes to stabilize luminescent signals prior to reading using a luminescence reader (EnVision Multilabel Plate Reader, PerkinElmer).

[1134] To evaluate the effect of the drug treatments, luminescent counts from wells containing untreated cells (100% viability) were used to normalize treated samples. A variable slope model was applied to fit a nonlinear regression curve to the data in GraphPad PRISM version 7.02 software. IC50 and Amax values were extrapolated from the resultant curves. The concentrations of treatment required to inhibit 50% of cell growth or survival (IC50) were calculated with representative IC50 values of the cell lines tested summarized in Table 21.

[1135] The representative cancer cell line was shown to be sensitive to the BCL-xL ADCs targeting EGFR with IC50 values ranging from 0.055 – 100+ nM activity. L11A-P21, L11A-P27, L11C-P19 and L11C-P25 were among the most potent BCL-xL ADCs tested on the NCI-H1650 cell line. These studies indicate that BCL-xL ADCs were capable of inhibiting cell proliferation on acancer cell line expressing EGFR.

Table 21: EGFR1 BCL-xL ADCs (IC50s)

Conjugate	NCI-H1650	
	IC50 (nM)	Amax (Span)
EGFR1-L1A-P1	<0.686	--
EGFR1-L1A-P2	<6.17	--
EGFR1-L1C-P3	<6.17	97.8
EGFR1-L3A-P1	<0.686	--
EGFR1-L3A-P1	0.6517	> 100
EGFR1-L3C-P3	1.162	100.6
EGFR1-L3C-P4	>500	--
EGFR1-L3C-P5	>500	--
EGFR1-L4A-P1	<0.686	--
EGFR1-L7A-P1	<0.686	--
EGFR1-L7A-P2		
EGFR1-L7C-P3	<6.17	--
EGFR1-L7C-P6	>500	--
EGFR1-L7C-P7	<55.56	--
EGFR1-L8A-P1	<2.06	--
EGFR1-L8C-P7		
EGFR1-L9A-P1		
EGFR1-L9A-P2	2.457	93.77
EGFR1-L9C-P4	>500	--
EGFR1-L9C-P5	>500	--
EGFR1-L109A-P1	<6.17	--
EGFR1-L10A-P1	0.276	86.4
EGFR1-L10A-P2	<6.17	--
EGFR1-L10C-P3	<18.52	--
EGFR1-L11A-P21	0.079	88.68
EGFR1-L11A-P27	0.055	97.5
EGFR1-L11C-P19	0.209	93.57
EGFR1-L11C-P25	0.084	93.28

Example 11. *In vitro* assessment of EGFR BCLxL ADCs in a cancer cell line

[1136] The BCL-xL antibody drug conjugates were tested against one endogenous cancer cell line in **NCI-H1650** (ATCC No. CRL-5883 cultured in RPMI-1640 + 10% FBS). One target was assessed: EGFR.

Inhibition of cell proliferation and survival

[1137] The ability of the BCL-xL antibody drug conjugates to inhibit cell proliferation and survival was assessed using the Promega CellTiter-Glo® proliferation assay.

[1138] Cell lines were cultured in media that is optimal for their growth at 5% CO_2, 37°C in a tissue culture incubator. Prior to seeding for the proliferation assay, the cells were split at least 2 days before the assay to ensure optimal growth density. On the day of seeding, adherent cells were lifted off tissue culture flasks using 0.25% trypsin. Cell viability and cell density were determined using a cell counter (Vi-Cell XR Cell Viability Analyzer, Beckman Coulter). Cells with higher than 85% viability were seeded for the assay.

[1139] The NCI-H1650 cell line was seeded in black, clear round bottom 384-well ultra-low attachment spheroid microplates (Corning cat. # 3830). Cells were seeded at a density of 3,000 cells per well in 45 µL of standard growth media. Plates were spun in a centrifuge for 5 minutes and 1,000 RPM. Plates were incubated at 5% CO_2, 37°C for 72 hours in a tissue culture incubator. On the day of dosing, EGFR targeting BCL-xL ADCs were prepared at 10X in standard growth media. The prepared drug treatments were then added to the cells resulting in final concentrations of 0.0025 – 50 nM and a final volume of 50 µL per well. Each drug concentration was tested in quadruplets. Plates were incubated at 5% CO_2, 37°C for 5 days in a tissue culture incubator.

[1140] Cell viability was assessed through the addition of 40 µL of CellTiter Glo® 3D Cell Viability Assay substrate (Promega, cat# G9681), a reagent which lyses cells and measures total adenosine triphosphate (ATP) content. Wells were mixed thoroughly and plates were incubated at room temperature for 30 minutes to stabilize luminescent signals prior to reading using a luminescence reader (EnVision Multilabel Plate Reader, PerkinElmer).

[1141] To evaluate the effect of the drug treatments, luminescent counts from wells containing untreated cells (100% viability) were used to normalize treated samples. A variable slope model was applied to fit a nonlinear regression curve to the data in GraphPad PRISM version 7.02 software. IC50 and Amax values were extrapolated from the resultant curves. The concentrations of treatment required to inhibit 50% of cell growth or survival (IC50) were calculated with representative IC50 values of the cell lines tested summarized in Table 22.

[1142] The representative cancer cell line was shown to be sensitive to the BCL-xL inhibitor ADCs targeting EGFR with IC50 values ranging from 0.042 – 0.069 nM activity. All nine ADCs tested demonstrated equivalent potency on the NCI-H1650 cell line model. These

studies indicate that BCL-xL ADCs were capable of inhibiting cell proliferation on a cancer cell line expressing EGFR.

Table 22: EGFR1 BCL-xL Inhibitor ADCs *In Vitro* Activity

Conjugate	NCI-H1650	
	IC50 (nM)	Span (Amax)
EGFR1-L30A-P21	0.043	92.1
EGFR1-L35A-P21	0.060	94.8
EGFR1-L36A-P21	0.052	91.7
EGFR1-L37A-P21	0.052	87.9
EGFR1-L38A-P21	0.042	89.6
EGFR1-L39A-P21	0.045	92.6
EGFR1-L40A-P21	0.053	101.8
EGFR1-L42A-P21	0.042	95.3
EGFR1-L42C-P25	0.069	97.7

Example 12. Evaluation of *in vitro* ADC activity in a panel of cancer cell lines

[1143] The antibody drug conjugates were tested against cancer cell lines obtained from ATCC (American Type Culture Collection) or from cell lines derived from patient xenograft models. The cells were cultured in media that is optimal for their growth at 5% CO2, 37°C in a tissue culture incubator. Prior to seeding for the proliferation assay, the cells were split at least 2 days before the assay to ensure optimal growth density. On the day of seeding, cells were lifted off tissue culture flasks using 0.25% trypsin. Cell viability and cell density were determined using a cell counter (Vi-Cell XR Cell Viability Analyzer, Beckman Coulter). Cells with higher than 85% viability were seeded in white clear bottom 384-well plates (Greiner cat # 781098) at a density of 1000 cells per well in 50 µL of standard growth media. Plates were incubated at 37°C overnight in a tissue culture incubator.

[1144] The ADCs were prepared in standard phosphate buffered solution to desired concentrations. A series of 10 dilutions were made for each ADC. The prepared drug treatments were then added to the cells resulting in final concentrations of 0.000005 – 300 nM. An acoustic transfer device (Echo555, Beckman Coulter) was used to add the ADCs to the cells. Each treatment was tested in triplicate assay plates. Plates were incubated at 37°C overnight or for 5 days in a tissue culture incubator. The ability of the ADCs to inhibit cell proliferation and survival was assessed using the Promega CellTiter-Glo® proliferation assay. Plates were incubated at room temperature for 20 minutes to stabilize luminescent signals prior to reading using a multimode plate reader (Pherastar, BMG). Luminescent counts of untreated cells were taken the day after seeding (Day 0 readings), and after 5 days

of treatment (Day 5 readings). The Day 5 readings of the untreated cells were compared to the Day 0 readings. Assays with at least one cell doubling during the incubation period were considered valid. To evaluate the effect of the drug treatments, luminescent counts from wells containing untreated cells (100% viability) were used to normalize treated samples. The concentrations of treatment required to inhibit 50% of cell growth or survival (GI50) were calculated using a four parameter logistic regression equation. The test results are shown in Tables 23, 24 and 25 below.

Table 23

Antibody target	IgG		AXL		CD74		EGFR-A		EGFR-C		ENPP3	
Linker Payload	L109A-P1		L109A-P1		L109A-P1		L109A-P1		L109A-P1		L109A-P1	
Cell Line Name	GI50 (uM)	Amax	GI50 (uM)	Amax	GI50 (uM)	Amax	GI50 (uM)	Amax	GI50 (uM)	Amax	GI50 (uM)	Amax
ags	0.086	40.74	0.103	34.54	0.101	37.92	0.098	45.15	0.153	47.34	0.031	26.55
aspc1	>0.3	95.97	>0.3	96.72	>0.3	104.88	>0.3	94.78	>0.3	85.16	>0.3	59.63
bicr22	>0.3	59.79	>0.3	52.24	>0.3	62.34	>0.3	91.68	0.084	36.1	0.218	41.46
bicr56	>0.3	50.76	>0.3	90.82	>0.3	100.24	>0.3	58.11	0.002	30.17	>0.3	94
c2bbe1	>0.3	100.4	>0.3	100.84	>0.3	115.64	>0.3	95.25	0.138	44.83	>0.3	111.98
c32	>0.3	95.83	>0.3	95.99	>0.3	98.46	>0.3	103.4	>0.3	97.63	>0.3	60.98
caki2	0.223	39.62	>0.3	91.73	>0.3	52.5	0.067	26.16	0.006	35.2	0.179	41.46
cal120	>0.3	97.32	>0.3	63.02	>0.3	102.04	>0.3	67.15	0.293	48.67	>0.3	96.96
calu3	0.091	-5.7	0.216	17.64	0.012	-46.22	0.092	-61.65	0.006	-60.45	0.168	12.49
calu6	>0.3	102.31	>0.3	101.43	>0.3	97.71	>0.3	102.48	>0.3	101.13	>0.3	100.19
capan2	0.287	48.41	>0.3	94.56	0.086	38.71	0.209	41.32	0.065	22.24	>0.3	59.57
cjm	>0.3	65.39	>0.3	99.91	>0.3	67.83	>0.3	78.46	>0.3	63.84	>0.3	96.4
cl40	>0.3	108.1	>0.3	84.98	>0.3	75.09	>0.3	100.28	>0.3	89.31	>0.3	76.75
colo201	>0.3	116.32	>0.3	85.83	>0.3	86.1	>0.3	87.12	>0.3	104.22	>0.3	104.91
colo741	>0.3	109.21	>0.3	74.24	>0.3	101.28	>0.3	103.44	>0.3	99.35	0.218	38.65
colo783	>0.3	99.51	>0.3	62.21	>0.3	64.86	>0.3	64.02	>0.3	57.96	0.268	43.63
colo792	>0.3	56.97	0.207	42.98	>0.3	83.87	>0.3	54.48	0.256	41.19	0.154	10.15
corl105	0.203	35.14	>0.3	64.37	0.006	22.24	0.178	18.27	0.008	-37.83	0.269	44.17
du4475	>0.3	102.46	>0.3	100.69	>0.3	111.16	>0.3	107.29	>0.3	103.62	>0.3	111.26
dv90	>0.3	76.92	>0.3	108.79	>0.3	105.55	>0.3	96.41	>0.3	79.07	>0.3	98.27
ebc1	>0.3	65.02	>0.3	89.81	>0.3	74.58	>0.3	86.76	>0.3	59.11	>0.3	66.71
ecc10	>0.3	90.23	>0.3	102.22	>0.3	94.05	>0.3	103.09	>0.3	93.14	>0.3	83.98
g361	>0.3	100.99	>0.3	100.83	>0.3	95.27	>0.3	105.04	>0.3	97.76	>0.3	69.44
g402	>0.3	96.95	>0.3	101.02	>0.3	101.15	>0.3	100.69	>0.3	98.93	>0.3	94.45
gss	0.106	-76.31	>0.1	68.38	>0.3	92.45	0.053	-83.73	0.072	-86.96	0.138	-85.26
hcc1143	>0.3	62.31	>0.3	98.63	>0.3	77.11	>0.3	52.97	>0.3	54.27	>0.3	62.29
hcc15	>0.3	100.83	>0.3	89.55	>0.3	101.16	>0.3	97.43	>0.3	98.21	>0.3	99.65
hcc1569	0.138	26.3	0.206	36.24	0.191	32.91	0.075	12.09	0.089	9.47	0.146	28.58

hcc1937	0.272	47.89	0.219	43.05	0.258	47.4	0.226	44.05	0.180	38.98	0.178	38.02
hcc2279	0.101	26.72	0.001	8.19	0.084	25.83	0.039	3.99	0.003	22.06	0.134	21.66
hcc2935	0.109	30.05	0.214	41.69	>0.3	58.84	0.181	34.81	0.006	21.58	>0.3	52.31
hcc38	0.067	11.78	0.088	23.75	0.119	23.07	0.079	-5.03	0.029	-21.41	0.119	5.18
hcc4006	>0.3	96.89	>0.3	100.71	>0.3	111.86	>0.3	99.85	>0.3	79.53	>0.3	96.82
hdqp1	0.269	46.27	>0.3	104.94	>0.3	107.3	0.283	45.96	0.001	36.04	>0.3	97.67
hec108	0.197	34.66	0.193	38.97	0.248	37.95	0.011	17.3	0.001	34.58	0.113	27.71
hec251	>0.3	96.97	>0.3	98.47	>0.3	103.09	>0.3	94.91	>0.3	92.8	>0.3	57.49
hpafii	>0.3	85.51	>0.3	106.47	>0.3	108.31	>0.3	102.84	0.238	47.31	>0.3	104.66
ht55	>0.3	103.54	>0.3	101.61	>0.3	96.63	>0.3	108.83	>0.3	97.2	>0.3	98.67
hucct1	>0.3	94.97	>0.3	95.41	>0.3	100.56	>0.3	98.51	>0.3	88.16	>0.3	98.64
ialm	>0.3	96.02	>0.3	110.94	>0.3	97.67	>0.3	93.64	>0.3	91.85	>0.3	95.52
jhoc5	>0.3	56.97	>0.3	65.36	>0.3	55.19	0.040	46.16	>0.3	52.96	>0.3	61.33
jhom2b	0.014	-2.34	>0.3	64.66	0.087	-9.61	>0.3	86.17	0.002	-48.22	>0.3	95.15
jhuem3	0.206	39.5	0.197	36.03	>0.3	55.33	0.111	28.08	0.093	4.84	0.050	49.07
jhuem7	0.057	25.04	0.096	23.04	0.222	45.78	0.060	21.43	0.034	21.52	0.089	23.67
ke39	>0.3	106.37	>0.3	92.29	>0.3	89.03	>0.3	97.58	>0.3	59.15	>0.3	101.24
lclc103h	>0.3	102.69	>0.3	90.63	>0.3	99.23	>0.3	103.08	>0.3	100.77	>0.3	67.16
lounh91	0.229	46.61	0.277	48.18	>0.3	54.67	0.207	42.61	0.223	45.39	0.241	43.45
ls123	0.068	4.51	0.084	34.01	0.146	17.49	0.067	-8.5	0.008	-33.61	0.120	15.77
ls513	>0.3	77.57	>0.3	87.01	>0.3	88.61	>0.3	72.45	>0.3	68.37	>0.3	80.81
melho	>0.3	97.13	>0.3	96.04	>0.3	101.96	>0.3	98.16	>0.3	98.44	>0.3	93.05
nccstck140	0.079	-45.72	0.065	21.38	0.188	21.77	0.032	24.75	0.000	-26.98	0.182	11.26
ncih1435	0.210	41.27	>0.3	90.43	>0.3	101.44	0.171	26.25	0.035	10.6	>0.3	94.38
ncih146	>0.3	71.51	>0.3	63.97	>0.3	76.34	>0.3	76.22	>0.3	59.69	>0.3	71.72
ncih1792	>0.3	102.01	>0.3	100.76	>0.3	103.83	>0.3	101.07	>0.3	95.88	>0.3	102.26
ncih1838	>0.3	96.77	>0.3	94.4	>0.3	98.78	>0.3	73.31	>0.3	56.68	>0.3	64.74
ncih2122	>0.3	101.63	>0.3	96.94	>0.3	104.63	>0.3	99.13	>0.3	100.02	>0.3	99.22
ncih2170	>0.3	56.33	>0.3	71.44	>0.3	66.31	0.214	36.55	0.179	39.44	>0.3	58.83
ncih2171	0.082	6.3	0.125	6.02	0.124	3.49	0.062	-65.03	0.053	-64.34	0.115	-11.98
ncih3255	>0.1	65.65	>0.3	67.65	>0.3	61.36	0.025	-15.88	0.000	-80.35	>0.3	95.85
ncih596	>0.3	86.2	>0.3	87.93	>0.3	96.68	>0.3	88.54	>0.3	69.56	>0.3	90.38
ncin87	0.135	43.14	>0.3	54.01	>0.3	68.28	0.151	27.72	0.122	40.03	>0.3	68.19
nvx0003132 92dplastic	>0.3	91.55	>0.3	91.45	>0.3	89.53	>0.3	65.43	>0.3	84.33	>0.3	64.5

nvx0009209 42dplastic	>0.3	84.37	>0.3	99.84	>0.3	99.2	0.230	46.99	0.002	1.51	>0.3	88.34
nvx0022484 92dplastic	>0.3	95.62	>0.3	103.57	>0.3	95.48	>0.3	98.08	>0.3	97.29	>0.3	67.61
nvx0051127 02dcollagen	>0.3	97.18	>0.3	103.64	>0.3	94.2	>0.3	85.64	0.003	8.92	>0.3	101
nvx0058467 62dplastic	>0.3	95.88	>0.3	94.1	>0.3	99.08	>0.3	102.4	>0.3	96.61	>0.3	96.83
nvx0062299 72dcollagen	>0.3	58.9	>0.3	99.45	>0.3	76.75	0.225	42.31	0.024	22.34	>0.3	62.97
ocum1	0.274	47.2	>0.3	84.72	>0.3	98.24	0.204	12.9	0.174	16.94	>0.3	91.86
ovise	0.130	20.45	>0.3	57.59	0.282	43.27	0.057	-8.68	0.029	-8.95	>0.3	103.77
panc0327	>0.3	50.91	>0.3	88.95	>0.3	63.54	0.198	38.04	0.051	21.19	>0.3	56.95
pdxcnvx1004	>0.3	66.14	>0.3	71.6	>0.3	105.17	>0.3	58.63	>0.3	57.35	>0.3	63.76
pdxcnvx1015	>0.3	101.18	>0.3	96.52	>0.3	105.36	>0.3	101.51	>0.3	97.25	>0.3	95.39
pdxcnvx1016	>0.3	101.19	>0.3	99.58	>0.3	100.5	>0.3	101.51	>0.3	98.67	>0.3	100.66
pdxcnvx1017	>0.3	97.73	>0.3	96.43	>0.3	99.86	>0.3	98.68	>0.3	97.57	>0.3	96.89
pdxcnvx1019	>0.3	99.07	>0.3	102.7	>0.3	99.14	>0.3	99.28	>0.3	92.91	>0.3	101.2
rcm1	>0.3	97.38	>0.3	96.94	>0.3	97.67	>0.3	61.45	0.294	49.76	>0.3	96.49
rerfgc1b	0.074	23.67	>0.3	98.86	>0.3	70.36	0.220	45.34	0.013	15.35	>0.3	96.7
rerflckj	>0.3	93.02	>0.3	99.21	>0.3	98.93	>0.3	103.36	0.013	23.27	>0.3	100.45
scc15	0.012	-26.32	0.009	19.07	0.036	15.16	0.000	-24.18	0.000	-11.3	0.003	1.49
skco1	0.165	21.85	0.231	34.1	0.227	32.87	0.136	9.95	0.039	-20.38	>0.3	98.53
skhep1	>0.3	55.53	0.161	43.82	>0.3	54.13	>0.3	51.38	0.206	46.11	>0.3	57.74
skmel1	>0.3	99.67	>0.3	107.08	>0.3	104.28	>0.3	107.42	>0.3	98.91	>0.3	98.74
skmel3	0.000	-83.09	0.003	-65.34	0.002	-61.4	0.002	-46.75	0.000	-54.76	0.001	-71
skmel31	0.181	46.31	0.016	36.09	0.048	36.51	0.165	37.42	0.165	32.87	>0.3	79.06
skmel5	>0.3	102.15	>0.3	95.39	>0.3	97.86	>0.3	96.85	>0.3	99.8	>0.3	95.77
sngm	0.123	38.05	>0.3	100.18	>0.3	63.54	>0.3	57.94	0.005	24.23	>0.3	68.89
snu1033	>0.3	95.42	>0.3	101.5	>0.3	104.8	>0.3	99.63	>0.3	62.07	>0.3	97.16
snu119	0.200	31.61	>0.3	87.88	>0.3	59.28	>0.3	78.56	0.083	22.58	>0.3	88.91
snu16	>0.3	109.49	>0.3	91.21	>0.3	65.83	>0.3	105.6	>0.3	91.14	>0.3	93.61
snu182	>0.3	89.01	>0.3	106.67	>0.3	78	>0.3	60.07	0.259	48.61	>0.3	52.4
snu216	>0.3	93.94	>0.3	105.22	>0.3	102.98	>0.3	98.74	>0.3	86.97	>0.3	97.75
snu283	0.002	-86.13	0.161	4.42	0.132	6.97	0.009	-91.72	0.000	-97.32	0.140	19.54
snu5	>0.3	105.89	>0.3	93.58	>0.3	99.62	>0.3	100.78	>0.3	94.98	>0.3	98.82
snu601	>0.3	98.39	>0.3	103.01	>0.3	101.85	>0.3	68.73	>0.3	65.52	>0.3	102.56

snu620	>0.3	100.68	>0.3	99.88	>0.3	97.12	>0.3	102.46	>0.3	101.02	>0.3	106.64
snu719	0.175	28.22	>0.3	55.47	>0.3	60.21	0.157	18.89	0.029	-30.14	0.283	48.65
snu81	>0.3	102.26	>0.3	90.49	>0.3	97.46	>0.3	97.15	>0.3	94.6	>0.3	99.51
snu878	0.079	23.97	0.173	41.31	0.172	34.66	0.004	27.82	0.025	22.28	0.123	32.2
sw1116	>0.3	80.3	>0.3	76.87	>0.3	98.43	>0.3	84.78	0.014	20.05	>0.3	107.68
sw1463	>0.3	93.14	>0.3	99.4	>0.3	96.38	>0.3	98.81	>0.3	97.07	>0.3	96.32
sw403	>0.3	86.99	>0.3	94.06	>0.3	102.1	>0.3	61.64	0.039	29.88	>0.3	97.4
t3m10	0.050	-48.54	0.078	-3.69	0.073	-29.75	0.052	-58.97	0.021	-61.56	0.078	5.76

Table 24

Antibody target	**EPCAM**		**F3**		**FOLR1**		**GPNMB**		**MET**		**MSLN**	
Linker Payload	**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**	
Cell Line Name	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**
ags	0.000	36.39	0.161	45.23	0.153	41.44	0.129	37.94	0.051	28.67	0.116	35.5
aspc1	>0.3	53.02	>0.3	104.07	>0.3	52.03	>0.3	92.61	>0.3	93.03	>0.3	108.67
bicr22	0.077	34.73	>0.3	59.18	>0.3	53.57	0.222	45.93	0.046	28.54	>0.3	100.1
bicr56	>0.3	62.63	>0.3	82.23	>0.3	94.35	>0.3	94.09	>0.3	87.26	>0.3	103.56
c2bbe1	>0.3	55.48	>0.3	112.67	>0.3	55.35	>0.3	91.08	>0.3	97.12	>0.3	110.23
c32	>0.3	67.59	>0.3	100.97	>0.3	60.58	>0.3	72.8	>0.3	71.92	>0.3	102.06
caki2	0.075	-11.37	0.221	34.72	>0.3	61.3	0.288	47.04	0.176	23.39	>0.3	52.99
cal120	>0.3	100.54	>0.3	52.02	>0.3	96.58	>0.3	94.5	0.192	45.11	>0.3	100.79
calu3	0.000	-77.03	0.001	-69.75	0.106	-3.64	0.144	33.45	0.068	-66.58	0.186	27.14
calu6	>0.3	94.47	>0.3	102.05	>0.3	97.58	>0.3	96.66	>0.3	98.13	>0.3	95.2
capan2	0.001	23.73	0.142	47.27	>0.3	56.44	>0.3	53.75	>0.3	50.94	>0.3	61.98
cjm	>0.3	59.23	>0.3	65.44	>0.3	66.11	>0.3	63.03	>0.3	101.8	>0.3	97.95
cl40	0.001	15.93	>0.3	79.12	>0.3	73.7	>0.3	132.3	>0.3	104.78	>0.3	80.11
colo201	>0.3	78.78	>0.3	120.64	>0.3	95.28	>0.3	111.56	>0.3	78	>0.3	91.43
colo741	0.129	29.27	>0.3	110.63	0.107	33.69	>0.3	61.4	>0.3	70.23	>0.3	94.46
colo783	0.082	19.45	>0.3	54.96	0.141	43	0.116	42.45	0.239	45.85	>0.3	61.47
colo792	0.043	18	0.261	47.07	0.084	29.72	0.223	46.71	0.059	18.44	>0.3	53.27
corl105	0.068	-1.4	0.168	34.61	0.257	45.34	>0.3	55.01	0.209	29.24	>0.3	73.12

du4475	>0.3	99.38	>0.3	109.42	>0.3	106.77	>0.3	73.9	>0.3	100.82	>0.3	110.07
dv90	0.201	39.67	>0.3	89.21	>0.3	96.09	>0.3	100.27	>0.3	95.73	>0.3	93.58
ebc1	0.148	30.14	>0.3	61.83	>0.3	96.39	>0.3	69.76	0.299	49.96	>0.3	73.7
ecc10	>0.3	98.6	>0.3	86.46	>0.3	93.48	>0.3	113.33	>0.3	90.9	>0.3	102.67
g361	>0.3	63.55	>0.3	107.24	>0.3	64.27	>0.3	98.26	>0.3	94.18	>0.3	96.3
g402	>0.3	94.51	>0.3	105.68	>0.3	96.19	>0.3	101.19	>0.3	97.93	>0.3	98.25
gss	0.000	-95.33	0.086	-88.29	>0.3	89.98	>0.1	59.1	0.060	-90.99	0.140	-71.06
hcc1143	0.287	49.73	>0.3	68.82	0.229	46.39	>0.3	58.03	>0.3	64.58	>0.3	75.85
hcc15	>0.3	65.11	>0.3	100.22	>0.3	99.23	>0.3	98.77	>0.3	94.4	>0.3	99.55
hcc1569	0.000	-1.59	0.144	15.85	0.162	29.11	0.167	25.07	0.097	10.96	0.214	43.24
hcc1937	0.008	11.71	0.000	28.62	0.210	44.52	>0.3	51.99	0.117	26.44	0.238	40.93
hcc2279	0.050	-0.52	0.000	24.45	0.188	36.21	0.204	38.41	0.057	0.03	0.254	48.25
hcc2935	0.016	27.24	>0.3	50.26	0.101	34.78	0.094	38.14	0.073	28.18	>0.3	54.99
hcc38	0.003	-33.19	0.094	-0.42	0.051	-8.79	0.087	13.97	0.036	-8.63	0.177	25.98
hcc4006	>0.3	108.23	>0.3	95.5	>0.3	100.49	>0.3	90.52	>0.3	100.85	>0.3	110.04
hdqp1	>0.3	78.39	>0.3	88.97	>0.3	95.31	>0.3	93.74	>0.3	94.25	>0.3	108.45
hec108	0.000	15.11	0.186	30.46	0.188	28.86	0.168	24.52	0.087	19.37	0.265	47.17
hec251	>0.3	65.81	>0.3	96.85	>0.3	65.64	>0.3	94.34	>0.3	79.6	>0.3	101.88
hpafii	0.005	27.63	0.001	47.08	>0.3	102.99	>0.3	96.91	>0.3	74.12	>0.3	108.97
ht55	>0.3	60.65	>0.3	103.37	>0.3	97.68	>0.3	71.63	>0.3	92.01	>0.3	88.84
hucct1	>0.3	99.41	>0.3	98.01	>0.3	98.22	>0.3	92.52	>0.3	98.37	>0.3	105.07
ialm	>0.3	89.45	>0.3	95.62	>0.3	94.77	>0.3	89.59	>0.3	96.72	>0.3	92.7
jhoc5	0.034	-49.14	0.211	27.77	0.256	40.71	>0.3	95.78	0.031	38.52	>0.3	56.1
jhom2b	0.000	-48.31	0.134	2.43	0.047	7.1	0.100	0.64	0.088	-22.65	>0.3	88.51
jhuem3	0.001	5.43	0.175	21.76	0.079	30.67	0.116	21.65	0.053	12.04	>0.3	103.44
jhuem7	0.011	23.68	0.011	33.59	0.058	20.29	0.082	26.52	0.107	19.87	0.235	46.14
ke39	>0.3	81.97	>0.3	103.28	>0.3	96.91	>0.3	98.71	>0.3	89.51	>0.3	87.37
lclc103h	>0.3	66.86	>0.3	94.62	>0.3	102.07	>0.3	95.91	>0.3	66.89	>0.3	98.11
lounh91	0.085	19.36	>0.3	53.77	0.175	39.06	>0.3	56.02	0.117	30.51	>0.3	57.26
ls123	0.001	-49.16	0.017	-11.04	0.099	19.08	0.122	24.77	0.067	-2.53	>0.3	54.87
ls513	0.026	15.84	>0.3	86.76	>0.3	98.62	>0.3	77.47	>0.3	89.15	>0.3	74.71
melho	>0.3	88.64	>0.3	104.56	>0.3	89.86	>0.3	88.44	>0.3	87.08	>0.3	95.99
nccstck140	0.008	-8.09	0.205	30.87	0.056	27.25	0.095	12.32	0.021	6.79	>0.3	51.71
ncih1435	0.262	48.17	>0.3	100.61	>0.3	56.02	>0.3	62.73	>0.3	91.17	>0.3	103.14
ncih146	0.013	-51.16	>0.3	67.32	>0.3	77.44	>0.3	86.34	0.093	-5.14	>0.3	79.09

ncih1792	>0.3	102.23	>0.3	102.94	>0.3	99.58	>0.3	98.33	>0.3	100.46	>0.3	102.14
ncih1838	>0.3	51.75	>0.3	66.62	>0.3	68.27	>0.3	69.33	>0.3	62.28	>0.3	100.95
ncih2122	>0.3	79.59	>0.3	96.01	>0.3	99.82	>0.3	97.88	>0.3	95.21	>0.3	106.88
ncih2170	0.028	32.46	0.273	46.71	>0.3	63.8	>0.3	60.02	0.290	48.47	>0.3	111.16
ncih2171	0.002	-92.38	0.043	-57.52	0.098	-9.72	0.074	-59.36	0.048	-83	0.119	2.83
ncih3255	0.036	27.77	>0.3	60.38	>0.3	54.27	>0.3	55.66	>0.3	74.93	>0.3	59.19
ncih596	>0.3	97.81	>0.3	81.85	>0.3	88.02	>0.3	80.23	>0.3	83.25	>0.3	103.09
ncin87	0.007	28.93	>0.3	82.47	>0.3	50.67	>0.3	57.35	>0.3	63.66	>0.3	96.87
nvx0003132 92dplastic	0.291	49.89	>0.3	99.52	>0.3	66.58	>0.3	55.26	>0.3	92.16	>0.3	68.02
nvx0009209 42dplastic	>0.3	54.64	0.094	44.35	>0.3	93.41	>0.3	105.63	0.041	12.82	>0.3	101.33
nvx0022484 92dplastic	>0.3	90.69	>0.3	108.04	>0.3	62.28	>0.3	95.35	>0.3	79.57	>0.3	88.8
nvx0051127 02dcollagen	0.022	30.94	>0.3	94.54	>0.3	76.96	>0.3	98.8	>0.3	94.33	>0.3	101.67
nvx0058467 62dplastic	>0.3	96.22	>0.3	100.35	>0.3	96.02	>0.3	96.02	>0.3	92.45	>0.3	97.79
nvx0062299 72dcollagen	>0.3	50.65	>0.3	57.57	>0.3	63.31	>0.3	71.28	>0.3	92.17	>0.3	93.1
ocum1	0.001	-33.39	0.179	21.54	>0.3	104.61	>0.3	109.51	0.159	10.74	>0.3	100.06
ovise	0.000	0.79	0.002	35.65	0.106	10.19	>0.3	82.25	0.110	1.49	>0.3	57.52
panc0327	0.018	30.91	0.000	40.28	>0.3	53.38	>0.3	54.77	>0.3	54.47	>0.3	70.23
pdxcnvx1004	0.137	32.02	>0.3	63.99	>0.3	54.44	>0.3	55.69	>0.3	51.55	>0.3	70.67
pdxcnvx1015	>0.3	102.05	>0.3	106.49	>0.3	90.92	>0.3	93.53	>0.3	95.82	>0.3	104.23
pdxcnvx1016	>0.3	98.41	>0.3	102.48	>0.3	104.45	>0.3	99.7	>0.3	96.45	>0.3	96.57
pdxcnvx1017	>0.3	98	>0.3	101.12	>0.3	94.93	>0.3	97.85	>0.3	95.65	>0.3	100.33
pdxcnvx1019	>0.3	98.89	>0.3	106.71	>0.3	100.92	>0.3	102.34	>0.3	95.18	>0.3	105.4
rcm1	0.072	38.61	>0.3	91.48	>0.3	96.17	>0.3	97.7	>0.3	102.09	>0.3	98.85
rerfgc1b	0.015	37.21	>0.3	56.24	>0.3	90.8	>0.3	73.29	>0.3	95.22	>0.3	97.01
rerflckj	>0.3	95.23	>0.3	93.16	>0.3	64.7	>0.3	87.2	>0.3	113.6	>0.3	106.61
scc15	0.000	2.57	0.000	-11.57	0.052	7.88	0.021	-15.61	0.002	-3.93	0.034	16.82
skco1	0.000	-53.34	0.147	3.03	0.225	36.47	0.236	36.65	0.113	6.94	0.193	24.2
skhep1	0.101	35.13	>0.3	57.79	>0.3	55.23	0.160	-1.34	0.138	46.82	>0.3	82.36
skmel1	>0.3	100.75	>0.3	103.5	>0.3	101.53	>0.3	98.96	>0.3	104.66	>0.3	103.84
skmel3	0.000	-61.74	0.001	-61.21	0.004	-63.56	0.001	-56.21	0.025	-19.9	0.000	-51.31
skmel31	>0.3	54.55	0.153	37.85	0.171	40.66	0.014	42.22	0.024	44.77	>0.3	96.3

skmel5	>0.3	91.09	>0.3	100.77	>0.3	90.59	>0.3	96.34	>0.3	94.63	>0.3	90.7
sngm	>0.3	61.72	>0.3	67.67	>0.3	56.79	>0.3	97.5	>0.3	67.51	>0.3	67.28
snu1033	>0.3	72.05	>0.3	93.77	>0.3	65.36	>0.3	91.38	>0.3	71.96	>0.3	106.76
snu119	0.028	6.09	>0.3	78.3	0.103	40.68	>0.3	51.25	>0.3	77.19	>0.3	105.04
snu16	>0.3	62.49	>0.3	97.24	>0.3	78.62	>0.3	108.53	>0.3	64.49	>0.3	72.26
snu182	0.137	37.35	>0.3	53.73	>0.3	52.16	>0.3	51.53	0.186	37.29	>0.3	81.86
snu216	>0.3	101.97	>0.3	94.1	>0.3	96.81	>0.3	93.86	>0.3	97.82	>0.3	103.51
snu283	0.000	-86.69	0.070	-67.03	0.053	-12.02	0.053	18.24	0.032	-44.95	>0.3	88.7
snu5	>0.3	97.56	>0.3	102.28	>0.3	96.78	>0.3	98	0.000	46.07	>0.3	103.63
snu601	>0.3	60.49	>0.3	101.04	>0.3	101.88	>0.3	97.53	>0.3	98.8	>0.3	100.87
snu620	>0.3	65.2	>0.3	107.02	>0.3	78.47	>0.3	98.74	>0.3	76.96	>0.3	102.68
snu719	0.002	15.05	0.251	41.76	0.253	45.96	0.226	41.48	0.157	40.99	>0.3	58.67
snu81	>0.3	86.3	>0.3	98.47	>0.3	99.47	>0.3	97.08	>0.3	91.55	>0.3	92.57
snu878	0.032	15.28	0.115	27.89	0.125	34	0.189	38.59	0.117	33.12	>0.3	51.59
sw1116	>0.3	74.89	>0.3	58.08	>0.3	103.4	>0.3	82.77	>0.3	94.28	>0.3	92.91
sw1463	>0.3	51.27	>0.3	97.22	>0.3	99.35	>0.3	89.53	>0.3	103.05	>0.3	92.47
sw403	>0.3	55.66	>0.3	89.54	>0.3	99.84	>0.3	92.07	>0.3	105.76	>0.3	97.15
t3m10	0.005	-69.52	0.001	-51.13	0.069	-27.15	0.057	-18.16	0.047	-29.2	0.102	-11.76

Table 25

Antibody target	**MUC16**		**NECTIN4**		**SLC34A2**		**SLC39A6**		**TACSTD2**		**TFRC**	
Linker Payload	**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**		**L109A-P1**	
Cell Line Name	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**	**GI50 (uM)**	**Amax**
ags	0.062	39.79	0.091	45.22	0.099	36.79	0.086	37.8	0.000	36.27	0.002	44.13
aspc1	>0.3	100.94	>0.1	96.28	>0.3	93.33	>0.3	100.64	>0.3	99.67	0.004	27.24
bicr22	>0.3	60.91	>0.1	91.66	0.214	37.72	0.202	37.25	0.121	43.55	0.002	-7.45
bicr56	>0.3	96.86	>0.1	96.4	>0.3	94.82	>0.3	93.1	>0.3	86.08	0.004	14.05
c2bbe1	>0.3	109.73	>0.1	96.06	>0.3	103.64	>0.3	103.47	>0.3	74.94	0.026	6.29
c32	>0.3	99.83	>0.1	94.18	>0.3	75.21	>0.3	72.94	>0.3	65.91	0.010	4.24
caki2	0.211	32.63	>0.1	96.71	>0.3	58.38	0.271	44.18	0.263	41.72	0.001	-81.37
cal120	>0.3	55.25	>0.1	97.53	>0.3	92.78	>0.3	96.45	>0.3	89.52	0.004	-61.17
calu3	0.095	-66.04	>0.1	86.18	0.113	28.08	0.112	-26.77	0.005	-52.14	0.002	-80.7

calu6	>0.3	100.88	>0.1	101.65	>0.3	100.3	>0.3	101.47	>0.3	98.25	0.019	29.36
capan2	0.271	45.88	>0.1	95.3	>0.3	64.63	>0.3	53.43	0.030	36.49	0.006	-40.52
cjm	>0.3	93.67	>0.1	98.53	>0.3	68.56	>0.3	63.13	>0.3	62.19	>0.3	68.61
cl40	>0.3	96.56	>0.1	103.67	>0.3	109.81	>0.3	143.49	>0.3	123.84	>0.3	67.07
colo201	>0.3	95.06	>0.1	104.47	>0.3	91.92	>0.3	107.31	>0.3	93.07	0.013	-48.03
colo741	>0.3	99.72	>0.1	100.59	>0.3	66.07	>0.3	101.14	>0.3	99.73	0.002	-66.74
colo783	>0.3	52.81	>0.1	63.35	0.185	40.69	0.241	44.04	0.109	32.37	0.002	-74.69
colo792	>0.3	52.46	0.085	45.35	0.215	33.98	0.149	33.21	0.134	41.67	0.001	-71.34
corl105	0.224	28.68	>0.1	93.08	>0.3	52.4	0.283	46.56	0.225	44.5	0.013	-51.59
du4475	>0.3	106.39	>0.1	102.71	>0.3	98.86	>0.3	100.82	>0.3	101.02	0.018	-66.13
dv90	>0.3	98.92	>0.1	84.13	>0.3	88.74	>0.3	116.27	>0.3	105.7	0.001	-86.75
ebc1	>0.3	72.6	>0.1	95.18	>0.3	63.91	>0.3	63.5	>0.3	66.18	0.004	-73.07
ecc10	>0.3	115.83	>0.1	101.08	>0.3	118.08	>0.3	129.04	>0.3	126.69	>0.3	77.05
g361	>0.3	101.98	>0.1	96.24	>0.3	71.61	>0.3	100.85	>0.3	101.86	0.002	2.71
g402	>0.3	102.87	>0.1	93.57	>0.3	94	>0.3	98.57	>0.3	100.88	0.002	-1.25
gss	0.083	-83.54	0.085	34.67	>0.1	69.06	0.088	-89.17	0.076	-73.75	0.001	-96.49
hcc1143	>0.3	69.36	>0.1	95.27	>0.3	61.26	>0.3	61.72	>0.3	62.09	0.010	14.85
hcc15	>0.3	99.96	>0.1	101.48	>0.3	97.89	>0.3	100.31	>0.3	97.45	0.010	-72.58
hcc1569	0.099	15.96	>0.1	58	0.162	26.04	0.110	10.96	0.112	18.32	0.000	-67.74
hcc1937	0.144	30.24	>0.1	65.93	0.042	45.18	0.223	37.87	0.024	38.82	0.003	-39.03
hcc2279	0.136	17.35	>0.1	61.13	0.157	30.56	0.122	20.26	0.150	32.73	0.001	-64.98
hcc2935	>0.3	56	>0.1	65.72	>0.3	55.11	0.220	39.43	0.020	37.93	0.005	-16.86
hcc38	0.094	4.03	0.073	43.76	0.090	22.25	0.090	8.9	0.001	18.68	0.001	-78.64
hcc4006	>0.3	105.96	>0.1	95.64	>0.3	94.32	>0.3	103.2	>0.3	99.42	0.005	26.67
hdqp1	>0.3	104.34	>0.1	97.86	>0.3	95.49	>0.3	104.49	>0.3	100.26	0.005	17.87
hec108	0.130	29.05	>0.1	70.18	0.172	32.77	0.153	24.58	0.118	23.14	0.001	-93.5
hec251	>0.3	98.57	>0.1	99.16	>0.3	89.43	>0.3	98.33	>0.3	80.62	0.004	-21.21
hpafii	>0.3	99.36	>0.1	89.61	>0.3	86.06	>0.3	103.42	>0.3	69.17	0.022	4.01
ht55	>0.3	88.42	>0.1	104.45	>0.3	91.59	>0.3	101.32	>0.3	83.54	0.016	-58.52
hucct1	>0.3	97.64	>0.1	94.91	>0.3	94.7	>0.3	99.9	>0.3	95.8	0.012	11.21
ialm	>0.3	98.15	>0.1	95.14	>0.3	91.87	>0.3	95.9	>0.3	95.62	0.053	45.85
jhoc5	0.156	25.82	>0.1	93.68	0.244	41.06	0.179	16.06	0.152	21.98	0.015	12.12
jhom2b	0.053	2.3	>0.1	79.33	0.143	-4.69	0.067	-2.03	>0.3	57.06	0.011	-80.11
jhuem3	0.123	46.93	>0.1	90.42	0.130	38.28	0.261	42.87	0.000	24.73	0.009	-10.96
jhuem7	0.087	30.38	0.057	38.38	0.004	22.52	0.074	21.19	0.027	16.07	0.006	-6.33

ke39	>0.3	79.32	>0.1	105.35	>0.3	96.36	>0.3	82.52	>0.3	77.83	0.006	19.79
lclc103h	>0.3	100.34	>0.1	102.82	>0.3	98.78	>0.3	103.77	>0.3	97.65	0.007	11.44
lounh91	>0.3	51.71	>0.1	58.49	>0.3	53.88	0.241	48	0.192	40.45	0.015	-17.22
ls123	0.066	18.65	>0.1	52.92	0.097	4.2	0.050	22.78	0.012	-6.93	0.001	-92.11
ls513	>0.3	84.45	>0.1	93.1	>0.3	80.48	>0.3	94.9	>0.3	82.67	0.001	-83.61
melho	>0.3	92.93	>0.1	95.68	>0.3	90.52	>0.3	95.21	>0.3	90.8	0.002	-77.13
nccstck140	0.038	24.1	0.021	16.41	0.055	10.82	0.115	22.63	0.001	-7.11	0.002	-11.84
ncih1435	>0.3	53.3	>0.1	88.17	>0.3	91.13	>0.3	62.69	>0.3	58.42	0.004	-36.52
ncih146	>0.3	52.95	>0.1	86.96	>0.3	60.21	>0.3	91.94	0.162	-13.45	0.002	-93.92
ncih1792	>0.3	99.91	>0.1	103.49	>0.3	101.79	>0.3	102.28	>0.3	99.48	0.002	16.27
ncih1838	>0.3	101.31	>0.1	96.86	>0.3	71.15	>0.3	97.06	>0.3	94.12	0.081	26.15
ncih2122	>0.3	101.91	>0.1	95.34	>0.3	97.61	>0.3	99.75	>0.3	85.38	0.003	-25.8
ncih2170	>0.3	50.6	>0.1	95.22	>0.3	91.32	>0.3	55.59	0.274	48.45	0.002	-54.05
ncih2171	0.049	-65.28	0.080	28.76	0.087	-21.66	0.058	-79.86	0.050	-69.36	0.003	-97.28
ncih3255	>0.3	55.93	>0.1	88.87	>0.3	55.9	>0.3	57.48	0.038	35.37	0.002	-32.44
ncih596	>0.3	95.85	>0.1	84.76	>0.3	85.44	>0.3	89.37	>0.3	86.59	0.009	43.87
ncin87	>0.3	54.41	>0.1	96.18	>0.3	79.1	0.295	49.35	0.031	36.97	0.002	-79.37
nvx0003132 92dplastic	>0.3	97.58	>0.1	89.1	>0.3	59	>0.3	56.29	>0.3	61.15	0.004	15.69
nvx0009209 42dplastic	>0.3	76.71	>0.1	73.24	>0.3	76.52	>0.3	79.68	>0.3	71.07	0.005	-3.13
nvx0022484 92dplastic	>0.3	102.16	>0.1	95.91	>0.3	89.19	>0.3	72.25	>0.3	98.66	>0.3	51.38
nvx0051127 02dcollagen	>0.3	92.74	>0.1	95.73	0.046	38.1	>0.3	108.7	>0.3	100.99	>0.3	77.35
nvx0058467 62dplastic	>0.3	98.74	>0.1	95.08	>0.3	92.41	>0.3	97.66	>0.3	95.8	>0.3	54.28
nvx0062299 72dcollagen	>0.3	66.34	>0.1	88.81	>0.3	66.91	>0.3	64.96	>0.3	65.26	0.018	10.97
ocum1	0.151	16.43	>0.1	98.17	>0.3	79.63	0.271	44.19	0.108	27.03	0.017	-84.91
ovise	0.148	9.67	>0.1	100.44	0.027	12.89	0.152	9.09	>0.3	99.9	0.004	-68.81
panc0327	>0.3	53.17	>0.1	91.94	>0.3	60.94	0.298	49.68	0.131	44.08	0.002	-62.22
pdxcnvx1004	>0.3	63.12	>0.1	66.52	>0.3	55.54	>0.3	51.75	0.294	49.21	0.069	25.39
pdxcnvx1015	>0.3	102.62	>0.1	96.42	>0.3	91.33	>0.3	95.4	>0.3	90.25	0.028	44.58
pdxcnvx1016	>0.3	91.67	>0.1	98.53	>0.3	97	>0.3	94.46	>0.3	91.82	>0.3	57.55
pdxcnvx1017	>0.3	102.38	>0.1	96.14	>0.3	95.33	>0.3	100.02	>0.3	99.24	0.061	46.69
pdxcnvx1019	>0.3	96.03	>0.1	103.81	>0.3	95.86	>0.3	99.63	>0.3	101.21	0.020	45.23

rcm1	>0.3	98.42	>0.1	98.89	>0.3	98.06	>0.3	98.13	>0.3	72.41	0.071	6.18
rerfgc1b	>0.3	51.28	>0.1	93.51	0.249	40.95	0.271	47.91	0.037	46.51	0.005	-38.31
rerflckj	>0.3	103.02	>0.1	97.46	>0.3	98.34	>0.3	103.12	>0.3	74.24	0.008	34.63
scc15	0.013	-6.21	0.039	23.91	0.016	-3.53	0.031	-4.68	0.000	-24.63	0.000	-36.73
skco1	0.138	3.74	>0.1	91.54	0.209	24.37	0.211	23.61	0.006	2.79	0.000	-70.72
skhep1	0.223	42.39	>0.1	92.39	>0.3	61.59	>0.3	59.22	>0.3	58.86	>0.3	53.56
skmel1	>0.3	102.83	>0.1	96.43	>0.3	95.4	>0.3	97.12	>0.3	97.73	0.003	-43.98
skmel3	0.005	-42.26	0.004	-56.83	0.001	-66.29	0.048	-39.94	0.004	-64.87	0.000	-74.1
skmel31	>0.3	56.21	>0.1	78.72	0.130	39.63	0.043	39.43	0.133	38.12	0.011	-4.49
skmel5	>0.3	93.53	>0.1	99.45	>0.3	96.46	>0.3	96.83	>0.3	91.98	0.006	8.12
sngm	>0.3	65.52	>0.1	77.42	0.003	-30.54	>0.3	63.67	>0.3	97.81	>0.3	62.32
snu1033	>0.3	103.91	>0.1	94.26	>0.3	92.17	>0.3	104.11	>0.3	92.37	0.004	-11.73
snu119	>0.3	105.49	>0.1	76.77	>0.3	62.39	>0.3	61.52	0.104	42.2	0.002	-41.17
snu16	>0.3	98.85	>0.1	96.95	>0.3	83.56	>0.3	102.82	>0.3	84.94	0.002	-78.46
snu182	>0.3	51.42	>0.1	92.25	>0.3	57.57	>0.3	62.15	>0.3	59.15	0.015	8.82
snu216	>0.3	100.56	>0.1	97.5	>0.3	95.59	>0.3	99.25	>0.3	97.81	0.003	18
snu283	0.029	-56.13	>0.1	76.32	0.112	-9.37	0.081	-3.9	0.016	-24.36	0.000	-98.84
snu5	>0.3	103.27	>0.1	98.9	>0.3	94.34	>0.3	101	>0.3	100.93	0.006	8.93
snu601	>0.3	100.31	>0.1	98.42	>0.3	95.59	>0.3	104.95	>0.3	93.34	0.002	-72.62
snu620	>0.3	100.07	>0.1	100.94	>0.3	75.3	>0.3	72.46	>0.3	68.49	0.003	-36.62
snu719	0.236	37.72	>0.1	64.98	0.249	41.63	0.178	31.44	0.206	43.1	0.001	0.37
snu81	>0.3	90.57	>0.1	97.18	>0.3	93.45	>0.3	90.53	>0.3	88.38	0.035	-6.02
snu878	0.130	31.61	0.084	47.55	0.161	25.45	0.142	30.17	0.146	32.69	0.001	-17.23
sw1116	>0.3	96.49	>0.1	87.87	>0.3	83.41	>0.3	89.76	>0.3	83.38	>0.3	91.78
sw1463	>0.3	71.51	>0.1	98	>0.3	98.82	>0.3	101.31	>0.3	89.06	0.019	3.11
sw403	>0.3	96.75	>0.1	94.52	>0.3	91.36	>0.3	100.97	>0.3	96.68	0.008	-68.6
t3m10	0.049	-48.78	0.036	28.93	0.069	-24.29	0.053	-39.48	0.056	-32.09	0.003	-83.42

Example 13. Evaluation of *in vitro* ADC activity with combination partners in a panel of cancer cell lines

[1145] Antibody drug conjugates (ADCs) targeting IgG, B7H3, CD56, DLK1, DLL3, EpCAM, and SEZ6 were tested against cancer cell lines obtained from ATCC (American Type Culture Collection) or from other commercial cell line vendors (corl279, ncih1436, ncih146, ncih211, ncih524). The cells were cultured in media that is optimal for their growth at 5% CO_2, 37°C in a tissue culture incubator. Prior to seeding for the proliferation assay, the cells were split at least 2 days before the assay to ensure optimal growth density. On the day of seeding, cells were lifted off tissue culture flasks using 0.25% trypsin. Cell viability and cell density were determined using a cell counter (Vi-Cell XR Cell Viability Analyzer, Beckman Coulter). Cells with higher than 85% viability were seeded in white clear bottom 384-well plates (Greiner cat # 781098) at a density of 1000 cells per well in 50 µL of standard growth media. Plates were incubated at 37°C overnight in a tissue culture incubator.

[1146] The ADCs were prepared in standard phosphate buffered solution to desired concentrations. A series of 10 dilutions were made for each ADC. The prepared drug treatments were then added to the cells resulting in final concentrations of 300 nM to 0.015 nM. Combination partners (Venetoclax and Topotecan) were added at fixed concentrations. Acoustic transfer devices (Echo525, Echo550, Beckman Coulter) were used to add the ADCs or combination partners to the cells. Each treatment was tested in triplicate assay plates. Plates were incubated at 37°C overnight or for 5 days in a tissue culture incubator. The ability of the ADCs to inhibit cell proliferation and survival was assessed using the Promega CellTiter-Glo® proliferation assay. Plates were incubated at room temperature for 20 minutes to stabilize luminescent signals prior to reading using a multimode plate reader (Pherastar, BMG). Luminescent counts of untreated cells were taken the day after seeding (Day 0 readings), and after 5 days of treatment (Day 5 readings). The Day 5 readings of the untreated cells were compared to the Day 0 readings. Assays with at least one cell doubling during the incubation period were considered valid. To evaluate the effect of the drug treatments, luminescent counts from wells containing untreated cells (100% viability) were used to normalize treated samples. The concentrations of treatment required to inhibit 50% of cell growth or survival (GI50) were calculated using a four parameter logistic regression equation. The test results are shown in Tables 26-29.

Table 26

				GI50 (uM)					GI Amax			
Antibody Target	**Antibody Name**	**Linker Payload**	**Combination partner**	**corl279**	**ncih1436**	**ncih146**	**ncih211**	**ncih524**	**corl279**	**ncih146**	**ncih211**	**ncih524**
IgG	3207	L11A-P21	Single Agent	0.22156	>0.3	0.031147	>0.3	0.010396	45.59	18.66	61.27	-0.05
B7H3	ABBV-155	L11A-P21	Single Agent	>0.3	0.103742	0.001524	>0.3	0.000699	60.43	11.53	67.23	16.67
B7H3	DS-5573a	L11A-P21	Single Agent	>0.3	>0.3	0.008362	>0.3	0.000334	53.79	-8.74	50.14	-10.7
CD56	Lorovtuzumab	L11A-P21	Single Agent	>0.3	>0.3	0.000386	>0.3	0.000244	54.31	-8.6	57.13	11.79
DLK1	DI-2-14	L11A-P21	Single Agent	0.065217	0.195712	0.006328	>0.3	0.00092	49.73	7.44	72.45	13.06
DLL3	Rovalpituzumab	L11A-P21	Single Agent	>0.3	>0.3	0.010364	>0.3	0.000152	56.41	-7.61	57.05	18.71
EpCAM	Oportuzumab	L11A-P21	Single Agent	>0.3	0.168594	0.000296	>0.3	0.004453	61.57	10.91	62.07	0.34
SEZ6	SC17.46	L11A-P21	Single Agent	0.241342	0.225471	0.010653	>0.3	0.004634	49.68	-43.86	59.98	-18.88

Table 27

				GI50 (uM)					GI Amax				
Antibody Target	**Antibody Name**	**Linker Payload**	**Combination partner**	**corl279**	**ncih1436**	**ncih146**	**ncih211**	**ncih524**	**corl279**	**ncih1436**	**ncih146**	**ncih211**	**ncih524**
IgG	3207	L11A-P21	10nM_Topotecan	0.052182	ND	ND	0.105416	0.008347	22.52	ND	ND	40.52	-3.81
IgG	3207	L11A-P21	50nM_Topotecan	0.000015	0.000019	0.000015	0.000015	0.000015	-92.14	24.23	-93.92	-94.95	-94.05
B7H3	ABBV-155	L11A-P21	10nM_Topotecan	0.029417	ND	ND	0.218435	0.000961	41.21	ND	ND	48.75	4.6
B7H3	ABBV-155	L11A-P21	50nM_Topotecan	0.000015	0.000686	0.000015	0.000015	0.000015	-90.17	35.68	-88.36	-93.94	-92.29
B7H3	DS-5573a	L11A-P21	10nM_Topotecan	0.015731	ND	ND	0.031265	0.000419	23.22	ND	ND	33.23	-9.57
B7H3	DS-5573a	L11A-P21	50nM_Topotecan	0.000015	0.000015	0.000015	0.000015	0.000015	-93.08	41.8	-88.12	-95.82	-94.48
CD56	Lorovtuzumab	L11A-P21	10nM_Topotecan	0.006884	ND	ND	0.038088	0.00021	32.75	ND	ND	38.96	-1.83
CD56	Lorovtuzumab	L11A-P21	50nM_Topotecan	0.000015	0.019917	0.000015	0.000015	0.000015	-91.72	35.32	-91.39	-97.41	-97.1
DLK1	DI-2-14	L11A-P21	10nM_Topotecan	0.000907	ND	ND	>0.3	0.000846	27.15	ND	ND	56.54	12.49
DLK1	DI-2-14	L11A-P21	50nM_Topotecan	0.000015	0.000135	0.000015	0.000015	0.000015	-93.93	11.59	-90.68	-93.66	-94.93

DLL3	Rovalpituzumab	L11A-P21	10nM_Topotecan	0.007625	ND	ND	0.115026	0.000376	32.04	ND	ND	42.65	10.54
DLL3	Rovalpituzumab	L11A-P21	50nM_Topotecan	0.000015	0.006516	0.000015	0.000015	0.000015	-91.33	27.35	-92.61	-97.39	-93.96
EpCAM	Oportuzumab	L11A-P21	10nM_Topotecan	0.008611	ND	ND	0.001667	0.000937	22.61	ND	ND	44.68	19.04
EpCAM	Oportuzumab	L11A-P21	50nM_Topotecan	0.000015	0.001371	0.000015	0.000015	0.000015	-93.04	19.68	-93.52	-96.46	-93.59
SEZ6	SC17.46	L11A-P21	10nM_Topotecan	0.01524	ND	ND	0.22293	0.001945	33.23	ND	ND	45.55	-33.78
SEZ6	SC17.46	L11A-P21	50nM_Topotecan	0.000015	0.010207	0.000015	0.000015	0.000015	-91	38.84	-92.54	-96.76	-92.64

Table 28

				GI50 (uM)					GI Amax				
Antibody Target	**Antibody Name**	**Linker Payload**	**Combination partner**	**corl279**	**ncih1436**	**ncih146**	**ncih211**	**ncih524**	**corl279**	**ncih1436**	**ncih146**	**ncih211**	**ncih524**
IgG	3207	L11A-P21	300nM_Venetoclax	0.121352	0.001641	0.001089	0.016593	0.011373	44.24	-91.08	-99.04	-69	-2.62
B7H3	ABBV-155	L11A-P21	300nM_Venetoclax	>0.3	0.000274	0.000162	0.001649	0.000716	67.5	-75.67	-98.2	-78.19	-22.07
B7H3	DS-5573a	L11A-P21	300nM_Venetoclax	0.124116	0.000103	0.000015	0.001046	0.000155	41.98	-89.58	-98.07	-91.47	-48.83
CD56	Lorovtuzumab	L11A-P21	300nM_Venetoclax	>0.3	0.000071	0.000034	0.000111	0.000204	59.87	-82.19	-98.48	-84.96	-15.81
DLK1	DI-2-14	L11A-P21	300nM_Venetoclax	0.003568	0.000167	0.000273	0.024861	0.000709	47.13	-89.16	-97.51	-61.36	-57.98
DLL3	Rovalpituzumab	L11A-P21	300nM_Venetoclax	>0.3	0.000066	0.00014	0.00302	0.000278	55.64	-87.2	-97.42	-80.84	-50.27
EpCAM	Oportuzumab	L11A-P21	300nM_Venetoclax	>0.3	0.000174	0.00015	0.000287	0.002017	60.8	-81.12	-97.37	-86.33	-12.12
SEZ6	SC17.46	L11A-P21	300nM_Venetoclax	>0.3	0.001719	0.000067	0.023578	0.001772	56.08	-88.4	-98.23	-67.26	-4.53

Table 29

	GI50 (uM)					GI Amax				
Code or Name	corl279	ncih1436	ncih146	ncih211	ncih524	corl279	ncih1436	ncih146	ncih211	ncih524
P21	0.002416	0.000508	0.000508	0.000508	0.000508	-88.27	-94.5	-98.38	-97.26	-97.52
Topotecan	0.015236	0.515484	0.003042	0.012528	0.008272	-95.95	-23.22	-91.77	-89.36	-83.85
Venetoclax	>10	4.919975	0.7886	3.920985	>10	98.71	13	-16.83	30.85	105.21

Example 14. Evaluation of *in vitro* combination activity of EGFR-AbA-L109A-P1 with inhibitors of the MAP Kinase pathway in a cancer cell lines

[1147] The EGFR-AbA-L109A-P1 antibody drug conjugate was tested against cancer cell lines obtained from ATCC (American Type Culture Collection) or alternative cell line vendor (KCLB, Korea.) The cells were cultured in media that is optimal for their growth at 5% CO2, 37°C in a tissue culture incubator. Prior to seeding for the proliferation assay, the cells were split at least 2 days before the assay to ensure optimal growth density. On the day of seeding, cells were lifted off tissue culture flasks using 0.25% trypsin. Cell viability and cell density were determined using a cell counter (Vi-Cell XR Cell Viability Analyzer, Beckman Coulter). Cells with higher than 85% viability were seeded in white clear bottom 384-well plates (Greiner cat # 781098) at a density of 1000 cells per well in 50 µL of standard growth media. Plates were incubated at 37°C overnight in a tissue culture incubator.

[1148] EGFR-AbA-L109A-P1 (KJ32-26EA) and combination partner compounds were prepared at 1000X in respective diluent. The combination partners included LTT462, Trametinib, and LXH254. A series of 7 to 10 dilutions were made for each compound, centering on a previously determined cell proliferation IC50. A dose matrix was created by combining serially diluted EGFR-Aba-L109A-P1 with the serial dilution of each partner compound. An acoustic transfer device (Echo555, Beckman Coulter) was used to add 50 nL of each dilution to the cells, resulting in final concentrations ranging from 0 – 10 µM. Each compound was also tested as a single agent or mixture for normalization purposes. Each treatment was tested in replicate assay plates.

[1149] Plates were incubated at 37°C overnight or for 5 days in a tissue culture incubator. The ability of the ADCs and partner compounds to inhibit cell proliferation and survival was assessed using the Promega CellTiter-Glo® proliferation assay. Plates were incubated at room temperature for 20 minutes to stabilize luminescent signals prior to reading using a multimode plate reader (Pherastar, BMG). Luminescent counts of untreated cells were taken the day after seeding (Day 0 readings), and after 5 days of treatment (Day 5 readings). The Day 5 readings of the untreated cells were compared to the Day 0 readings. Assays with at least one cell doubling during the incubation period were considered valid. To evaluate the effect of the drug treatments, luminescent counts from wells containing untreated cells (100% viability) were used to normalize treated samples. The percent inhibition and growth inhibition were calculated as a relative response to untreated cells after 5 days of growth. Both normalized datasets were fit using a sigmoidal response model and the Combination effect (SS) was measured as a sum of the activity over the Loewe dose additivity model as described in Lehar et al. *Nature*

Biotechnology (2009), 27(7), 659-666. The results are shown in FIG. 8A and FIG. 8B. As shown in FIGs. 8A and 8B, the EGFR-AbA-L109A-P1 Bcl-xli ADC induced a dose dependent decrease in the viability in HPAF-II, Panc 03.27, and SNU-601 cells. The activity of the EGFR-AbA-L109A-P1 ADC was significantly improved when combined with Trametinib and other inhibitors of the MAP Kinase pathway.

Example 15. *In vivo* efficacy of EGFR2-Bcl-xL inhibitor-ADC in combination with docetaxel against the H1650 human non-small cell lung carcinoma (NSCLC) model in mice

[1150] Efficacy of EGFR2-L109A-P1 ADC was evaluated in the H1650, non-small cell lung carcinoma (NSCLC) model, *in vivo* after combining treatment with docetaxel.

Methods

Synthesis of anti-EGFR2 CysMab DANAPA-L11A-P27 and anti-EGFR2 CysMab DANAPA-L109A-P1 ADCs

[1151] 25 mg of antibody (0.17 µmoles, 1.0 equiv.) was incubated with 2.5 ml of settled RMP Protein A resin (GE Lifesciences, 17513803) and agitated for 15 minutes. Cysteine HCl monohydrate was added to a final concentration of 20 mM and incubated with agitation for 30 min at room temperature to allow the reactive cysteines to be deblocked. The resin was washed rapidly with 50 column volumes PBS on a vacuum manifold. The resin was then resuspended in an equal volume PBS containing 250 nM $CuCl_2$. Reformation of antibody interchain disulfides was monitored by taking time points. At each time point, 25 µL of resin slurry was removed, 1 µL of 20 mM MC-valcit-PAB-MMAE was added, and the tube flicked several times. The resin was spun down, supernatant removed, and then eluted with 50 µL Antibody elution buffer (Thermo Scientific, 21004). The resin was pelleted and the supernatant analyzed by reverse phase chromatography using an Agilent PLRP-S 4000A 5um, 4.6x50mm column (Buffer A is water, 0.1% TFA, Buffer B Acetonitrile, 0.1% TFA, column held at 80 C, Flowrate 1.5 ml/min; Gradient 0 minutes – 30%B, 5 minutes – 45%B, 6.5 min – 100%B, 8 minutes – 100%B, 10 minutes – 30%). At 90 minutes after addition of $CuCl_2$, $CuCl_2$ was removed by washing with 50 column volumes of PBS on a vacuum manifold and then 2.5 ml of PBS was added to resuspend. To this slurry of resin and antibody respective Linker-Payload (102 µl of a 20 mM solution in DMSO, 1.63 µmoles, 12 equiv.) was added. The resulting mixture was then incubated at ambient temperature for 3 hours. The resin was then washed with 50 column volumes PBS. The ADC was eluted from the resin with Antibody elution buffer (Thermo Scientific, 21004). The ADC was then buffer exchanged into 1X PBS (20X PBS,

TeknovaP0191) by dialysis and preparative size exclusion chromatography eluted in Dulbecco's PBS pH 7.2 (Hyclone SH30028.03) to remove aggregates was performed with a HiLoad 16/600 Superdex 200 pg (GE Healthcare, 28989335). The material was then concentrated using a centrifugal concentrator using an Amicon Ultra-15, 50KDa, regenerated cellulose (Millipore, UFC0905024), to 4.5 mg/ml and filtered sterilely through 0.22 µm sterile PVDF Filter, 25mm (Millapore, SLGV013SL) and stored at 4°C. The final yield was 17.1 mg (0.114 µmol) The following analyses were performed: analytical size-exclusion chromatography (SEC) to determine percent monomer, mass spectroscopy (MS) to determine DAR, LAL test to determine endotoxin load and protein concentration determined by A280 utilizing extinction coefficient and molecular weight of antibody. HRMS data (protein method) indicated a dominant mass of the heavy chain+2 species, giving a DAR of ~4.0 was calculated by comparing MS intensities of peaks for DAR1 DAR2 and DAR3 species. SEC indicated 1.8% aggregation, as determined by comparison of the area of the high-molecular-weight peak absorbance at 210 and 280 nm with the area of the peak absorbance for monomeric ADC.

[1152] General Methodology (1): Drug-to-antibody ratio (DAR) of exemplary ADCs was determined by liquid chromatography-mass spectrometry (LC/MS) according to the following method. For all LC methods, mobile phase A was purified MS grade water (Honeywell, LC015-1), mobile phase B was MS grade 80% Isopropanol (Honeywell LC323-1): 20% acetonitrile (Honeywell, LC015-1), LC323-1), supplemented with 1 % of formic acid (FA) (Thermo Scientific, 85178). The column temperature was set at 80°C. A general MS method was optimized for all ADCs synthesized. The column used for analysis was an Agilent PLRP-S 4000 A; 2.1x150mm, 8um (Agilent, PL1912-3803). Flowrate used was 0.3 ml/min. The gradient used was 0-0.75 minute 95%A, 0.76 -1.9 minute 75%A, 1.91-11.0 minute 50%A, 11.01-11.50 10%A, 11.51-13.50 minute 95%A,13.51-18 minute 95%A on an Acuity Bio H-Class Quaternary UPLC (Waters). MS system was Xevo G2-XS QToF ESI mass spectrometer (Waters) and data acquired from 1.5-11 minutes and masses were analyzed between 15000-80000 daltons. DAR was determined from the deconvoluted spectra or UV chromatogram by summing the integrated MS (total ion current) or UV (280 nm) peak area of unconjugated and conjugated given species (mAb or associated fragment), weighted by multiplying each area by the number of drug attached. The summed, weighted areas were divided by the sum of total area and the results produced a final average DAR value for the full ADC.

[1153] Size exclusion chromatography (SEC) (1) was performed to determine the quality of the ADCs and aggregation percentage (%) after purification. The analysis was performed on analytical column Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) in isocratic

conditions 100% PBS pH 7.2 ((Hyclone SH30028.03)), flow 0.45 ml/min for 8 minutes. The % aggregate fraction of the ADC sample was quantified based on the peak area absorbance at 280 nm. Calculation was based on the ratio between the high molecular weight eluent at 280 nm divided by the sum of peak area absorbance at the same wavelength of the high molecular weight and monomeric eluents multiplied by 100%. Data was aquired on an Agilent Bio-Inert 1260 HPLC outfitted with a Wyatt miniDAWN light scattering and Treos refractive index detectors (Wyatt Technologies, Santa Barbara, CA)

[1154] All exemplified ADCs were characterized by analytical size exclusion chromatography Superdex 200 Increase 5/150 GL (GE Healthcare, 28990945) to determine monomer percentage and LC-MS for DAR determination. The average DAR values were determined using the above LC/MS methods (LC/MS I) and percentage aggregation was determined using the above SEC methods (SEC I).

***In vivo* testing**

[1155] H1650 cells were cultured at 37 °C in an atmosphere of 5% CO_2 in air in RPMI1640 (BioConcept Ltd. Amimed, # 1-41F01-I) supplemented with 10 % FCS (BioConcept Ltd. Amimed, # 2-01F30-I), 2 mM L-glutamine (BioConcept Ltd. Amimed, #5-10K00-H) and 1mM sodium pyruvate (BioConcept Ltd. Amimed, #5-60F00-H), 10 mM HEPES (Gibco #11560496) and D-glucose 1.25g/500mL medium (Gibco, # A24940-01). To establish H1650 xenografts, cells were harvested and re-suspended in HBSS (Gibco, #14175) / Matrigel (Corning # 354234) (1:1 v/v) before injecting 100 µL containing 5 x 10^6 cells subcutaneously in the flanks of female SCID mice (Taconic, Europe). Tumor growth was monitored regularly post cell inoculation and animals were randomized into treatment groups (n = 6) with a mean tumor volume of about 150 mm^3. Control groups and EGFR2 CysMab DANAPA-L109A-P1-ADC were dosed as indicated in FIG. 9. The ADCs were administered intravenously (i.v.) once at the start of treatment at 30 mg/kg in combination with 7.5 mg/kg docetaxel (Zentiva: 1ml/20mg) which was administered i.v. once 24h after the ADCs. Doses were adjusted to individual mouse body weights. The i.v. dose volume was 10 ml/kg and each ADC was dissolved in 0.9% (w/v) NaCl in water and the docetaxel stock solution was diluted to appropriate concentration with 5% (w/v) sterile glucose solution prior to administration.

[1156] Tumor volume data on day 21 and 28 post treatment initiation were analyzed for statistical difference relative to vehicle control group and EGFR-Bcl-xLi (L109A-P1) ADC, using one way ANOVA post hoc Tukey's multiple comparisons test (Indigo Software). Results are presented as mean ± SEM.

[1157] As a measure of efficacy the %T/C value was calculated on day 21 according to:

(Δtumor volume treated/Δtumor volume control)*100

Tumor regression was calculated according to:

-(Δtumor volume treated/tumor volume treated at start)*100

Where Δtumor volumes represent the mean tumor volume on the evaluation day minus the mean tumor volume at the start of the experiment.

Results: efficacy and tolerability

[1158] EGFR2 CysMab DANAPA-L109A-P1 ADC at 7.5 mg/kg in combination with docetaxel (7.5 mg/kg) significantly ($p<0.05$) reduced the growth of H1650 tumors compared with the vehicle control group on day 21 (FIG. 9 and Table 30). On day 28 post treatment initiation, EGFR2 CysMab DANAPA-L109A-P1-ADC had induced significantly ($p<0.05$) greater tumor growth inhibition than Isotype IgG CysMab DANAPA-L109A-P1 ADC in combination with docetaxel (FIG. 9 and Table 30). All treatment schedules were well tolerated based on body weight changes (FIG. 10).

Table 30 Summary of the antitumor effect of EGFR2-CysMab DANAPA-L109A-P1-ADC in combination with docetaxel. Delta tumor volumes and T/C% values were calculated on day 21 and are presented as means. Statistical analysis on day 21 (vs vehicle control group) and on days 21 and 28 (vs IgG CysMab DANAPA-L109A-P1 ADC + docetaxel) was performed using one way ANOVA post hoc Tukey's multiple comparisons test (Indigo InLife with results analysis in TIBCO Spotfire).

Test agent, dose schedule and route	ΔTumor volume (mm^3) D21	Response on day 21		Maximum regression (day)	Significance		
		T/C (%)	Regression		On D21 vs Vehicle group	On D21 vs Isotype-DANAPA-L109A-P1-ADC group	On D28 vs Isotype-DANAPA-L109A-P1-ADC group
Vehicle i.v. + vehicle i.v.	782.02 ± 158.78	100	-	N/A	N/A	P<0.05 (growth)	N/A
Naked EGFR2 CysMab DANAPA 30 mg/kg iv qdx1 + vehicle iv 24h later	884.59 ± 119.38	113	-	N/A	NS	P<0.05 (growth)	N/A
Vehicle iv + docetaxel 7.5 mg/kg qdx1 iv 24h later	396.59 ± 32.96	51	-	-1(D7)	NS	P<0.05 (growth)	N/A
IgG CysMab DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv 24h later	437.20 ± 48.62	56	-	N/A	NS	P<0.05 (growth)	N/A
EGFR2 CysMab DANAPA-L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv 24h later	410.52 ± 93.18	52	-	N/A	NS	P<0.05 (growth)	N/A
IgG CysMab DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv 24h later	88.62 ± 14.42	11	-	-42 (D7)	P<0.05	N/A	N/A
EGFR2 CysMab DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv 24h later	-96.95 ± 14.07	-	-68	-83 (D14)	P<0.05	NS	P<0.05 (reduction)

N/A, not applicable

Example 16: *In vivo* efficacy of EGFR1-Bcl-xL inhibitor-ADCs with different linker payloads against the H1650 human non-small cell lung carcinoma (NSCLC) model in mice

[1159] Efficacy of five (5) EGFR-Bcl-xL inhibitor (Bcl-xLi) ADCs with different linker payloads (L11A-P21, L11A-P27, L11C-P19, L11C-P25 and L109A-P1) was evaluated in the H1650, non-small cell lung carcinoma (NSCLC) model *in vivo* by combining treatment with docetaxel. The EGFR antibody is also labeled as EGFR1 CysMab (see Example 6)

Methods

[1160] H1650 cells were cultured at 37 °C in an atmosphere of 5 % CO_2 in air in RPMI1640 (BioConcept Ltd. Amimed) supplemented with 10 % FCS (BioConcept Ltd. Amimed, # 2-01F30-I), 2 mM L-glutamine (BioConcept Ltd. Amimed, #5-10K00-H) and 1mM sodium pyruvate (BioConcept Ltd. Amimed, #5-60F00-H), 10 mM HEPES (Gibco #11560496) and D-glucose 1.25g/500mL medium (Gibco, # A24940-01). To establish H1650 xenografts, cells were harvested and re-suspended in HBSS (Gibco, #14175) / Matrigel (Corning # 354234) before injecting 100 µL containing 5 x 10^6 cells subcutaneously in the flanks of female SCID mice (Taconic, Europe). Tumor growth was monitored regularly post cell inoculation and animals were randomized into treatment groups (n = 7) with a mean tumor volume of about 150 mm^3. Control groups and various EGFR-Bcl-xLi ADCs with different linker payloads (L11A-P21, L11A-P27, L11C-P19, L11C-P25 and L109A-P1) were dosed as indicated in FIG. 11. The ADCs were administered intravenously (i.v.) once at the start of treatment at 7.5 mg/kg in combination with 7.5 mg/kg docetaxel (Zentiva: 1ml/20mg), which was administered i.v. once 24h after the ADCs. Doses were adjusted to individual mouse body weights. The i.v. dose volume was 10 ml/kg and each ADC was dissolved in PBS (BioConcept Ltd. Amimed, # 3-05F29-I), and the docetaxel stock solution was diluted to appropriate concentration with 5% (w/v) sterile glucose solution (Braun, # 19029) prior to administration.

[1161] Tumor volume data on day 21 and 46 post treatment initiation were analyzed for statistical difference relative to vehicle control group and EGFR-Bcl-xLi (L109A-P1) ADC, using one way ANOVA post hoc Tukey's multiple comparisons test (Indigo Software). Results are presented as mean ± SEM in FIG. 11 and Table 31.

[1162] As a measure of efficacy, the %T/C value was calculated on day 21 according to:

(Δtumor volume treated/Δtumor volume control)*100

Tumor regression was calculated according to:

-(Δtumor volume treated/tumor volume treated at start)*100

Where Δtumor volumes represent the mean tumor volume on the evaluation day minus the mean tumor volume at the start of the experiment.

Results: efficacy and tolerability

[1163] All ADCs at 7.5 mg/kg in combination with docetaxel (7.5 mg/kg) significantly ($p<0.05$) reduced the growth of H1650 tumors compared with the vehicle control group on day 21 (FIG. 11 and Table 31). On day 21 and 46 post treatment initiation with EGFR-L11A-P21, EGFR-L11C-P19 and EGFR-L11C-P25 tumor growth was significantly reduced relative to treatment with EGFR-L109A-P1 in combination with docetaxel (FIG. 11 and Table 31). All treatment schedules were well tolerated based on body weight changes (FIG. 12).

Table 31: Summary of the antitumor effect of EGFR-Bcl-xLi-ADCs with different linker payloads in combination with docetaxel. Delta tumor volumes and T/C% values were calculated for day 21 and are presented as means. Statistical analysis on day 21 (vs vehicle control group) and on days 21 and 46 (vs EGFR-L109A-P1) was performed using one way ANOVA post hoc Tukey's multiple comparisons test; Indigo InLife Results analysis in TIBCO Spotfire.

Test agent, dose schedule and route	ΔTumor volume (mm^3) D21	T/C (%) D21	Regression (%)	Statistics		
				D21 vs Vehicle gp	21 vs EGFR-L109A-P1	D46 vs EGFR-L109A-P1
Vehicle i.v. + vehicle i.v.	499 ± 74	100	N/A	N/A	P<0.05	N/A
EGFR naked Ab 7.5 mg/kg i.v.+ docetaxel 7.5 mg/kg iv 24h later	295 ± 29	59	N/A	NS	P<0.05	N/A
Isotype-L11A-P21 i.v.+ docetaxel 7.5 mg/kg iv 24h later	300 ± 33	60	N/A	NS	P<0.05	N/A
Isotype-L11C-P25 i.v.+ docetaxel 7.5 mg/kg iv 24h later	203 ± 18	41	N/A	P<0.05	NS	N/A
EGFR-L11A-P21 i.v.+ docetaxel 7.5 mg/kg iv 24h later	-16 ± 20	N/A	-8	P<0.05	NS	P<0.05
EGFR-L11A-P27 i.v.+ docetaxel 7.5 mg/kg iv 24h later	14 ± 18	3	N/A	P<0.05	NS	NS

EGFR-L11C-P19 i.v.+ docetaxel 7.5 mg/kg iv 24h later	-52 ± 21	N/A	-35	P<0.05	P<0.05	P<0.05
EGFR-L11C-P25 i.v.+ docetaxel 7.5 mg/kg iv 24h later	-76 ± 17	N/A	-50	P<0.05	P<0.05	P<0.05
EGFR-L109A-P1 i.v.+ docetaxel 7.5 mg/kg iv 24h later	73 ± 17	15	N/A	P<0.05	N/A	N/A

Example 17. *In vivo* testing of EpCAM ADCs in EBC-1 cells

[1164] EBC-1 cells were cultured at 37°C (atmosphere of 5% CO_2) in DMEM (Gibco 11965-084) supplemented with 10% FBS (HI-FBS #134K19, Tet-free). Treatment with 0.25% Trypsin (Gibco 25200-056) was used for sub-culturing. To establish EBC-1 xenografts, cells were harvested and re-suspended in a 1:1 v/v mixture of phosphate buffered saline and Matrigel. A total of 5 x 10^6 cells in a volume of 150 µL were injected subcutaneously in the flanks of female nude mice (Charles River, USA). Tumor growth was monitored regularly post cell inoculation and animals were randomized into treatment groups (n = 8) with a mean tumor volume of about 210 mm^3. EpCAM-DANAPA-L11C-P25 ADC, 3207-DANAPA-L11C-P25 isotype control ADC, and EpCAM-DANAPA CysMab control antibody were all dosed in combination with paclitaxel (LC Laboratories, Woburn, MA, Cat#: P-9600) as indicated in FIG. 13. The ADCs and CysMab antibody were administered intravenously (i.v.) once at the start of treatment at 30 mg/kg. Paclitaxel was administered i.v. once at 12.5 mg/kg, 24h after the ADC or CysMab dose. All reagents were dosed at 10 mL/kg based on the individual mouse body weight. The ADCs and CysMab were formulated at 3 mg/mL in PBS on the day of treatment. Paclitaxel was reconstituted in 50% Ethanol + 50% Cremophor EL (Kolliphor EL) at a concentration of 6 mg/mL, and then further diluted to 1.25 mg/mL with sterile saline prior to administration on the day of treatment.

[1165] Tumor volume data were analyzed for statistical difference relative to the EpCAM-DANAPA-L11C-P25 ADC + paclitaxel combination group. Unpaired two-tailed T-tests were used to make comparisons between groups.

[1166] As a measure of efficacy, %T/C values were calculated according to the formula:

(Δtumor volume treated/Δtumor volume control)*100.

Tumor regression was calculated according to the formula:

-(Δtumor volume treated/tumor volume treated at start)*100.

Δtumor volumes represent mean tumor volumes on the measurement day minus the mean tumor volume at the start of treatment. The Δtumor volume control value specified above refers

to mean tumor volume changes in the vehicle group. Results are presented in Table 32 as mean ± SEM.

Results: efficacy and tolerability

[1167] EpCAM-DANAPA-L11C-P25 ADC dosed at 30 mg/kg in combination with paclitaxel at 12.5 mg/kg significantly ($p<0.05$) reduced the growth of EBC-1 tumors compared to the vehicle and docetaxel alone groups. This ADC also had significantly greater anti-tumor activity than the 3207-DANAPA-L11C-P25 isotype control ADC, and the EpCAM-DANAPA CysMab control antibody, when combined with paclitaxel. EpCAM-DANAPA-L11C-P25 induced tumor regression in combination with paclitaxel, leading to complete responses in 4/8 animals by day 32 post-first dose. However, EpCAM-DANAPA-L11C-P25 ADC alone was also able to induce tumor regression. The depth of response was slightly less than that achieved by the combination with paclitaxel, although the difference was not statistically significant ($p=0.168$). All treatments were well tolerated based on percent body weight changes calculated post-first dose (FIG. 14).

Table 32. Summary of the antitumor activity of EpCAM-DANAPA-L11C-P25 ADC in combination with paclitaxel. All ΔTumor volume, %T/C, and %Regression values are presented as means, based off tumor measurements collected on the days post-treatment specified below. T/C and regression values were calculated using formulas specified in the methods. Complete responders were identified as animals with tumors that had regressed to 0 mm^3 by the specified dates. Statistical analyses were performed by comparing each treatment group to the EpCAM-DANAPA-L11C-P25 ADC + paclitaxel combination group using unpaired two-tailed T-tests.

Test agent (Dose, schedule, and route)	**ΔTumor volume (mm^3±SEM) (Day of Evaluation)**	**%T/C (Day of Evaluation)**	**%Regression (Day of Evaluation)**	**Complete Responders (Day of Evaluation)**	**Significance vs EpCAM-DANAPA-L11C-P25 ADC on Day 9 (Day of Evaluation)**
Untreated	1501.1 ± 174.1 (Day 9)	100% (Day 9)	N/A	0/8 (Day 32)	P=2.7e-7 (Day 9)
Paclitaxel Alone	510.8 ± 158.5 (Day 10)	34% (Day 10)	N/A	0/8 (Day 32)	P=0.0014 (Day 10)
3207-DANAPA-L11C-P25 isotype control ADC (30 mg/kg, SD,iv) + Paclitaxel (12.5 mg/kg, SD, iv)	238.0 ± 55.9 (Day 10)	16% (Day 10)	N/A	0/8 (Day 32)	P=0.0001 (Day 10)
EpCAM-DANAPA CysMab (30 mg/kg, SD, iv) + Paclitaxel (12.5 mg/kg, SD, iv)	96.7 ± 24.4 (Day 9)	6% (Day 9)	N/A	0/8 (Day 32)	P=0.0002 (Day 9)
EpCAM-DANAPA-L11C-P25 ADC (30 mg/kg, SD, iv)	-42.9 ± 45.6 (Day 9)	N/A	20% (Day 9)	2/8 (Day 32)	P=0.168 (Day 9)
EpCAM-DANAPA-L11C-P25 ADC (30 mg/kg, SD, iv) + Paclitaxel (12.5 mg/kg, SD, iv)	-126.1 ± 34.3 (Day 9)	N/A	59% (Day 9)	4/8 (Day 32)	N/A

N/A, not applicable

Example 18. *In vivo* therapeutic effect of several CD7-targeting ADCs in ALL-SIL T-cell Acute Lymphoblastic Leukemia xenografts after intravenous (IV) administration

[1168] The *in vivo* therapeutic effect of several CD7-targeting ADCs formulated in Phosphate-Buffered Saline (PBS) was determined in ALL-SIL T-cell Acute Lymphoblastic Leukemia xenografts after intravenous (IV) administration.

Materials and methods

[1169] ALL-SIL cells, obtained from DSMZ, were cultured in RPMI supplemented with 20% FBS. Cells were resuspended in 100% matrigel (BD Biosciences) and 0.1ml containing 5x106 cells were subcutaneously inoculated into the right flank of female NSG mice, provided by Jax. When tumors reached the appropriate volume, mice were randomized, 6 animals per group, using Easy stat software. IgG1 DANAPA-L9A-P21, Ab D DANAPA_L9A-P1, Ab D DANAPA_L9A-P21, Ab D DANAPA_L9C-P25, Ab D DANAPA_L9A-P33 and Ab D DANAPA_L9C-P40 (2.5 and/or 7.5 mg/kg) were injected once IV in PBS. Mice body weight was monitored three times a week and tumor size measured using electronic calipers. Tumor volume was estimated by measuring the minimum and maximum tumor diameters using the formula: (minimum diameter)2(maximum diameter)/2. Tumor growth inhibition on day 17 was calculated using the formula:

$$\left(1-\frac{\text{Median (DTV at Dx in treated group)}}{\text{Median (DTV at Dx in Control group)}}\right)\times 100$$

With DTV (Delta Tumor Volume) at Dx, calculated being TV at Dx - TV at Randomization. Mice were sacrificed at the first measurement for which tumor volume exceeded 2000 mm^3 or at the first signs of animal health deterioration. All experiments were conducted in accordance with the French regulations in force in 2018 after approval by Servier Research Institute (IdRS) Ethical Committee. NSG mice were maintained according to institutional guidelines.

Results

[1170] The efficacy of several anti-CD7 ADCs on ALL-SIL xenografts is illustrated in Figure 15. Treatment was started 7 days post tumor cells inoculation (median size: 240 mm^3). IgG1 DANAPA-L9A-P21 (non-targeting ADC Fc silent), Ab D DANAPA_L9A-P1, Ab D DANAPA_L9A-P21, Ab D DANAPA_L9C-P25, Ab D DANAPA_L9A-P33 and Ab D DANAPA_L9C-P40 (CD7-targeting ADC Fc silent) were administered once IV at 2.5 and/or 7.5 mg/kg.

[1171] The non-targeting ADC Fc silent had no effect on tumor growth, with a Tumor Growth Inhibition (%TGI) on day 17 of -101.72%, as depicted in FIG. 15 and Table 33. On the contrary, all CD7-targeting ADCs induced complete and long-lasting tumor regression, with %TGI on d17

ranging from 108.59 to 124.37% ($p \leq 0.001$ as compared to untreated control group). No clinically relevant body weight loss or other clinical signs due to the treatment were observed (FIG. 16).

Table 33: ALL-SIL tumor growth inhibition upon treatment with IgG1 DANAPA-L9A-P21, Ab D DANAPA_L9A-P1, Ab D DANAPA_L9A-P21, Ab D DANAPA_L9C-P25, Ab D DANAPA_L9A-P33 and Ab D DANAPA_L9C-P40 (2.5 and/or 7.5 mg/kg, administered once IV, n=6

Treatment	Dose/Schedule	%TGI (d17)
IgG1 DANAPA-L9A-P21	7.5 MK QD, IV	-101.72
Ab D DANAPA_L9A-P1	7.5 MK QD, IV	108.59
Ab D DANAPA_L9A-P21	2.5 MK QD, IV	116.54
Ab D DANAPA_L9A-P21	7.5 MK QD, IV	111.07
Ab D DANAPA_L9C-P25	2.5 MK QD, IV	116.6
Ab D DANAPA_L9C-P25	7.5 MK QD, IV	124.37
Ab D DANAPA_L9A-P33	2.5 MK QD, IV	124.07
Ab D DANAPA_L9A-P33	7.5 MK QD, IV	123.33
Ab D DANAPA_L9C-P40	2.5 MK QD, IV	122.43
Ab D DANAPA_L9C-P40	7.5 MK QD, IV	125.52

CLAIMS

1. An antibody-drug conjugate of Formula (1):

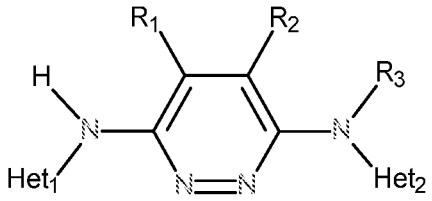
$$Ab\text{-}(L\text{-}D)_p \qquad (1)$$

wherein Ab is an antibody or an antigen-binding fragment thereof;

L is a linker that covalently attaches Ab to D;

p is an integer from 1 to 16; and

D is a Bcl-xL inhibitor compound of Formula (I) or Formula (II) covalently attached to the linker L:



(I)

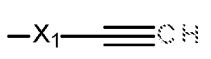
,

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- R_1 and R_2 independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally substituted by a a linear or branched C_1-C_6alkyl group;

  or R_1 and R_2 form with the carbon atoms carrying them a C_3-C_6cycloalkylene group,

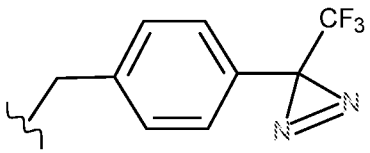
- R_3 represents a group selected from: hydrogen; C_3-C_6cycloalkyl; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; -X_1-O-R_c; -X_1-$COOR_c$; -X_1-$PO(OH)_2$; -X_1-$SO_2(OH)$; -X_1-N_3 and :



,

- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

the group:



or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;

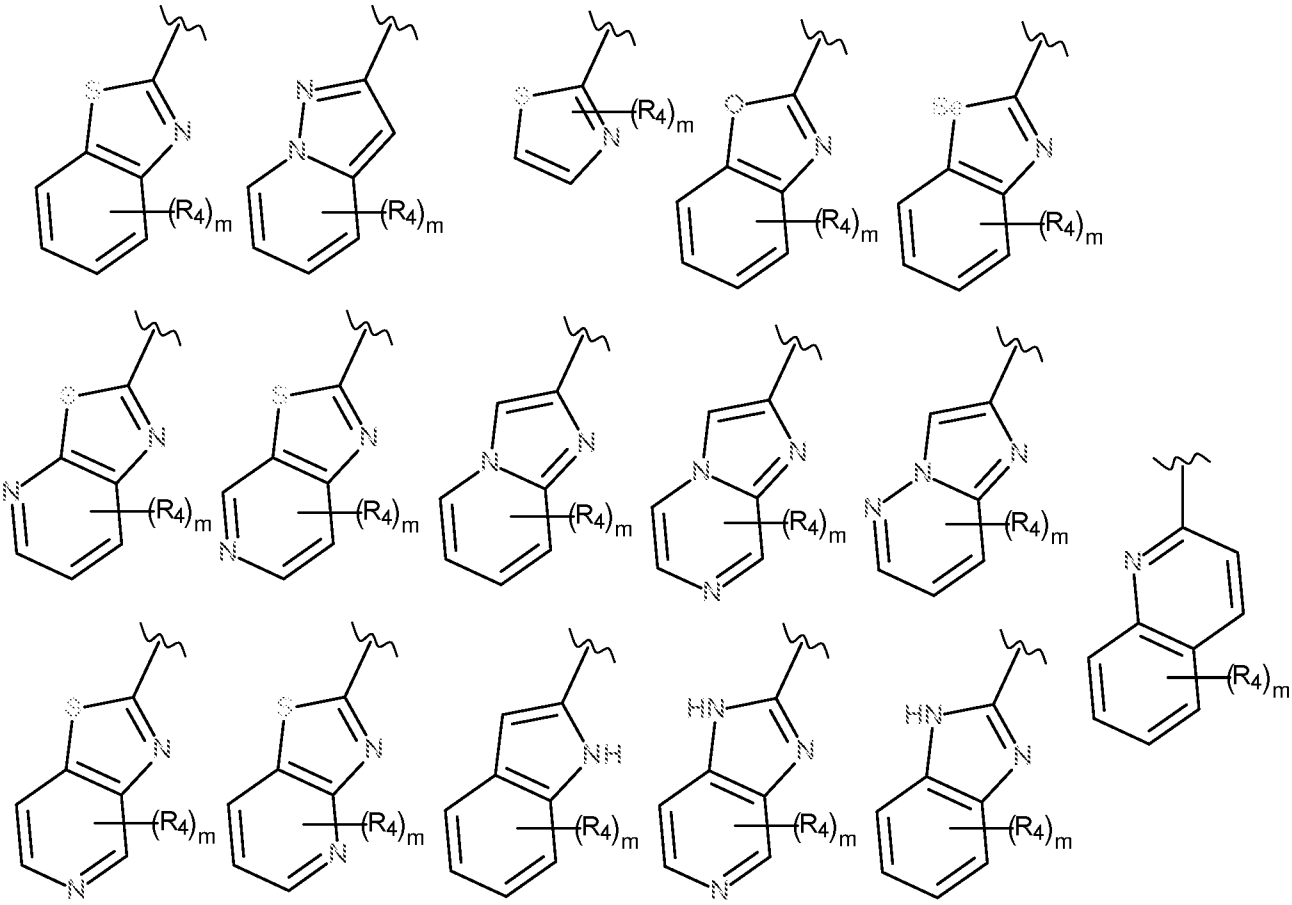
or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,

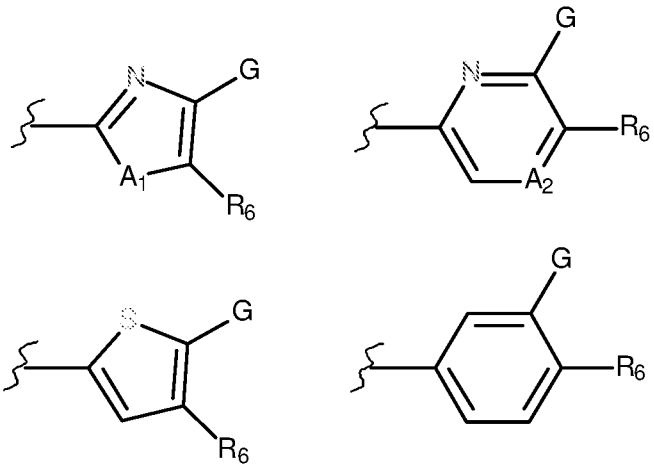
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,

  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



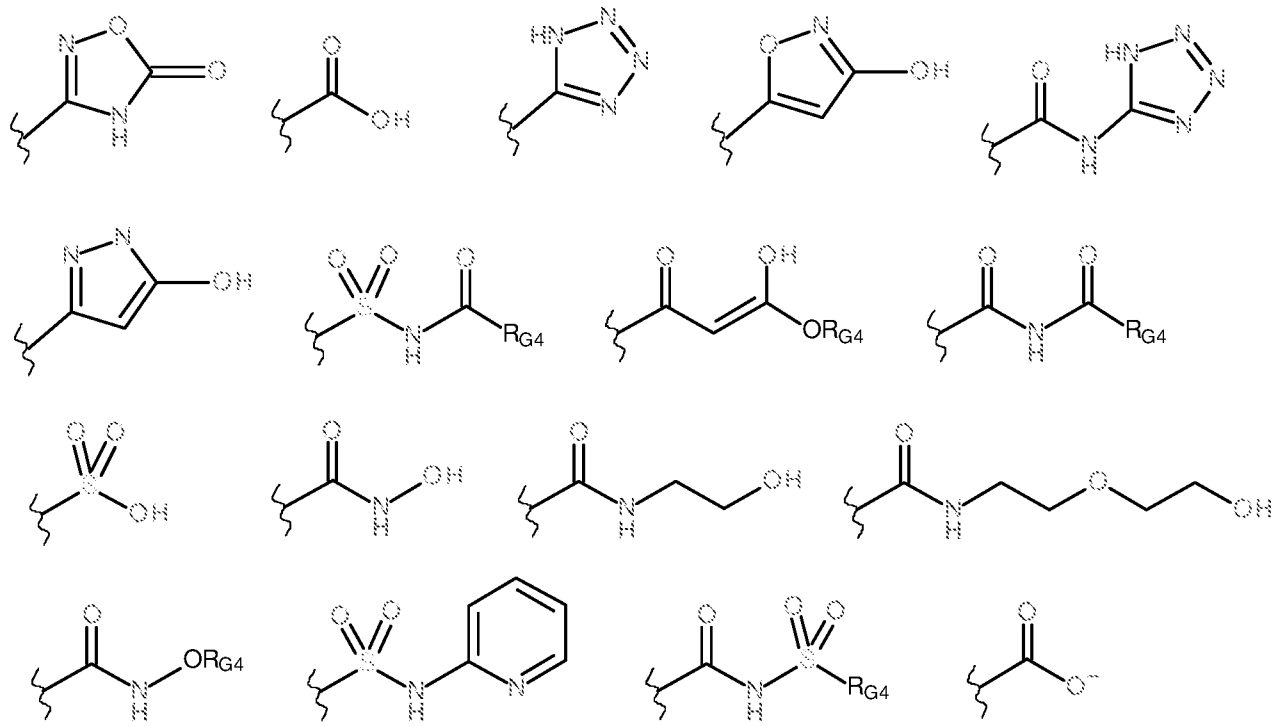
- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

$-C(O)OR_{G3}$, $-C(O)NR_{G1}R_{G2}$, $-C(O)R_{G2}$, $-NR_{G1}C(O)R_{G2}$, $-NR_{G1}C(O)NR_{G1}R_{G2}$, $-OC(O)NR_{G1}R_{G2}$, $-NR_{G1}C(O)OR_{G3}$, $-C(=NOR_{G1})NR_{G1}R_{G2}$, $-NR_{G1}C(=NCN)NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2NR_{G1}R_{G2}$, $-S(O)_2R_{G3}$, $-S(O)_2NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2R_{G2}$, $-NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, $-C(=S)NR_{G1}R_{G2}$, $-C(=NR_{G1})NR_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, $-NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen

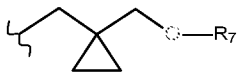
atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,

- R_6 represents a group selected from:

  hydrogen;

  -C_2-C_6alkenyl;

  -X_2-O-R_7;

  
  ;

  -X_2-NSO_2-R_7;

  -C=C(R_9)-Y_1-O-R_7;

  C_3-C_6cycloalkyl;

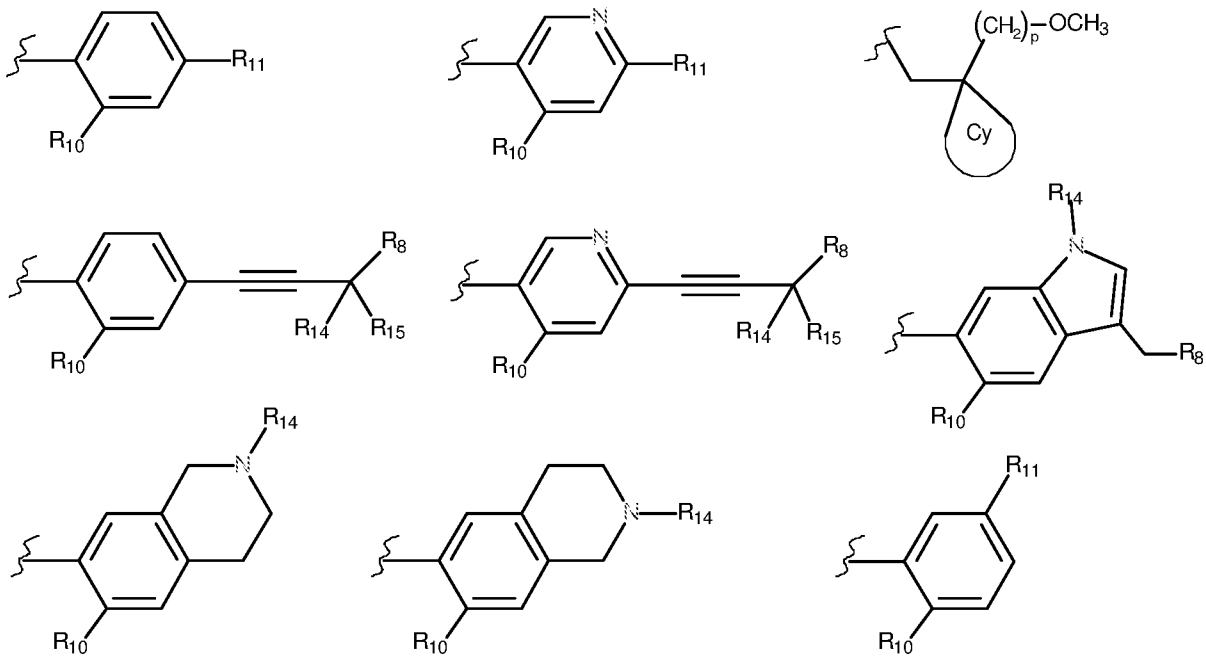
  C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

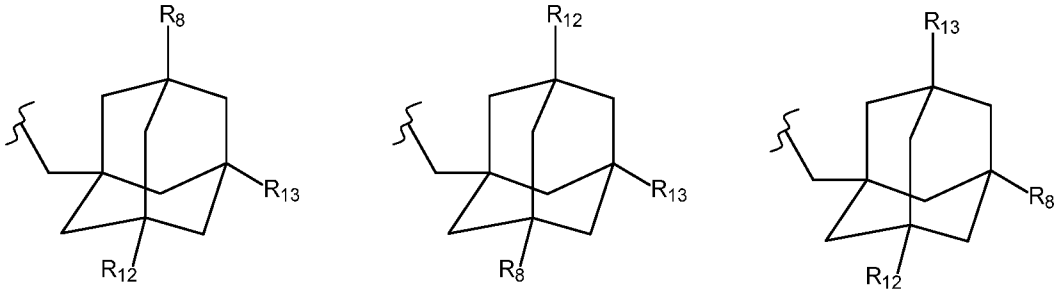
  C_3-C_6cycloalkylene-Y_2-R_7 ;

  C_3-C_6heterocycloalkylene-Y_2-R_7 group,

  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

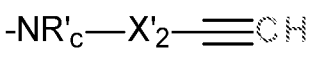
- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:





wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -NR'$_a$R'$_b$; -NR'$_a$-CO-OR'$_c$; -NR'$_a$-CO-R'$_c$; -N$^+$R'$_a$R'$_b$R'$_c$; -O-R'$_c$; -NH-X'$_2$-N$^+$R'$_a$R'$_b$R'$_c$; -O-X'$_2$-NR'$_a$R'$_b$, -X'$_2$-NR'$_a$R'$_b$, -NR'$_c$-X'$_2$-N_3 and :

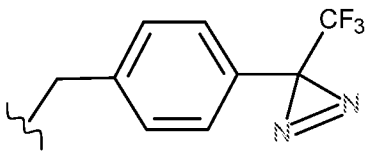


,

- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,

- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH;
  C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,

- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,

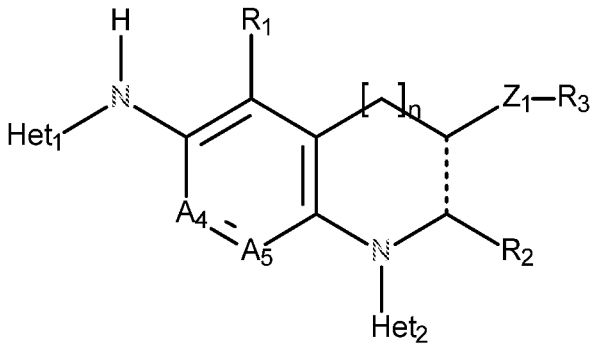
- m=0, 1 or 2,

- p=1, 2, 3 or 4,

- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl

group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl,

wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceeded by virtue of one or more substituents bonded thereto; or

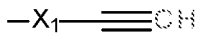


(II),

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- n=0, 1 or 2,
- ------ represents a single or a double bond.
- A_4 and A_5 independently of one another represent a carbon or a nitrogen atom,
- Z_1 represents a bond, -N(R)-, or –O-, wherein R represents a hydrogen or a linear or branched C_1-C_6alkyl,
- R_1 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally substituted by a a linear or branched C_1-C_6alkyl group;
- R_2 represents a hydrogen or a methyl;
- R_3 represents a group selected from: hydrogen; linear or branched C_1-C_4alkyl; -X_1-NR_aR_b;

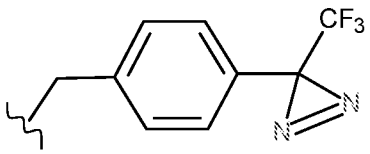
$-X_1-N^+R_aR_bR_c$; $-X_1-O-R_c$; $-X_1-COOR_c$; $-X_1-PO(OH)_2$; $-X_1-SO_2(OH)$; $-X_1-N_3$ and :



,

- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; $-SO_2$-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

  the group:



  or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;

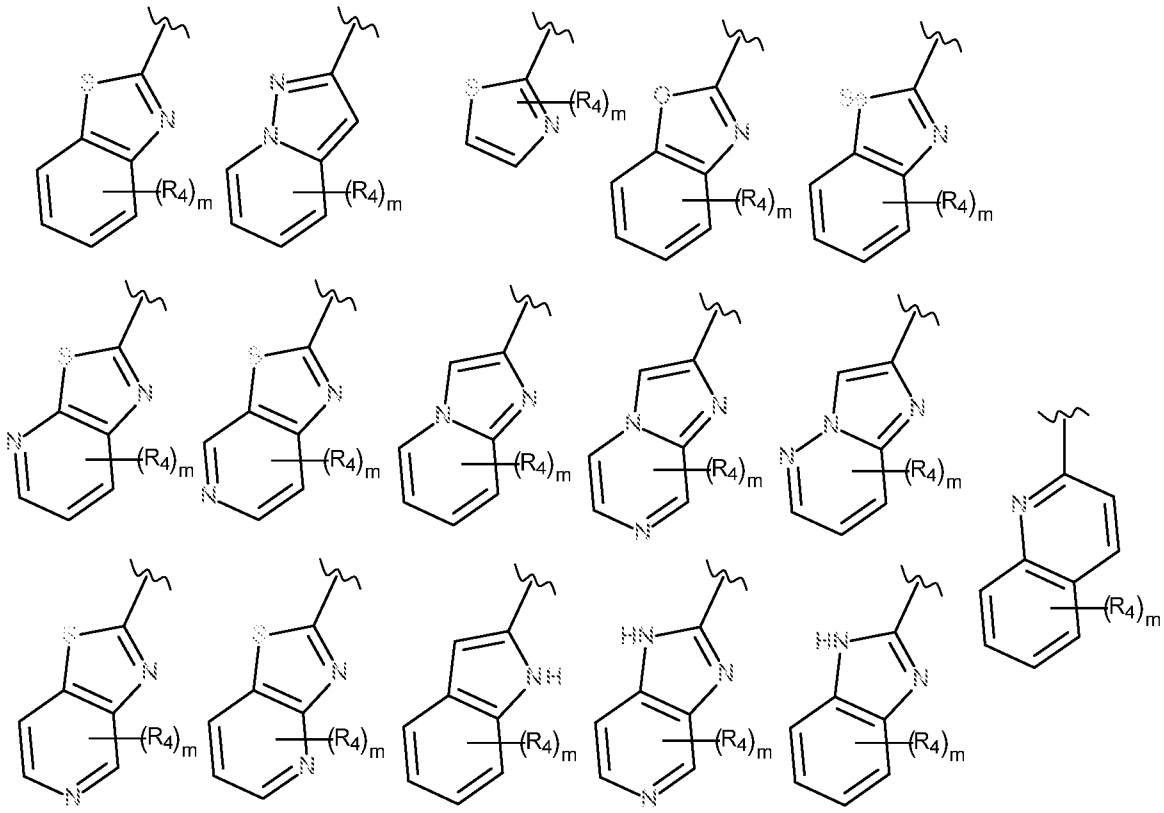
  or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,

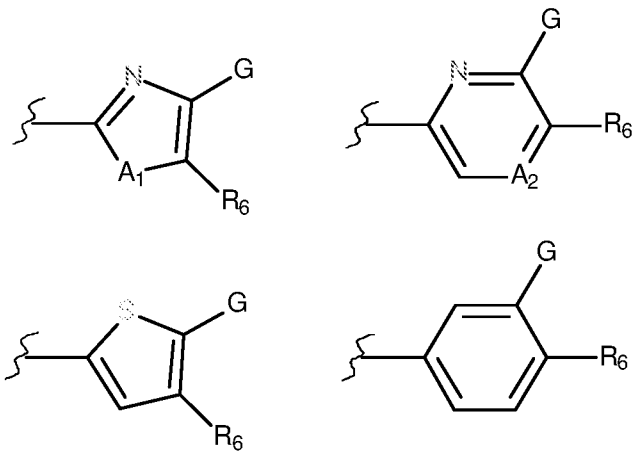
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,

  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

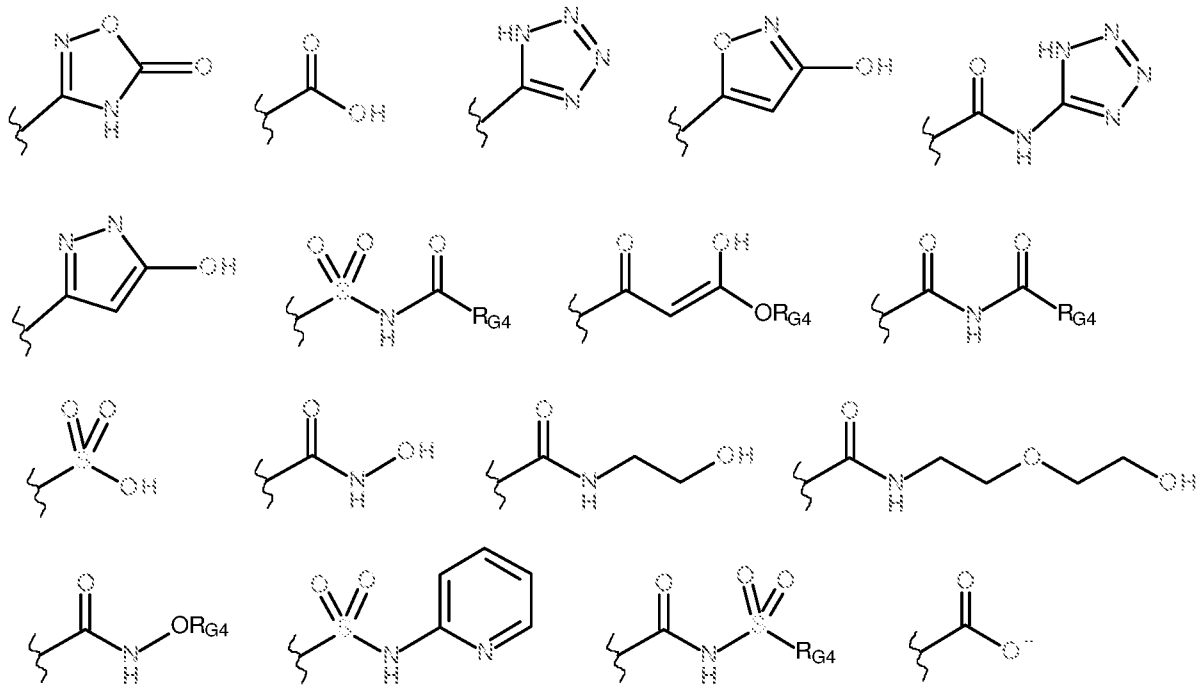
  -C(O)OR_{G3}, -C(O)N$R_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)N$R_{G1}R_{G2}$,

-$OC(O)NR_{G1}R_{G2}$, -$NR_{G1}C(O)OR_{G3}$, -$C(=NOR_{G1})NR_{G1}R_{G2}$, -$NR_{G1}C(=NCN)NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2NR_{G1}R_{G2}$, -$S(O)_2R_{G3}$, -$S(O)_2NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2R_{G2}$, -$NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, -$C(=S)NR_{G1}R_{G2}$, -$C(=NR_{G1})NR_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, -NO_2, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen

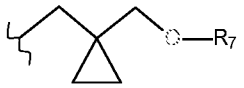
atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,

- R_6 represents a group selected from:

  hydrogen;

  -C_2-C_6alkenyl;

  -X_2-O-R_7;

  ;

  -X_2-NSO_2-R_7;

  -C=C(R_9)-Y_1-O-R_7;

  C_3-C_6cycloalkyl;

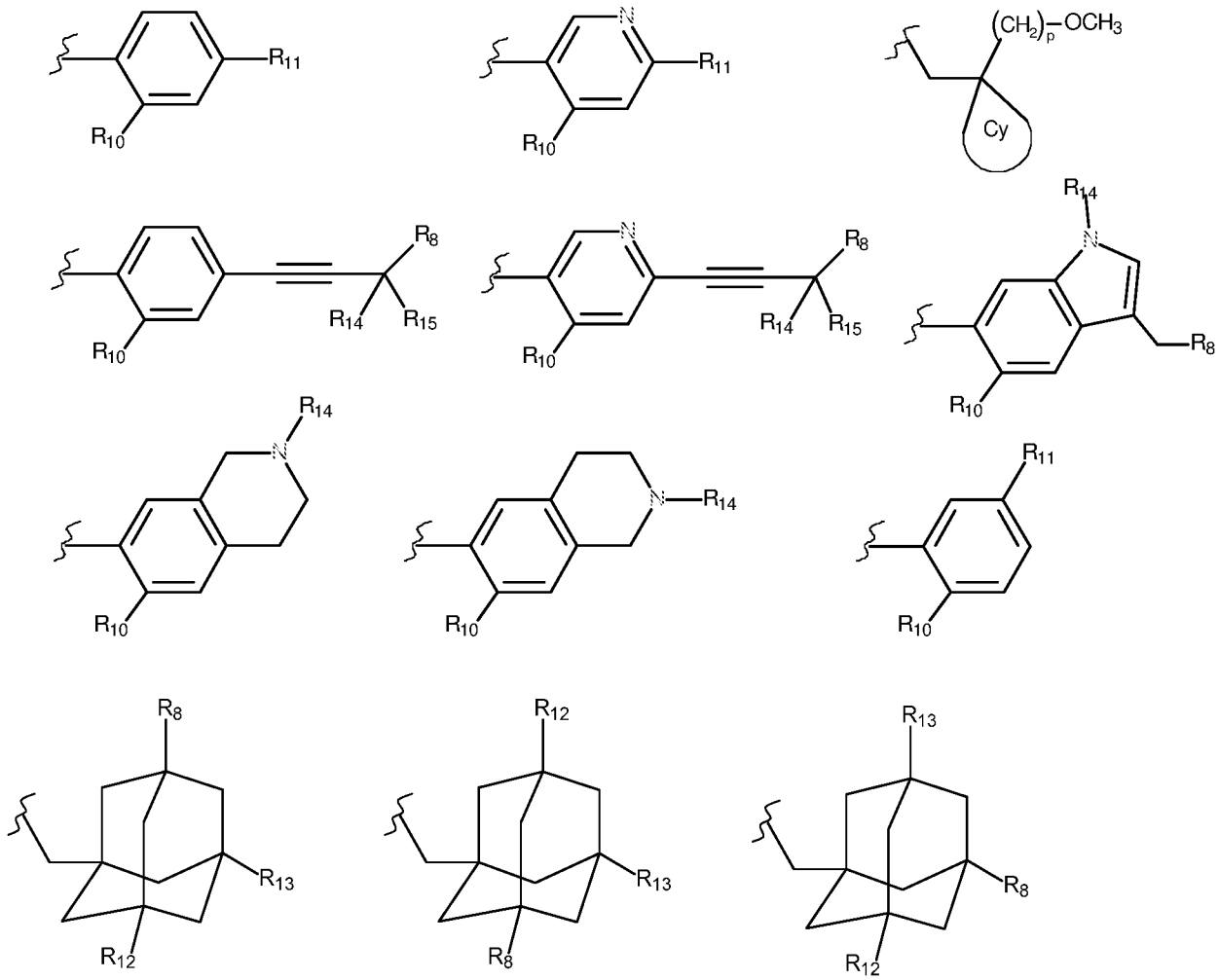
  C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

  C_3-C_6cycloalkylene-Y_2-R_7 ;

  C_3-C_6heterocycloalkylene-Y_2-R_7 group,

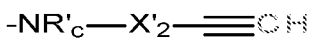
  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:



wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -NR'$_a$R'$_b$; -NR'$_a$-CO-OR'$_c$; -NR'$_a$-CO-R'$_c$; -N$^+$R'$_a$R'$_b$R'$_c$; -O-R'c; -NH-X'$_2$-N$^+$R'$_a$R'$_b$R'$_c$; -O-X'$_2$-NR'$_a$R'$_b$, -X'$_2$-NR'$_a$R'$_b$, -NR'$_c$-X'$_2$-N$_3$ and :



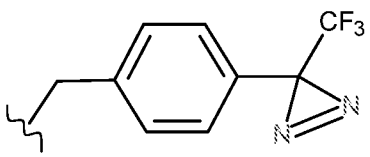
,

- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, halogen, C_1-C_3alkylene-R_8, -O-C_1-

C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,

- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 represents a linear or branched C_1-C_4alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X_2 represents a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged

C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,

- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,

- m=0, 1 or 2,

- p=1, 2, 3 or 4,

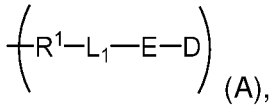
- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, –NH_2, oxo or piperidinyl,

  wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceeded by virtue of one or more substituents bonded thereto.

2. The antibody-drug conjugate of claim 1, wherein *p* is an integer from 1 to 6 or from 2 to 4, or p is 2 or 4; or *p* is determined by liquid chromatography-mass spectrometry (LC-MS).

3. The antibody-drug conjugate of claim 1 or 2, wherein L comprises:
an attachment group;
at least one bridging spacer group; and
at least one cleavable group, optionally at least one cleavable group comprising a pyrophosphate group and/or a self-immolative group.

4. The antibody-drug conjugate of claim 3, wherein -(L-D) is of the formula (A):



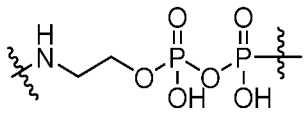
(A),

wherein:

R^1 is an attachment group;

L_1 is a bridging spacer group;

E is a cleavable group.

5. The antibody-drug conjugate of claim 3 or 4, wherein the cleavable group comprises a pyrophosphate group or the cleavable group comprises 
.

6. The antibody-drug conjugate of claim 3 or 4, wherein the bridging spacer group comprises:

(i) a polyoxyethylene (PEG) group;

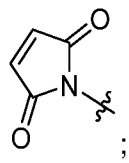
(ii) a PEG group selected from, PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, and PEG15;

(iii) a -CO-CH_2-CH_2-PEG12- group;

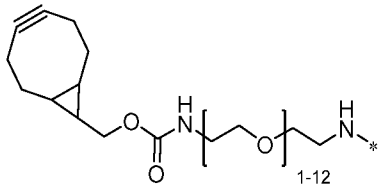
(iv) a butanoyl, pentanoyl, hexanoyl, heptanoyl, or octanoyl group; or

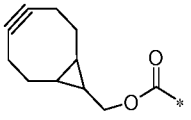
(v) a hexanoyl group
.

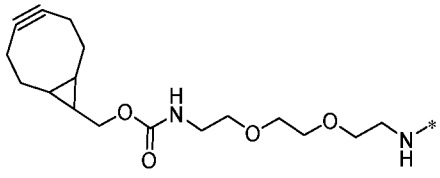
7. The antibody-drug conjugate of claim 6, wherein (i) the attachment group is formed from at least one reactive group selected from a maleimide group, thiol group, cyclooctyne group, and an azido group; optionally wherein:

a) the maleimide group has the structure:  ;

b) the azido group has the structure: -$N=N^+=N^-$;

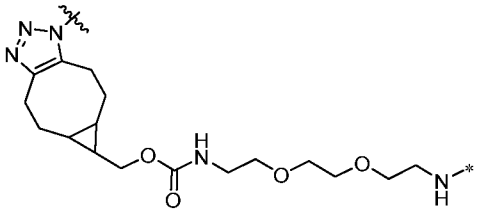
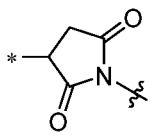
c) the cyclooctyne group has the structure:  or

 , and wherein —* is a bond to the antibody; or

d) the cyclooctyne group has the structure:  , and

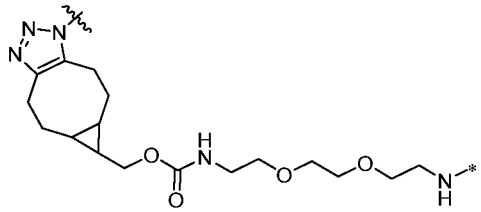
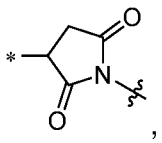
wherein —* is a bond to the antibody; or

(ii) the attachment group has a formula comprising:

 or  , and

wherein —* is a bond to the antibody..

8. The antibody-drug conjugate of claim 7, wherein the antibody is joined to the linker (L) by an attachment group selected from:

 and  ,

wherein —* is a bond to the antibody, and wherein  is a bond to the bridging spacer group .

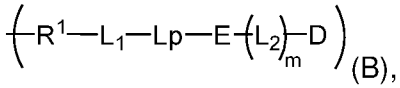
9. The antibody-drug conjugate of claim 8, wherein the bridging spacer group is -CO-CH_2-CH_2-PEG12-.

10. The antibody-drug conjugate of claim 8 or 9, wherein the bridging spacer group is joined to a cleavable group; optionally the cleavable group is -pyrophosphate-CH_2-CH_2-NH_2-.

11. The antibody-drug conjugate of any one of claims 8 to 10, wherein the cleavable group is joined to the Bcl-xL inhibitor (D).

12. The antibody-drug conjugate of any one of claims 1 to 3, wherein the linker comprises:
an attachment group,
at least one bridging spacer group,
a peptide group, and
at least one cleavable group.

13. The antibody-drug conjugate of claim 12, wherein -(L-D) is of the formula (B):



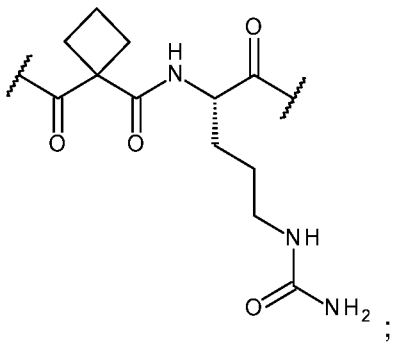
(B),

wherein:

R^1 is an attachment group;

L_1 is a bridging spacer;

Lp is a peptide group comprising 1 to 6 amino acid residues or Lp comprises a group



;

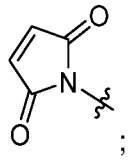
E is a cleavable group

L_2 is a bridging spacer;

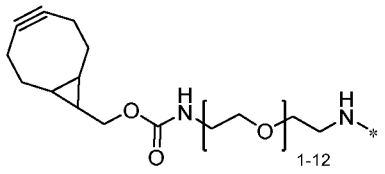
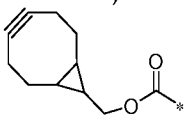
m is 0 or 1; and

D is a Bcl-xL inhibitor.

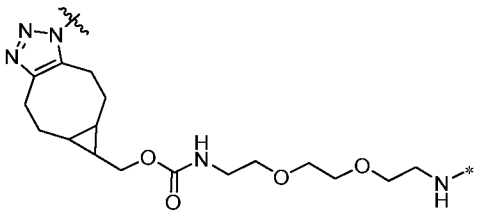
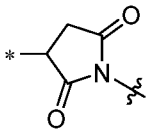
14. The antibody-drug conjugate of claim 12 or 13, wherein (i) the attachment group is formed from at least one reactive group comprising a maleimide group, thiol group, cyclooctyne group, and/or an azido group, optionally wherein:

a) the maleimide group has the structure:  ;

b) the azido group has the structure: -N=N$^+$=N$^-$;

c) the cyclooctyne group has the structure:  or  , and wherein —* is a bond to the antibody; or

(ii) the attachment group has a formula comprising:

 or  , and

wherein —* is a bond to the antibody.

15. The antibody-drug conjugate of any one of claims 12 to 14, wherein:

(i) at least one bridging spacer comprises a PEG group, optionally the PEG group is selected from, PEG1, PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, and PEG15; or

(ii) at least one bridging spacer is selected from *-C(O)-CH_2-CH_2-PEG1-**, *-C(O)-CH_2-PEG3-**, *-C(O)-CH_2-CH_2-PEG12**, *-NH-CH_2-CH_2-PEG1-**, a polyhydroxyalkyl group, *-C(O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(O)-**, and *-C(O)-CH_2-CH_2-PEG12-NH-C(O)CH_2-CH_2-**, wherein ** indicates the point of direct or indirect attachment of the at least one bridging spacer to the attachment group and * indicates the point of direct or indirect attachment of the at least one bridging spacer to the peptide group.

16. The antibody-drug conjugate of any one of claims 12 to 15, wherein L_1 is selected from *-C(O)-CH_2-CH_2-PEG1-**, *-C(O)-CH_2-PEG3-**, *-C(O)-CH_2-CH_2-PEG12**, *-NH-CH_2-CH_2-PEG1-**, and a polyhydroxyalkyl group, wherein ** indicates the point of direct or indirect attachment of L_1 to R^1 and * indicates the point of direct or indirect attachment of L_1 to Lp.

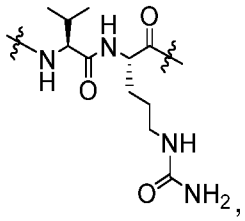
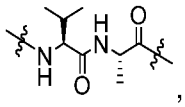
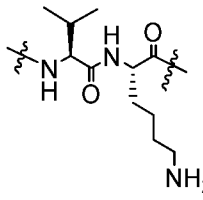
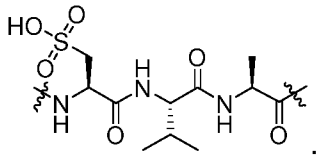
17. The antibody-drug conjugate of any one of claims 12 to 16, wherein m is 1 and L_2 is -C(O)-N(CH_3)-CH_2-CH_2-N(CH_3)-C(O)-.

18. The antibody-drug conjugate of any one of claims 12 to 17, wherein

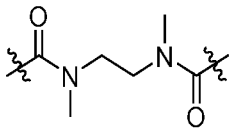
(i) the peptide group comprises 1 to 6, 1 to 4, 1 to 3 or 1 to 2 amino acid residues, optionally the amino acid residues are selected from L-glycine (Gly), L-valine (Val), L-citrulline (Cit), L-cysteic acid (sulfo-Ala), L-lysine (Lys), L-isoleucine (Ile), L-phenylalanine (Phe), L-methionine (Met), L-asparagine (Asn), L-proline (Pro), L-alanine (Ala), L-leucine (Leu), L-tryptophan (Trp), and L-tyrosine (Tyr);

(ii) the peptide group comprises Val-Cit, Val-Ala, Val-Lys, and/or sulfo-Ala-Val-Ala;

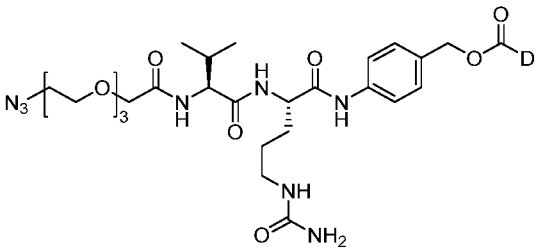
(iii) the peptide group is selected from:

 ,  ,  , and  .

19. The antibody-drug conjugate of any one of claims 12 to 18, wherein (i) the cleavable group comprises a pyrophosphate and/or a self-immolative group; (ii) the cleavable group comprises a self-immolative group; or (iii) the cleavable group comprises a self-immolative group comprising para-aminobenzyl-carbamate, para-aminobenzyl-ammonium, para-amino-(sulfo)benzyl-ammonium, para-amino-(sulfo)benzyl-carbamate, para-amino-(alkoxy-PEG-alkyl)benzyl-carbamate, para-amino-(polyhydroxycarboxytetrahydropyranyl)alkyl-benzyl-carbamate, or para-amino-(polyhydroxycarboxytetrahydropyranyl)alkyl-benzyl-ammonium.

20. The antibody-drug conjugate of any one of claims 13 to 19, wherein m is 0 or 1 or m is 1 and the bridging spacer comprises  .

21. The antibody-drug conjugate of any one of claims 13-20, wherein -(L-D) is formed from a compound selected from:



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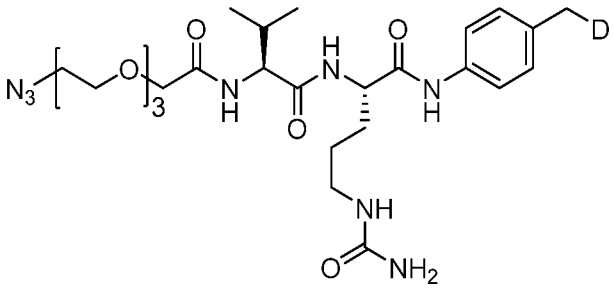

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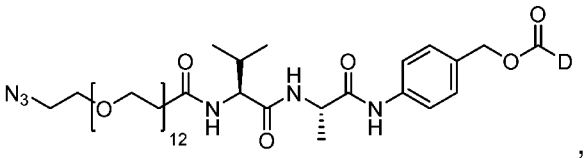

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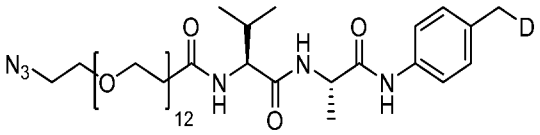

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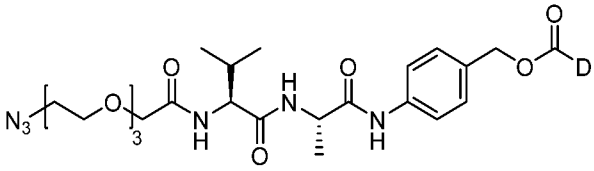

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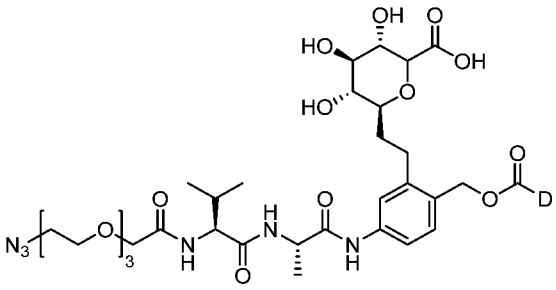

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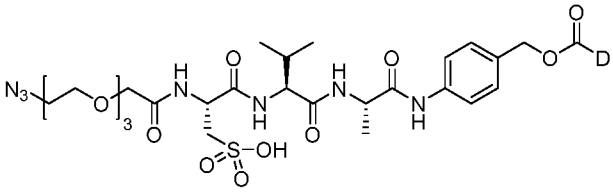

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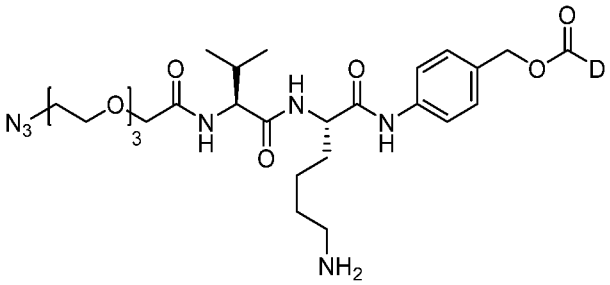

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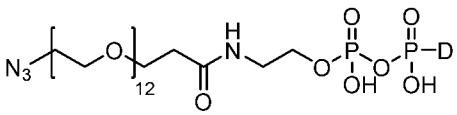

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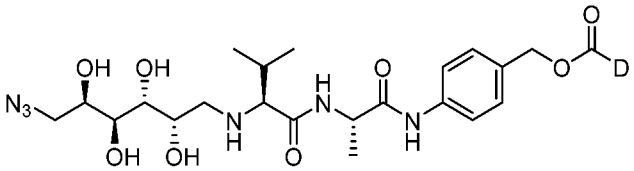

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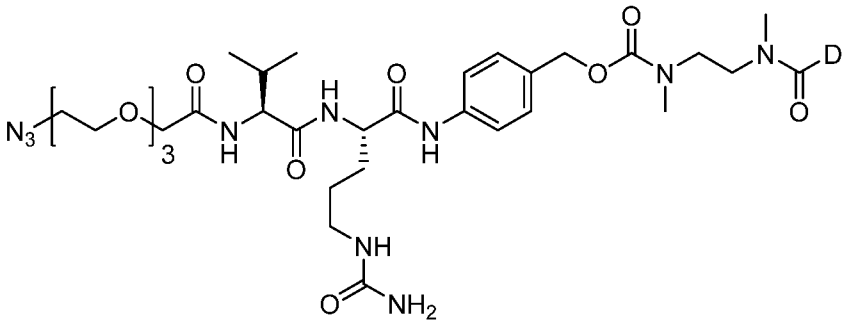

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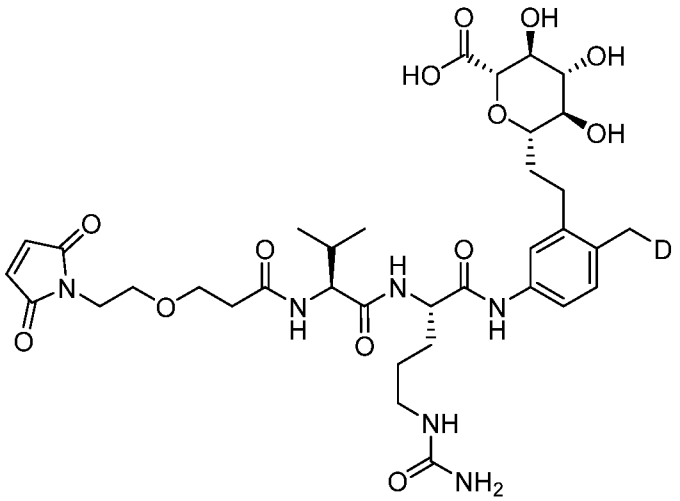

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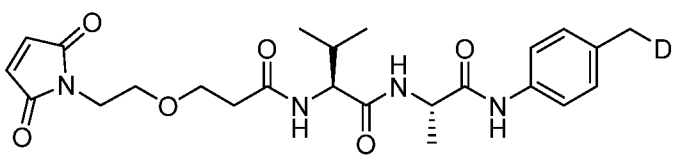

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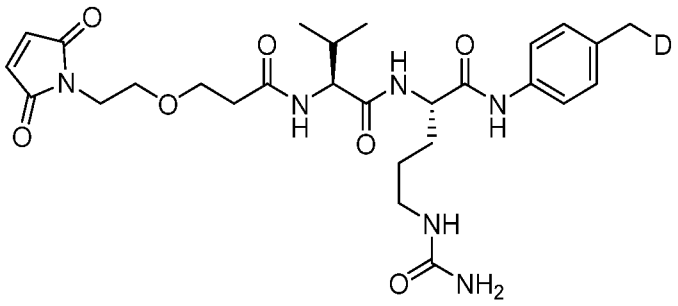

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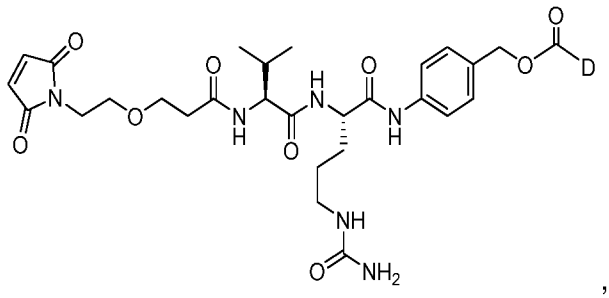

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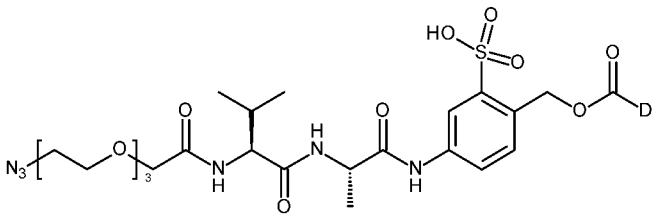

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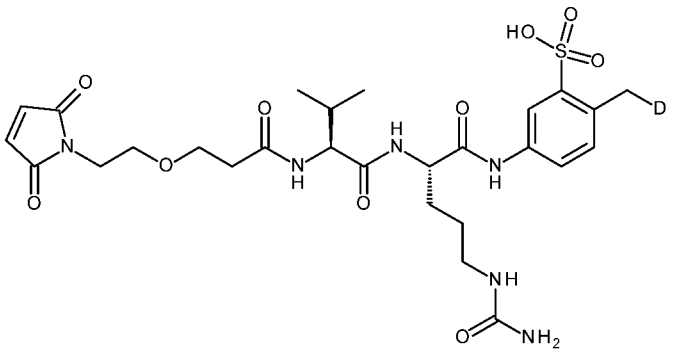

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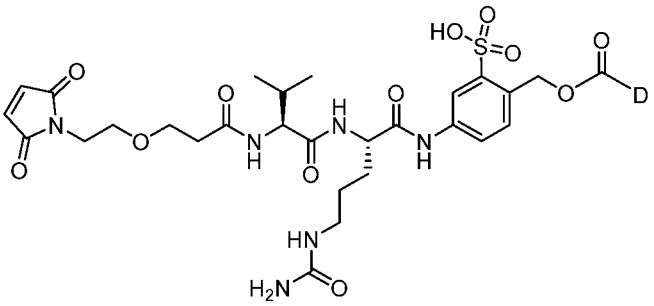

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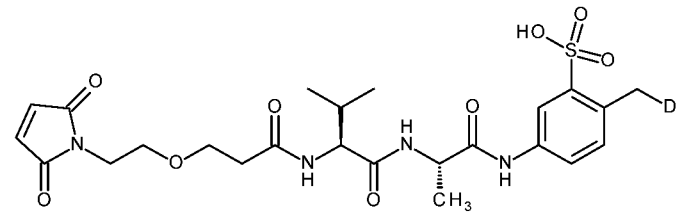

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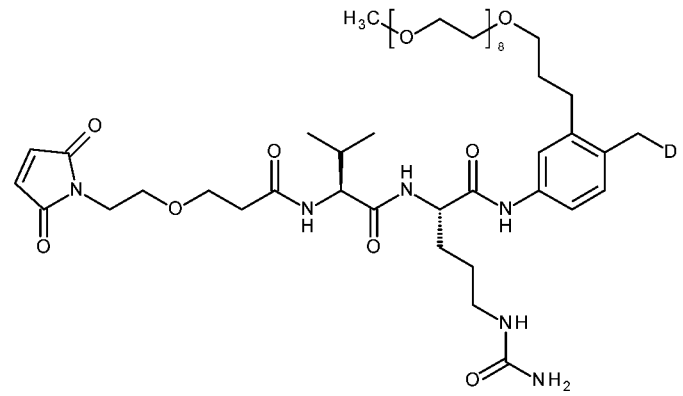

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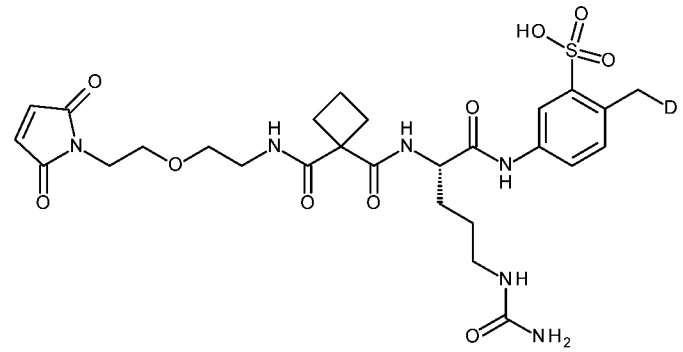

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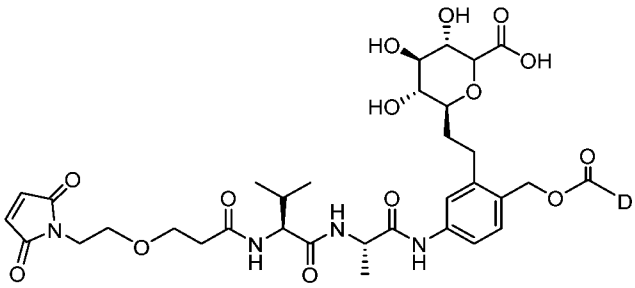

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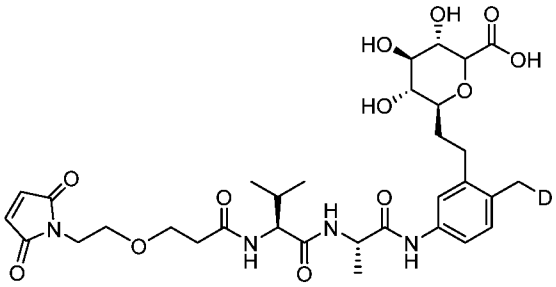

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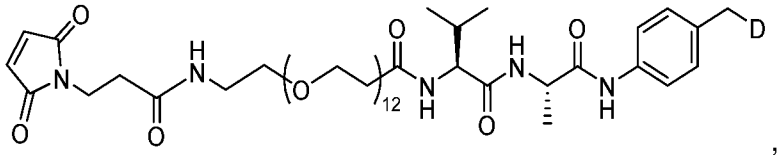

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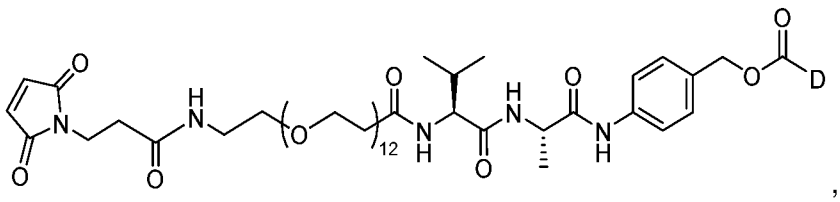

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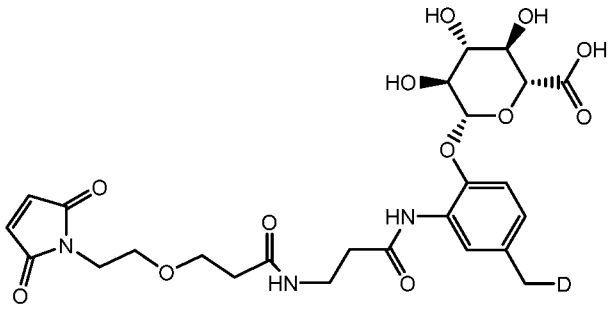

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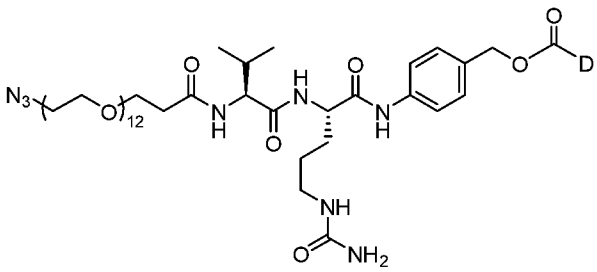

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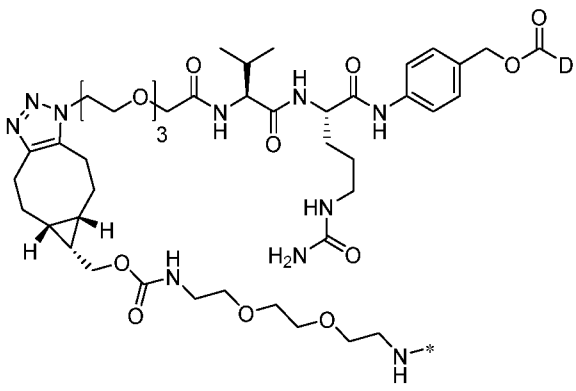
22. The antibody-drug conjugate of any one of claims 13-21, wherein -(L-D) comprises a formula selected from:



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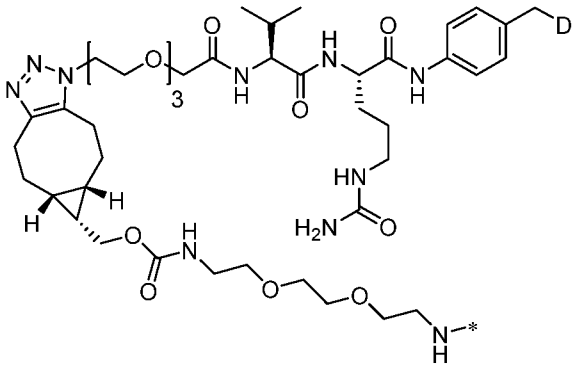

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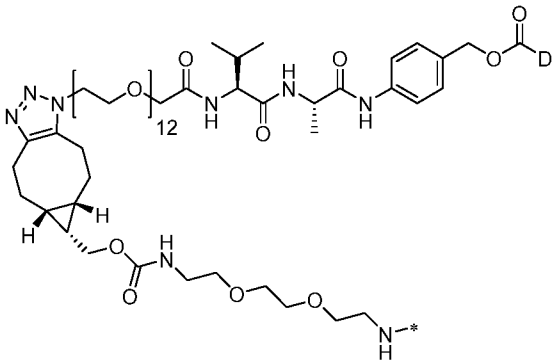

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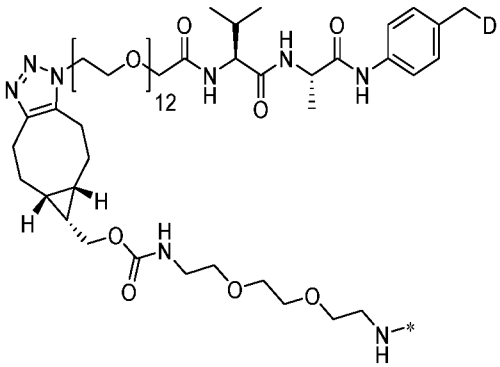

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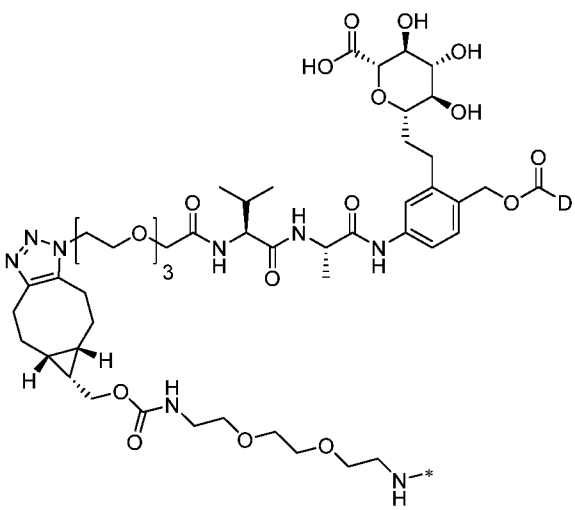

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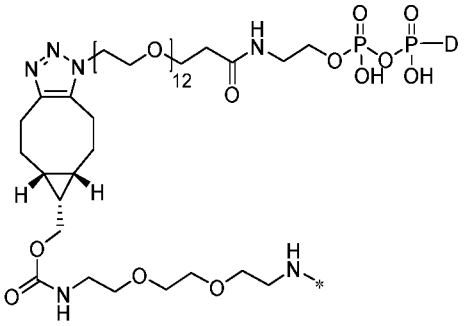

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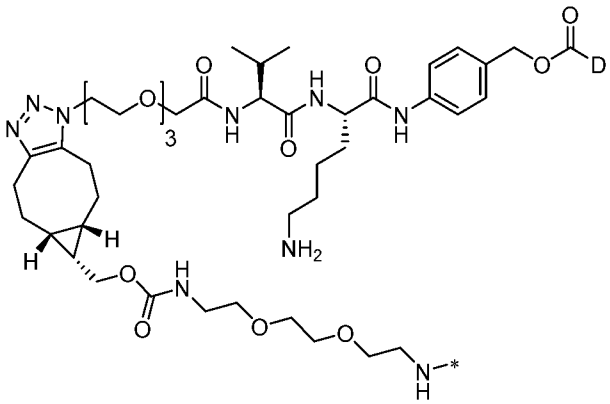

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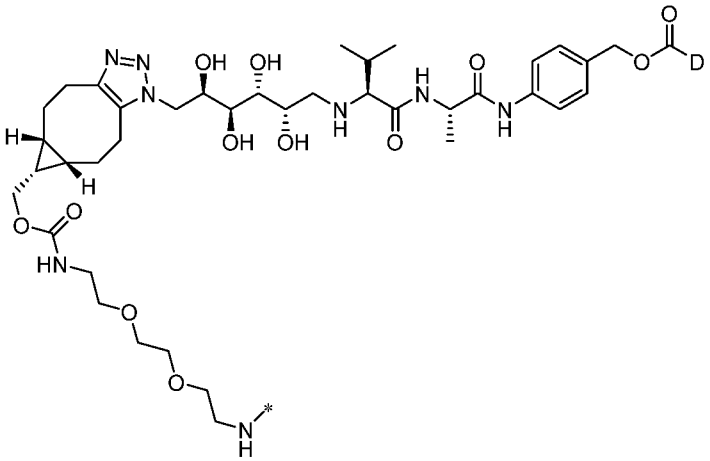

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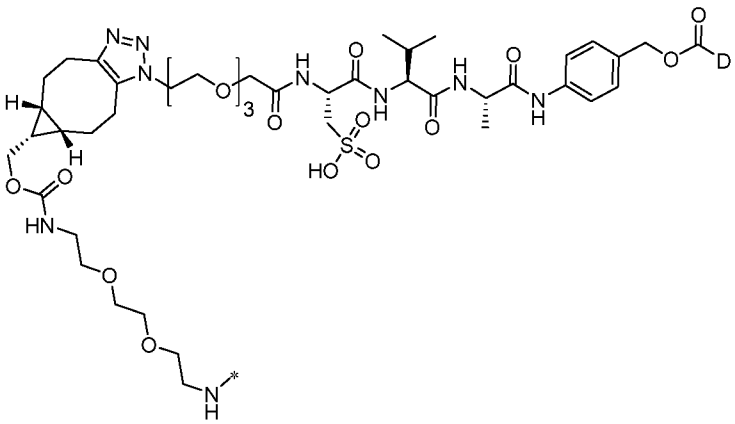

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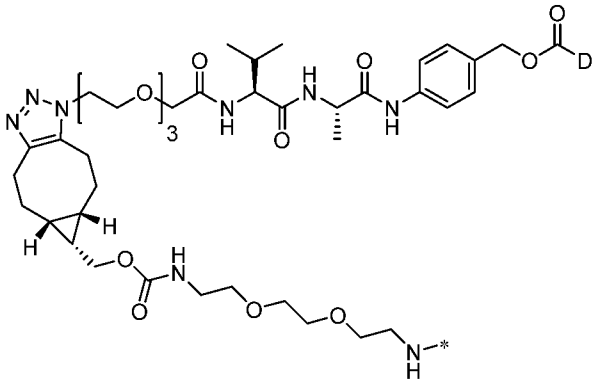

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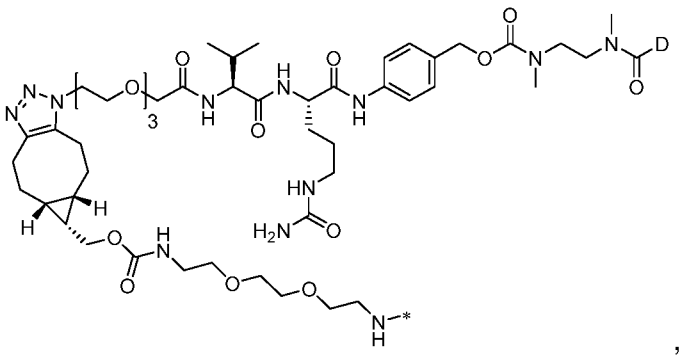

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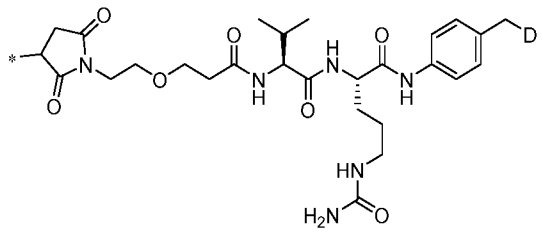

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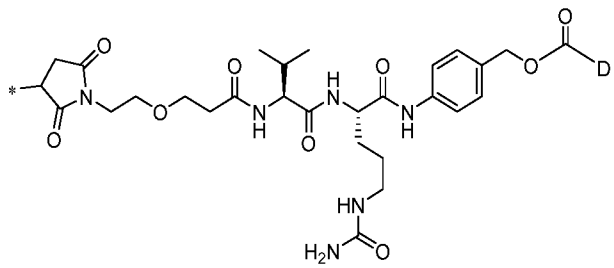

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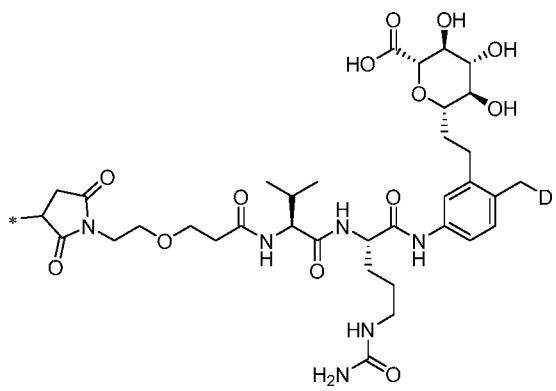

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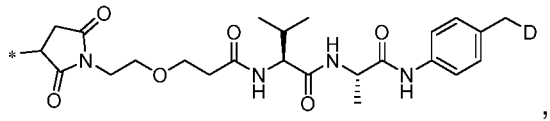

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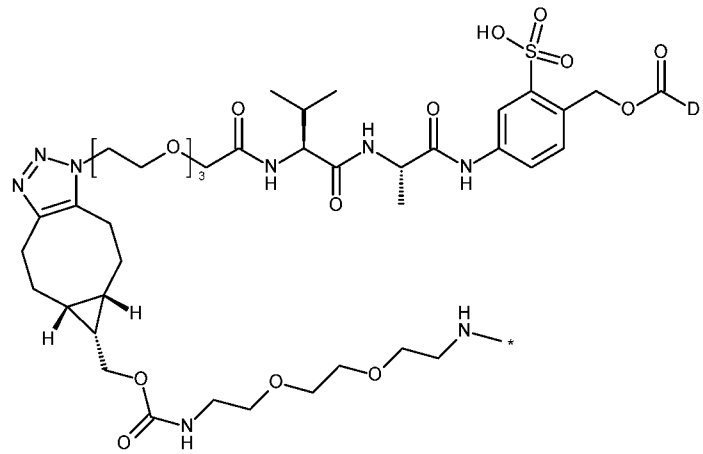

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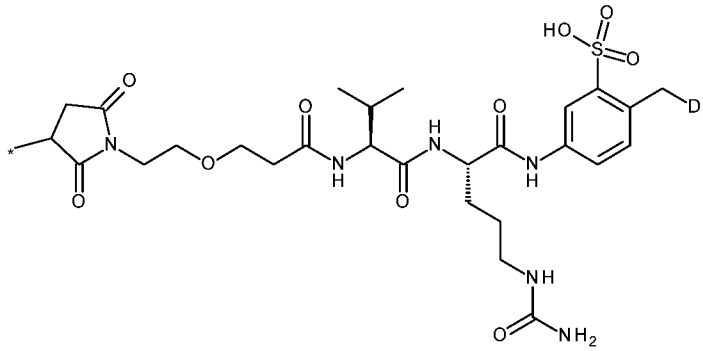

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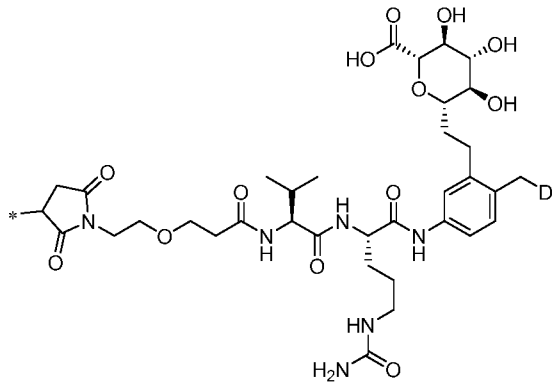

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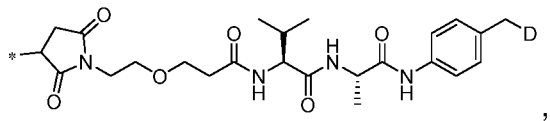

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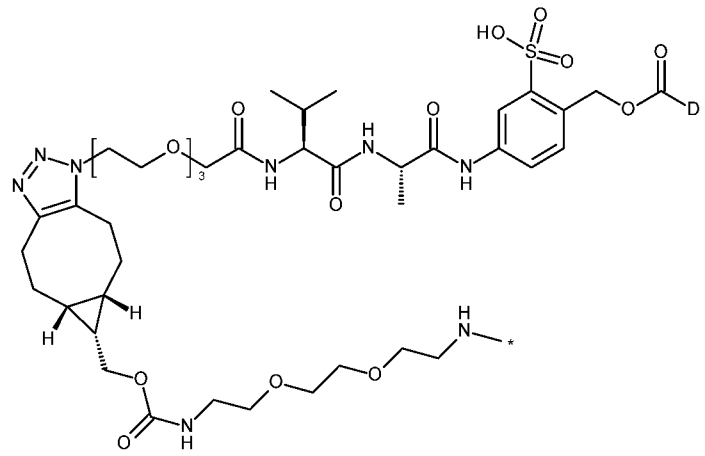

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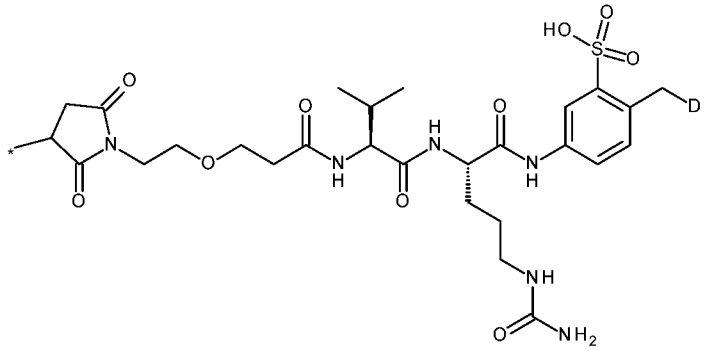

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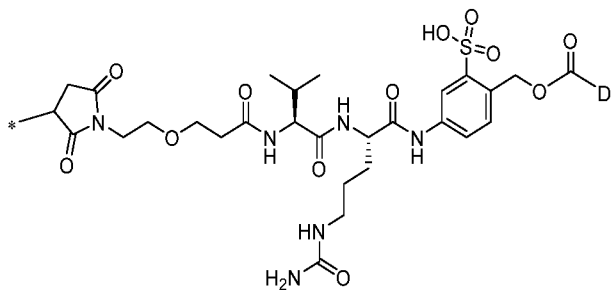

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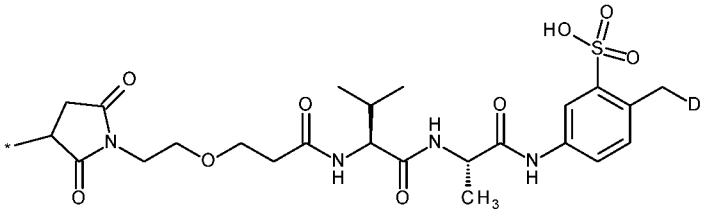

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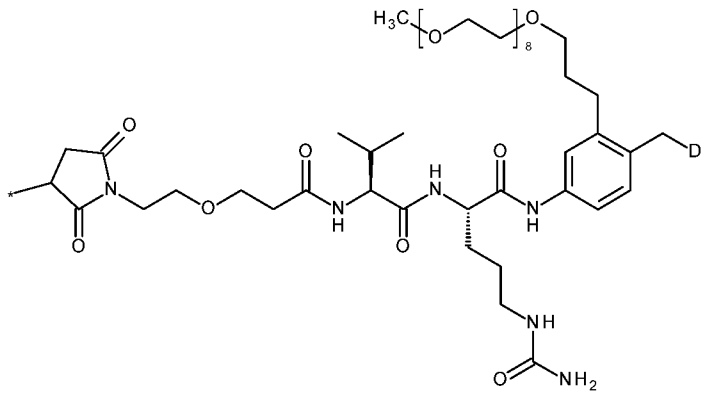

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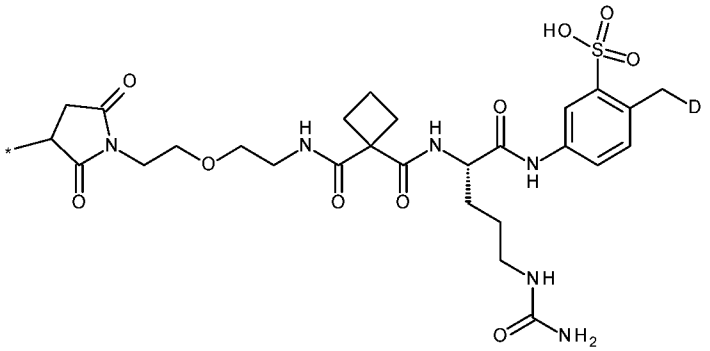

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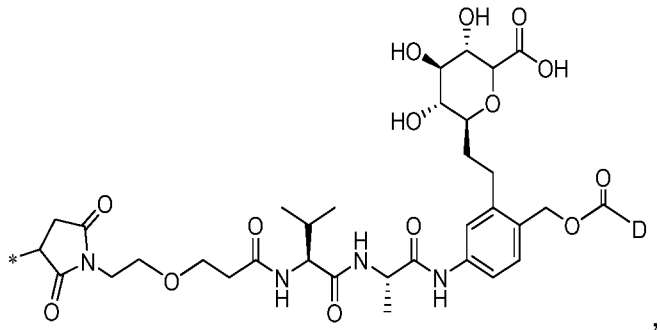

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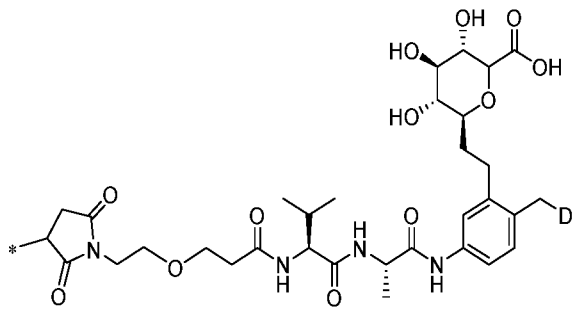

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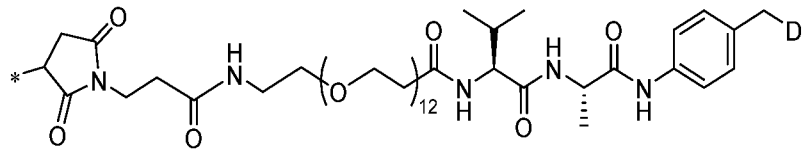

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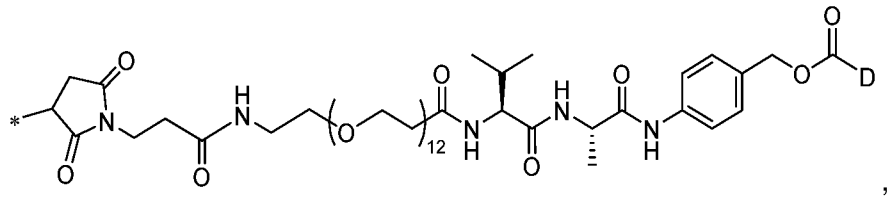
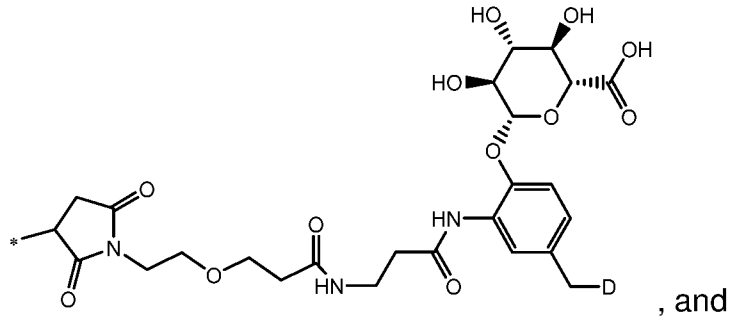
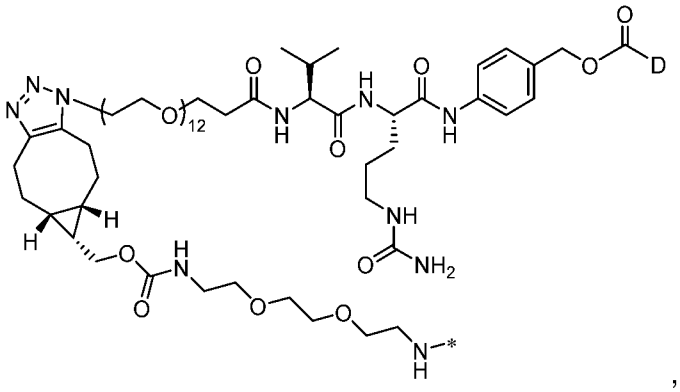


,



, and

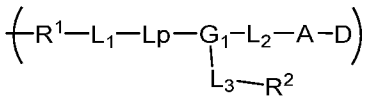


,

and

wherein —* is a bond to the antibody.

23. The antibody-drug conjugate of claim 1 or 2, wherein -(L-D) is of the formula (C):



(C),

wherein:

R^1 is an attachment group;

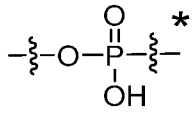
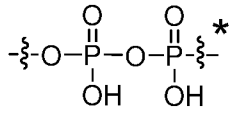
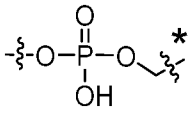
L_1 is a bridging spacer;

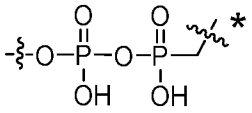
L_p is a peptide group comprising 1 to 6 amino acids;

D is a Bcl-xL inhibitor;

G_1-L_2-A is a self-immolative spacer;

L_2 is a bond, a methylene, a neopentylene or a C_2-C_3 alkenylene;

A is a bond, -OC(=O)-*,  ,  ,  ,

 , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or

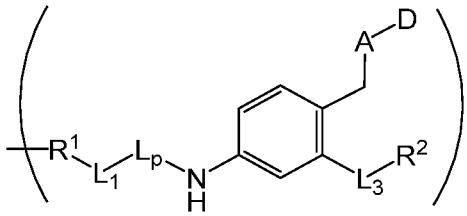
-OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*,

wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety; and

R^2 is a hydrophilic moiety.

24. The antibody-drug conjugate of claim 23, or pharmaceutically acceptable salt thereof, wherein -(L-D) is of Formula (D):

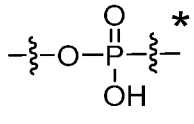
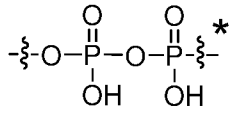
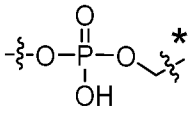
 (D)

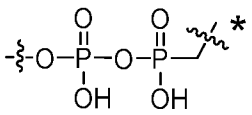
wherein:

R^1 is an attachment group;

L$_1$ is a bridging spacer;

Lp is a peptide group comprising 1 to 6 amino acids;

A is a bond, -OC(=O)-*,  ,  ,  ,

 , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or

-OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*,

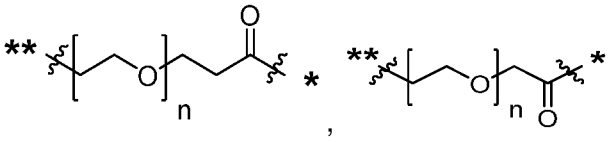
wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

L$_3$ is a spacer moiety; and

R^2 is a hydrophilic moiety.

25. The antibody-drug conjugate of claim 23 or 24, wherein:

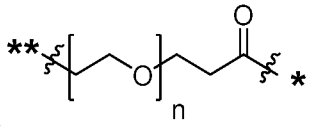
(1) L_1 comprises:

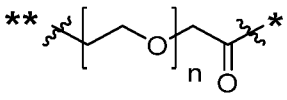


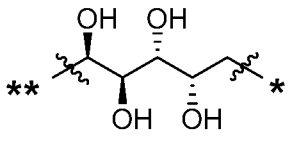
, or

*-CH(OH)CH(OH)CH(OH)CH(OH)-**,

wherein each n is an integer from 1 to 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1;

(2) L_1 is  , and n is an integer from 1 to 12 or n is 1 or n is 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1;

(3) L_1 is  , and n is an integer from 1 to 12, wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1;

(4) L_1 comprises  , wherein the * of L_1 indicates the point of direct or indirect attachment to Lp, and the ** of L_1 indicates the point of direct or indirect attachment to R^1;

(5) L_1 is a bridging spacer comprising:

*-C(=O)$(CH_2)_m$O$(CH_2)_m$-**; *-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$-**;
*-C(=O)NH(($(CH_2)_m$O)$_t$$(CH_2)_n$-**;
*-C(=O)O$(CH_2)_m$SSC(R^3)$_2$$(CH_2)_m$C(=O)N$R^3$$(CH_2)_mNR^3$C(=O)$(CH_2)_m$-**;
*-C(=O)O$(CH_2)_m$C(=O)NH$(CH_2)_m$-**; *-C(=O)$(CH_2)_m$NH$(CH_2)_m$-**;
*-C(=O)$(CH_2)_m$NH$(CH_2)_n$C(=O)-**; *-C(=O)$(CH_2)_m$$X_1$$(CH_2)_m$-**;
*-C(=O)(($(CH_2)_m$O)$_t$$(CH_2)_n$$X_1$$(CH_2)_n$-**; *-C(=O)$(CH_2)_m$NHC(=O)$(CH_2)_n$-**;

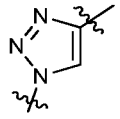
*-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$NHC(=O)(CH$_2$)$_n$-**;

*-C(=O)(CH$_2$)$_m$NHC(=O)(CH$_2$)$_n$X$_1$(CH$_2$)$_n$-**;

*-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$NHC(=O)(CH$_2$)$_n$X$_1$(CH$_2$)$_n$-**;

*-C(=O)((CH$_2$)$_m$O)$_t$(CH$_2$)$_n$C(=O)NH(CH$_2$)$_m$-**; *-C(=O)(CH$_2$)$_m$C(R^3)$_2$-** or

*-C(=O)(CH$_2$)$_m$C(=O)NH(CH$_2$)$_m$-**, wherein the * of L$_1$ indicates the point of direct or indirect attachment to Lp, and the ** of L$_1$ indicates the point of direct or indirect attachment to R^1;

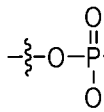
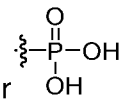
X$_1$ is  ; and

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

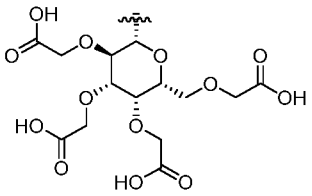
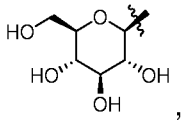
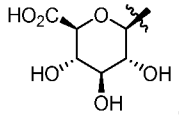
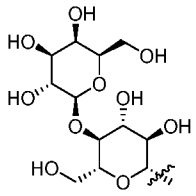
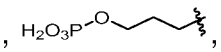
each n is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and

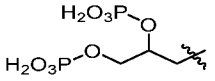
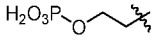
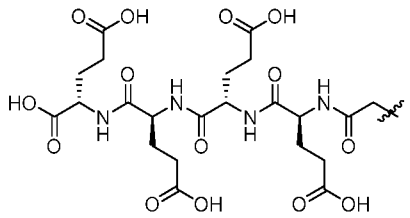
each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30;

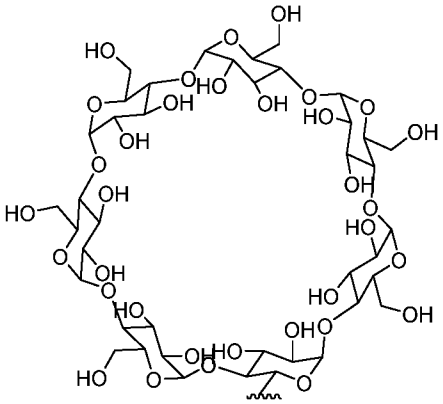
and each R^3 is independently selected from H and C$_1$-C$_6$alkyl.

26. The antibody-drug conjugate of any one of claims 23 to 25, wherein R^2 is a hydrophilic moiety comprising polyethylene glycol, polyalkylene glycol, a polyol, a polysarcosine, a sugar, an oligosaccharide, a polypeptide, C$_2$-C$_6$ alkyl substituted with 1 to 3  or  , or C$_2$-C$_6$alkyl substituted with 1 to 2 substituents independently selected from -OC(=O)NHS(O)$_2$NHCH$_2$CH$_2$OCH$_3$, -NHC(=O)C$_{1-4}$alkylene-P(O)(OCH$_2$CH$_3$)$_2$ and -COOH groups.

27. The antibody-drug conjugate of any one of claims 23 to 26, wherein R^2 is

 ,  ,  ,  ,  ,

 ,  ,  ,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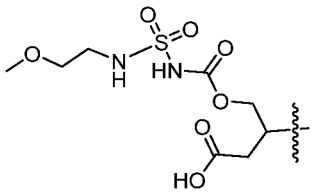
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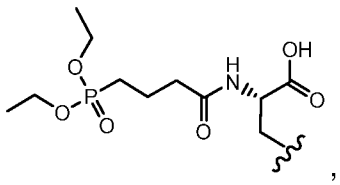
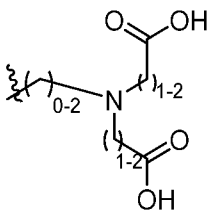

,

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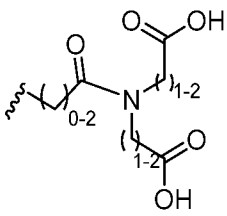
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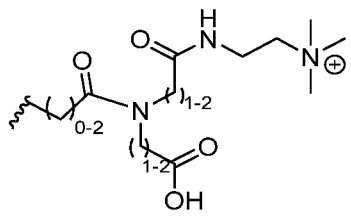
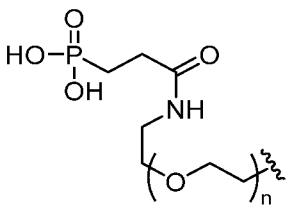
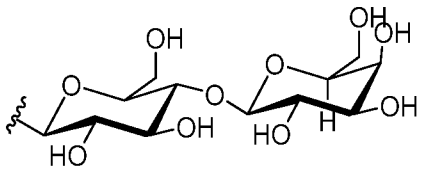
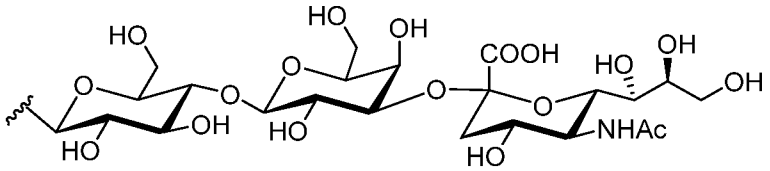
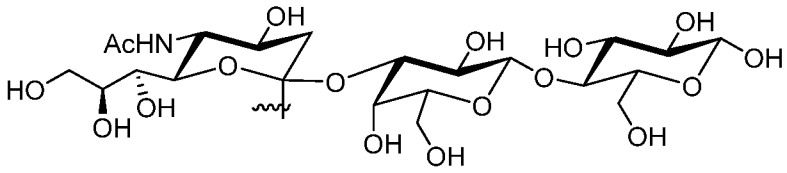

,

wherein n is an integer between 1 and 6,

,

, or

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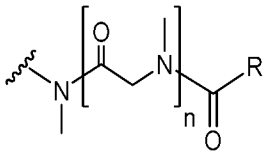
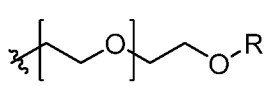
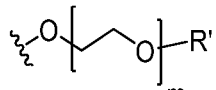
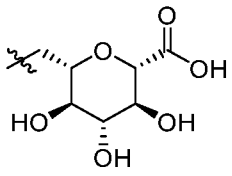
28. The antibody-drug conjugate of claim 23 or 24, wherein the hydrophilic moiety comprises:

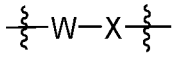
(i) a polysarcosine with the following moiety:


, wherein n is an integer between 3 and 25; and R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$; or

(ii) a polyethylene glycol of formula:  or  ,wherein R is H, -CH_3, $CH_2CH_2NHC(=O)OR_a$, $-CH_2CH_2NHC(=O)R_a$, or $-CH_2CH_2C(=O)OR_a$, R' is OH, -OCH_3, $-CH_2CH_2NHC(=O)OR_a$, $-CH_2CH_2NHC(=O)R_a$, or $-OCH_2CH_2C(=O)OR_a$, in which R_a is H or C_{1-4} alkyl optionally substiltuted with either OH or C_{1-4} alkoxyl, and each of m and n is independently an integer between 2 and 25.

29. The antibody-drug conjugate of any one of claims 23 to 27, wherein the hydrophilic moiety comprises  .

30. The antibody-drug conjugate of any one of claims 23 to 29, wherein:

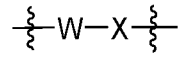
(i) L_3 is a spacer moiety having the structure  ,

wherein:

W is -CH_2-, -CH_2O-, $-CH_2N(R^b)C(=O)O$-, $-NHC(=O)C(R^b)_2NHC(=O)O$-, $-NHC(=O)C(R^b)_2NH$-, $-NHC(=O)C(R^b)_2NHC(=O)$-, $-CH_2N(X\text{-}R^2)C(=O)O$-, $-C(=O)N(X\text{-}R^2)$-, $-CH_2N(X\text{-}R^2)C(=O)$-, $-C(=O)NR^b$-, -C(=O)NH-, $-CH_2N\ R^b\ C(=O)$-, $-CH_2NR^b\ C(=O)NH$-, $-CH_2NR^bC(=O)NR^b$-, -NHC(=O)-, -NHC(=O)O-, -NHC(=O)NH-, -OC(=O)NH-, $-S(O)_2NH$-, $-NHS(O)_2$-, -C(=O)-, -C(=O)O- or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl, and C_3-C_8 cycloalkyl; and

X is a bond, triazolyl, or -CH_2-triazolyl-,

wherein X is connected to R^2; or

(ii) L_3 is a spacer moiety having the structure  ,

wherein:

W is -CH_2-, -CH_2O-, $-CH_2N(R^b)C(=O)O$-, $-NHC(=O)C(R^b)_2NHC(=O)O$-, $-NHC(=O)C(R^b)_2NH$-, $-NHC(=O)C(R^b)_2NHC(=O)$-, $-CH_2N(X\text{-}R^2)C(=O)O$-, $-C(=O)N(X\text{-}R^2)$-, $-CH_2N(X\text{-}R^2)C(=O)$-, $-C(=O)NR^b$-, -C(=O)NH-, $-CH_2NR^bC(=O)$-, $-CH_2NR^bC(=O)NH$-, $-CH_2NR^bC(=O)NR^b$-, -NHC(=O)-, -NHC(=O)O-, -NHC(=O)NH-, -OC(=O)NH-,

-$S(O)_2NH$-, -$NHS(O)_2$-, -C(=O)-, -C(=O)O- or -NH-, wherein each R^b is independently selected from H, C_1-C_6alkyl, and C_3-C_8 cycloalkyl; and

X is -CH_2-triazolyl-C_{1-4} alkylene-$OC(O)NHS(O)_2NH$-, -C_{4-6} cycloalkylene-$OC(O)NHS(O)_2NH$-, -$(CH_2CH_2O)_n$-$C(O)NHS(O)_2NH$-, -$(CH_2CH_2O)_n$-$C(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-, -CH_2-triazolyl-C_{1-4} alkylene-$OC(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-, -C_{4-6}cycloalkylene-$OC(O)NHS(O)_2NH$-$(CH_2CH_2O)_n$-,wherein each n independently is 1, 2, or 3,

wherein X is connected to R^2.

31. The antibody-drug conjugate of any one of claims 3 to 30, wherein the attachment group is formed by a reaction comprising at least one reactive group.

32. The antibody-drug conjugate of any one of claims 3 to 31, wherein the attachment group is formed by reacting:

a first reactive group that is attached to the linker, and

a second reactive group that is attached to the antibody or is an amino acid residue of the antibody, wherein optionally,

(i) at least one of the reactive groups comprises:

a thiol,

a maleimide,

a haloacetamide,

an azide,

an alkyne,

a cyclcooctene,

a triaryl phosphine,

an oxanobornadiene,

a cyclooctyne,

a diaryl tetrazine,

a monoaryl tetrazine,

a norbornene,

an aldehyde,

a hydroxylamine,

a hydrazine,

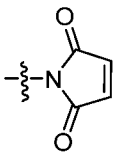
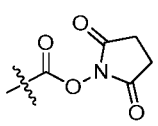
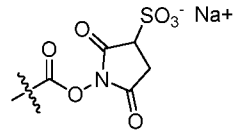
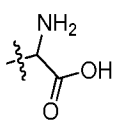
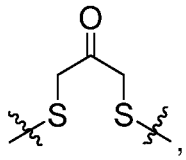
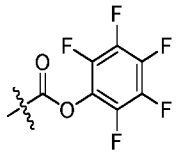
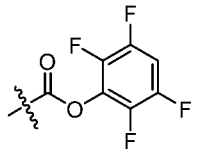
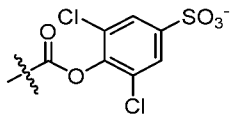
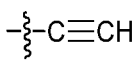
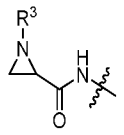
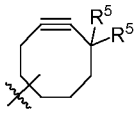
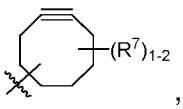
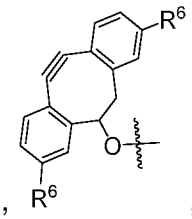
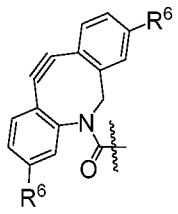
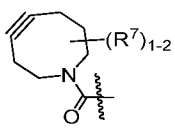
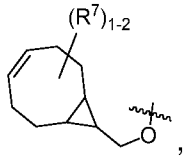
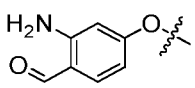
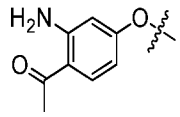
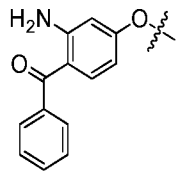
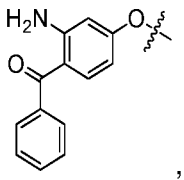
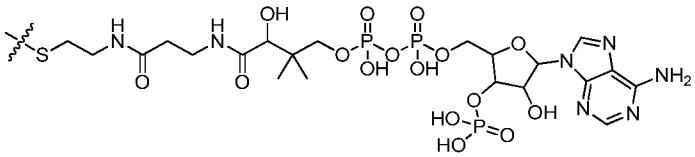
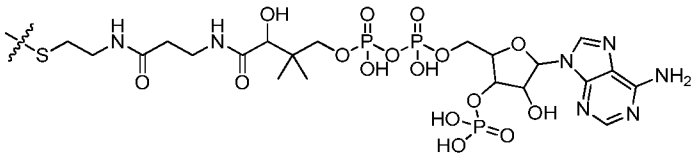
NH_2-NH-C(=O)-,

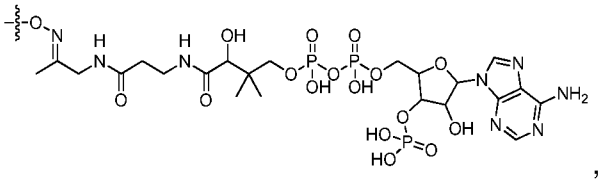
a ketone,

a vinyl sulfone,

an aziridine,

an amino acid residue,

 , -ONH_2, -NH_2,  ,  ,  ,  ,  ,  ,  , -N_3,  , -SH, -SR^3, -SSR^4, -$S(=O)_2(CH=CH_2)$, -$(CH_2)_2S(=O)_2(CH=CH_2)$, -$NHS(=O)_2(CH=CH_2)$, -$NHC(=O)CH_2Br$, -$NHC(=O)CH_2I$,  , -$C(O)NHNH_2$,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,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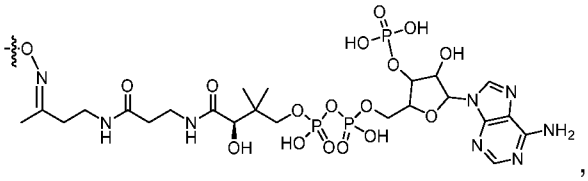
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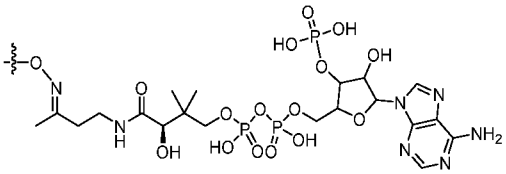
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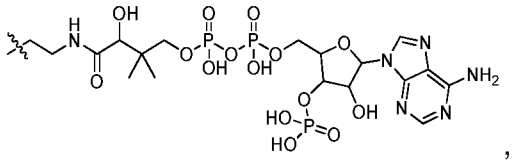
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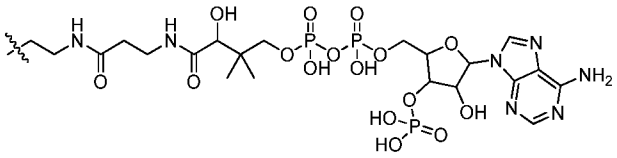
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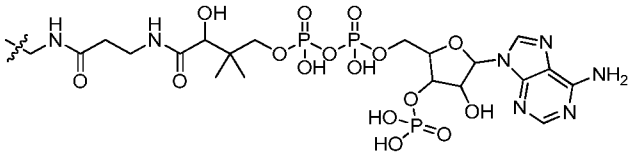

;

wherein:

each R^3 is independently selected from H and C_1-C_6alkyl;

each R^4 is 2-pyridyl or 4-pyridyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and –OH;

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH; and/or

(ii) the first reactive group and second reactive group comprise:

a thiol and a maleimide,

a thiol and a haloacetamide,

a thiol and a vinyl sulfone,

a thiol and an aziridine,

an azide and an alkyne,

an azide and a cyclooctyne,

an azide and a cyclooctene,

an azide and a triaryl phosphine,

an azide and an oxanobornadiene,

a diaryl tetrazine and a cyclooctene,

a monoaryl tetrazine and a nonbornene,

an aldehyde and a hydroxylamine,

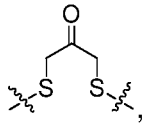
an aldehyde and a hydrazine,

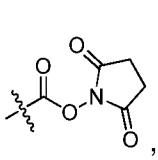
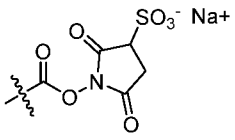
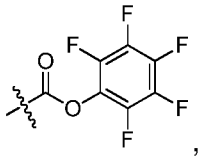
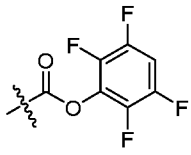
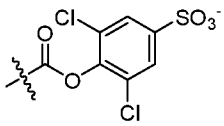
an aldehyde and NH_2-NH-C(=O)-,

a ketone and a hydroxylamine,

a ketone and a hydrazine,

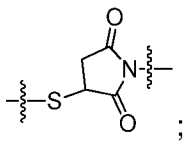
a ketone and NH_2-NH-C(=O)-,

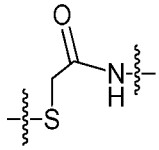
a hydroxylamine and  ,

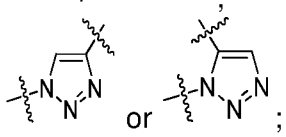
an amine and  ,  ,  ,  , or  , or

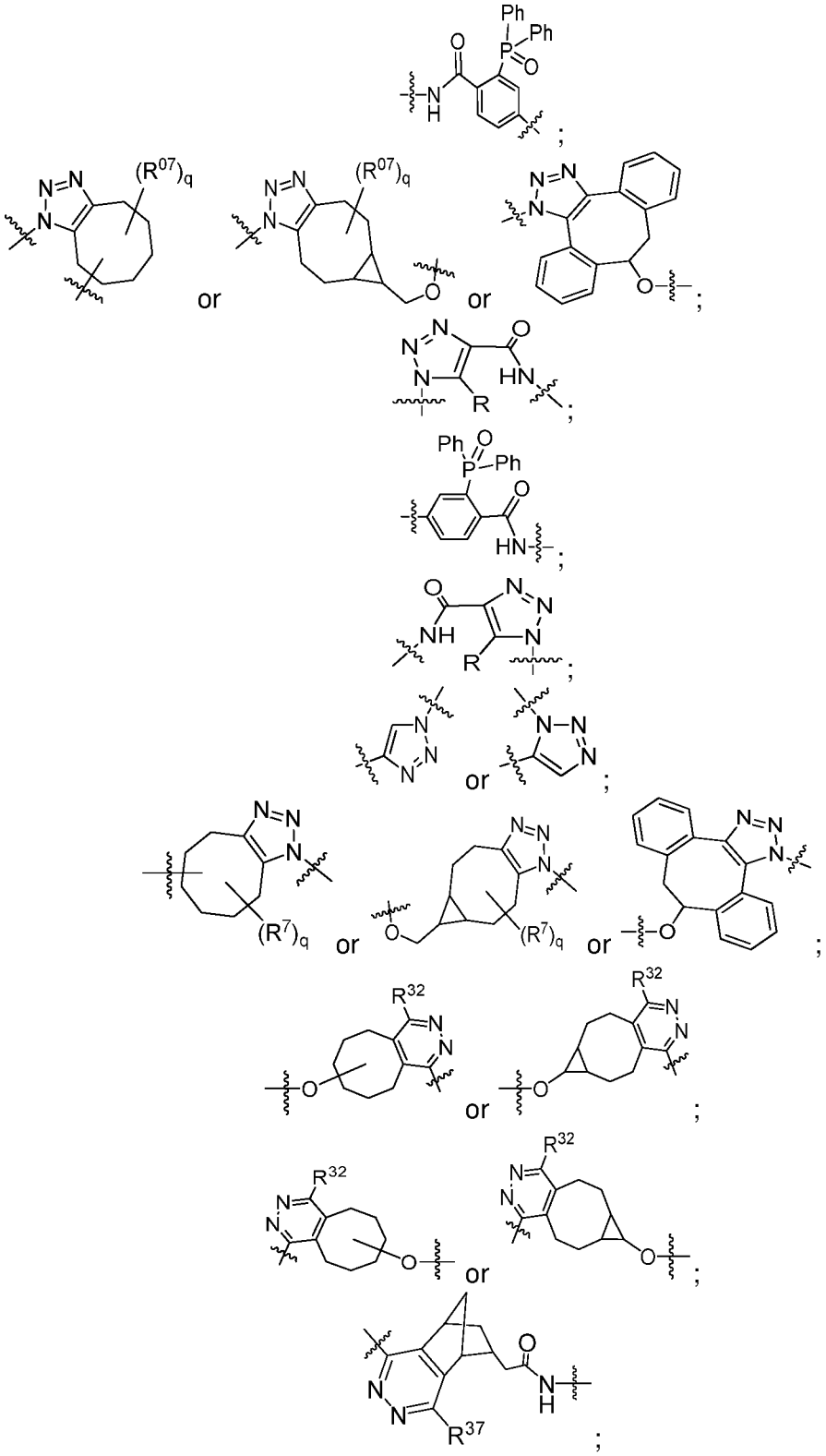
a CoA or CoA analogue and a serine residue.

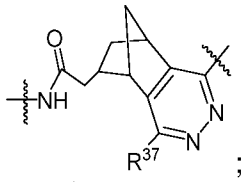
33. The antibody-drug conjugate any one of claims 3 to 32, where the attachment group comprises a group selected from:

 ;

 ;

 ;

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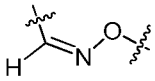
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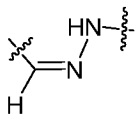
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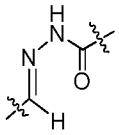
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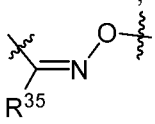
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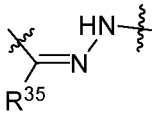
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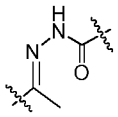
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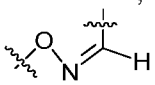
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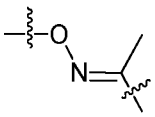
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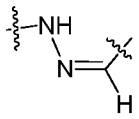
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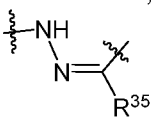
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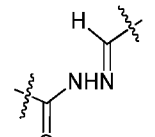
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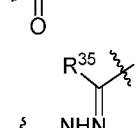
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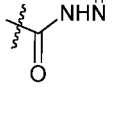
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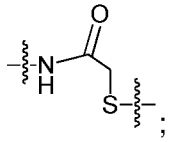
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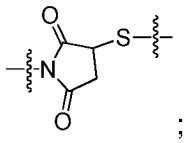
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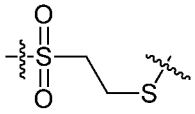
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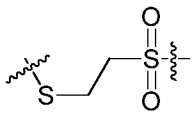
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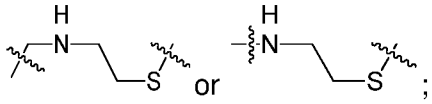
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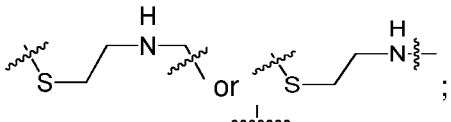
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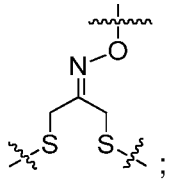
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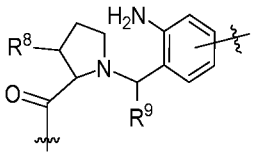
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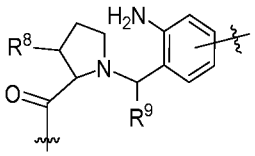
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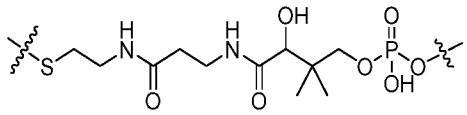
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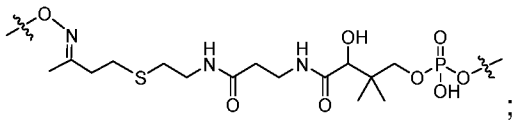
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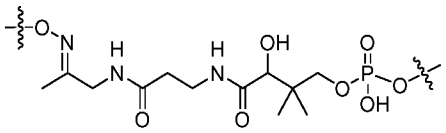
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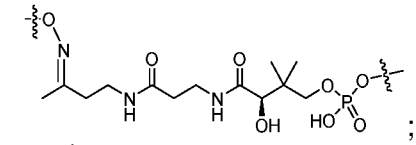
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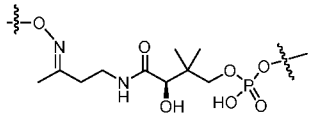
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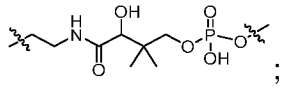
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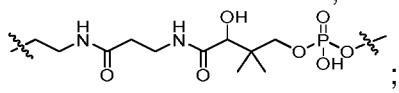
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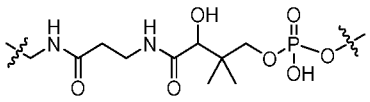

; and

disulfide,

wherein:

R^{32} is H, C_{1-4} alkyl, phenyl, pyrimidine or pyridine;

R^{35} is H, C_{1-6} alkyl, phenyl or C_{1-4} alkyl substituted with 1 to 3 –OH groups;

each R^7 is independently selected from H, C_{1-6} alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4} alkoxy substituted with –C(=O)OH and C_{1-4} alkyl substituted with –C(=O)OH;

R^{37} is independently selected from H, phenyl and pyridine;

q is 0, 1, 2 or 3;

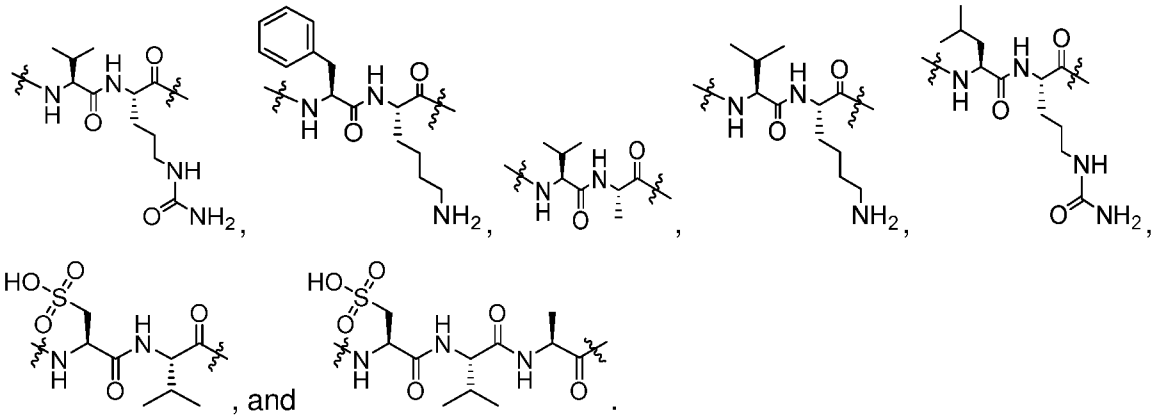
R^8 is H or methyl; and

R^9 is H, $-CH_3$ or phenyl.

34. The antibody-drug conjugate any one of claims 23 to 33, wherein the peptide group comprises 1 to 4 or 1 to 3 or 1 or 2 amino acid residues, optionally the amino acid residues are selected from L-glycine (Gly), L-valine (Val), L-citrulline (Cit), L-cysteic acid (sulfo-Ala), L-lysine (Lys), L-isoleucine (Ile), L-phenylalanine (Phe), L-methionine (Met), L-asparagine (Asn), L-proline (Pro), L-alanine (Ala), L-leucine (Leu), L-tryptophan (Trp), and L-tyrosine (Tyr) .

35. The antibody-drug conjugate any one of claims 23 to 33, wherein the peptide group comprises Val-Cit, Phe-Lys, Val-Ala, Val-Lys, Leu-Cit, sulfo-Ala-Val, and/or sulfo-Ala-Val-Ala.

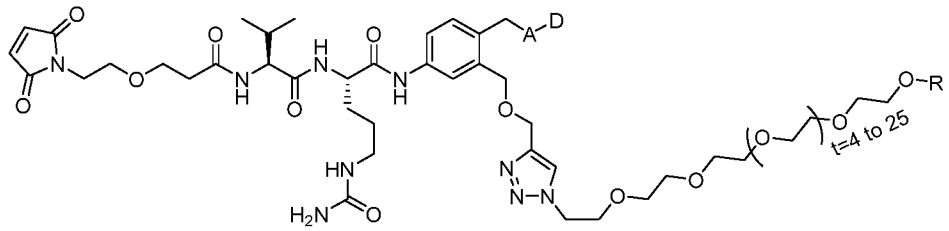
36. The antibody-drug conjugate any one of claims 23 to 35, wherein Lp is selected from:



, and .

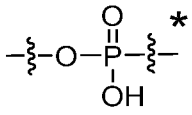
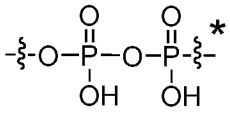
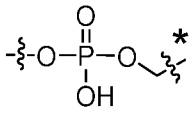
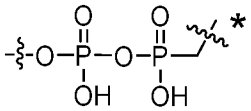
37. The antibody-drug conjugate of any one of claims 23 to 36, wherein:

-(L-D) comprises or is formed from a compound of formula:



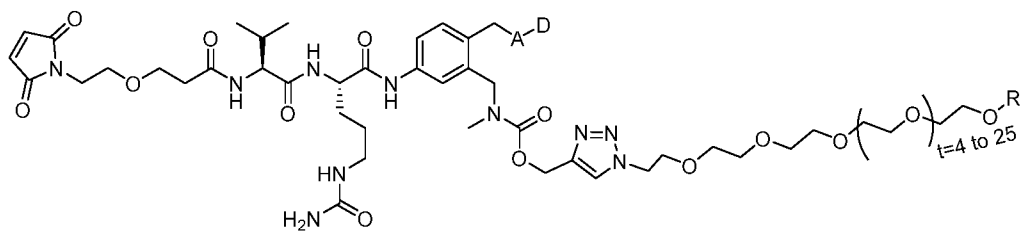
(1) , wherein:

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  ,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*,

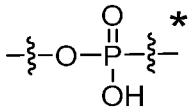
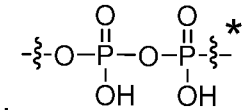
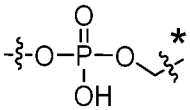
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

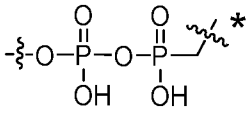
D is a Bcl-xL inhibitor;



(2) , wherein:

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

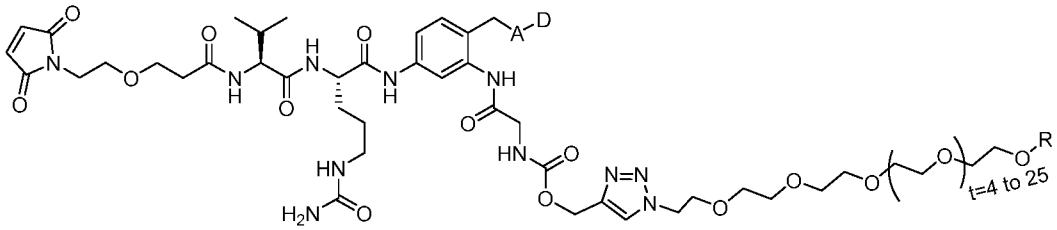
A is a bond, -OC(=O)-*,  ,  ,  ,

 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

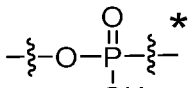
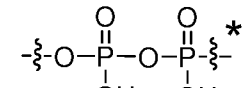
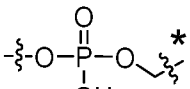
-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

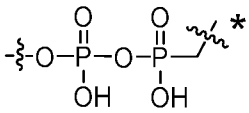
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(3)  , wherein:

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

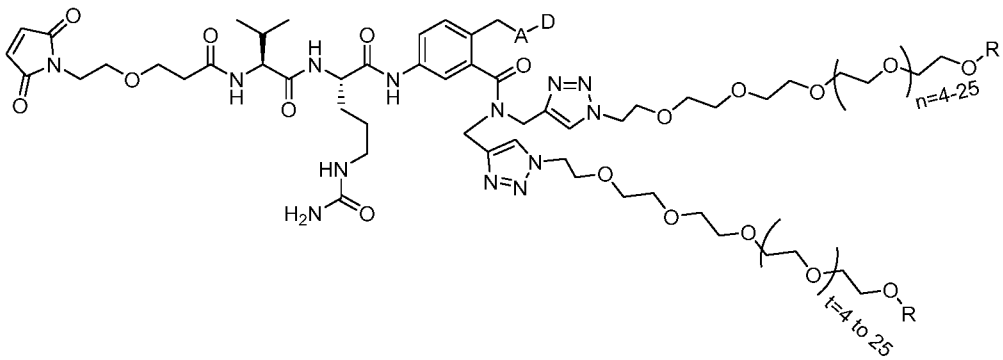
A is a bond, -OC(=O)-*,  ,  ,  ,

 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

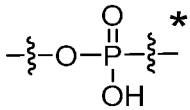
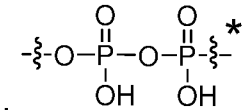
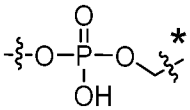
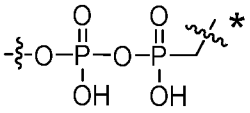
-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

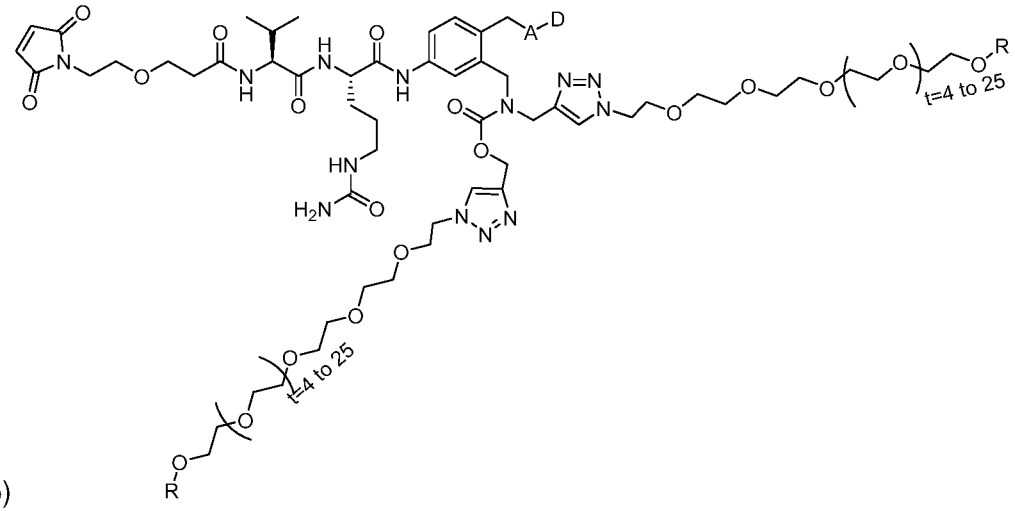
(4)  , wherein:

each R is independently selected from H, $-CH_3$, and $-CH_2CH_2C(=O)OH$;

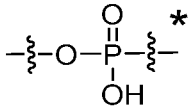
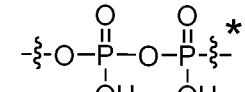
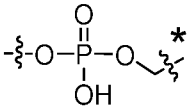
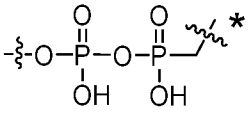
A is a bond, -OC(=O)-*,  ,  ,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

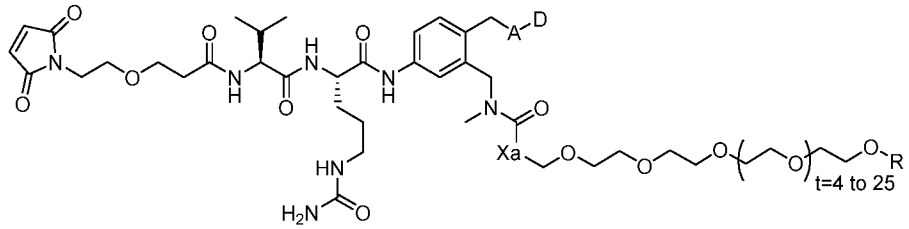
(5)  , wherein:

each R is independently selected from H, $-CH_3$, and $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  ,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$,

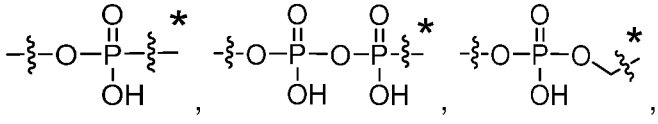
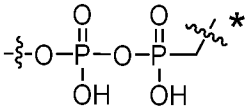
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(6) 

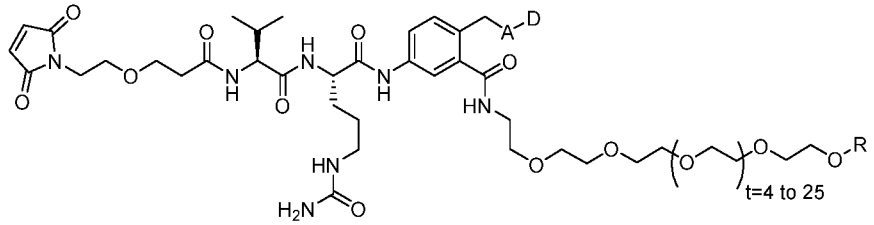
, wherein:

Xa is $–CH_2-$, $-OCH_2-$, $-NHCH_2-$ or $–NRCH_2-$ and each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, $-OC(=O)-*$,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$,

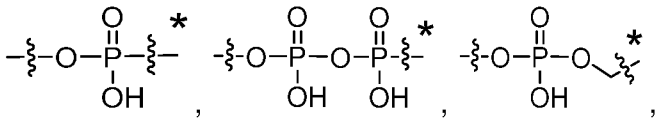
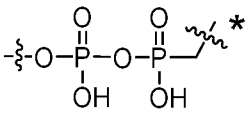
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(7) 

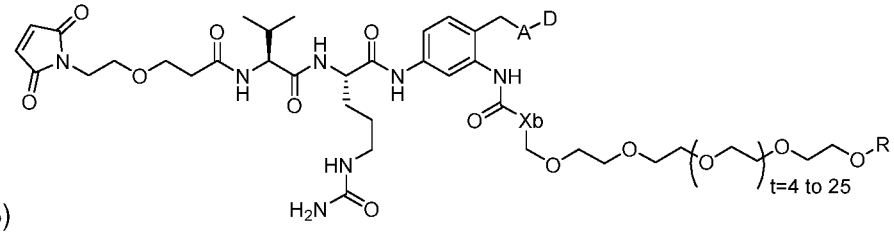
, wherein:

R is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

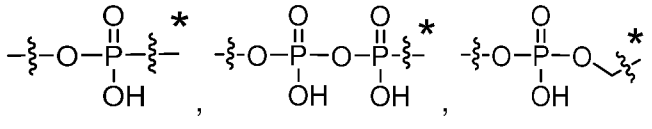
A is a bond, $-OC(=O)-*$,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-*$ or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)-*$,

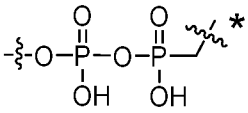
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(8)  , wherein:

Xb is -CH_2-, -OCH_2-, -$NHCH_2$- or –$NRCH_2$- and each R independently is H, -CH_3 or -$CH_2CH_2C(=O)OH$;

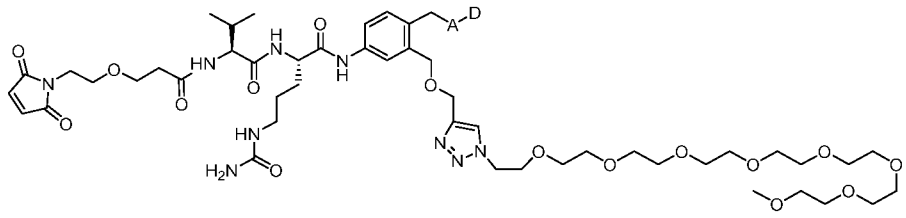
A is a bond, -OC(=O)-*,  ,

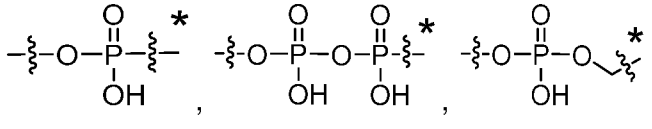
 , -$OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or

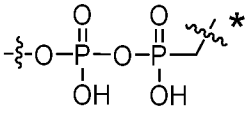
-$OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(9)  , wherein:

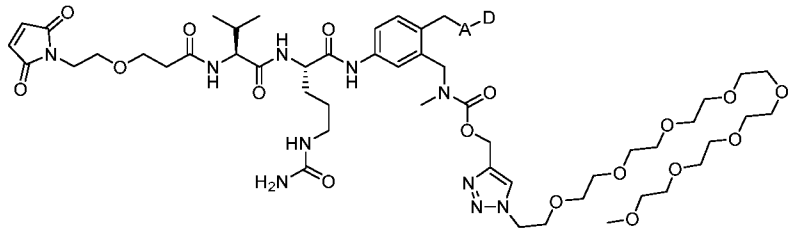
A is a bond, -OC(=O)-*,  ,

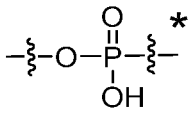
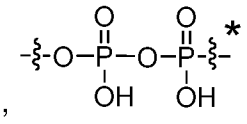
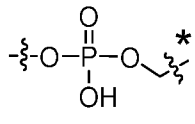
 , -$OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or

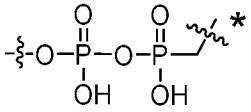
-$OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(10)  , wherein:

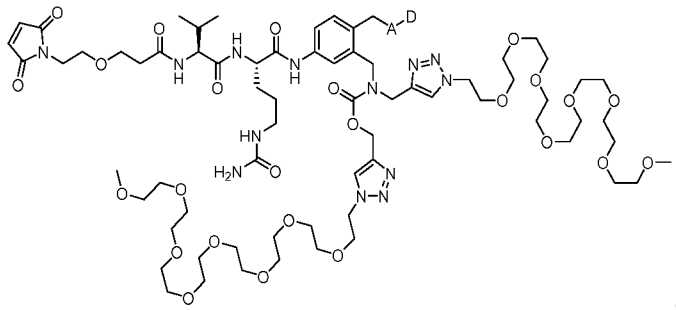
A is a bond, -OC(=O)-*,  ,  ,  ,

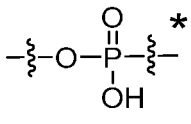
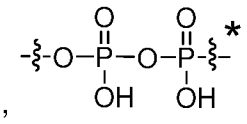
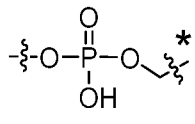
 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

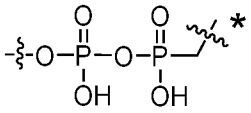
-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(11)  , wherein:

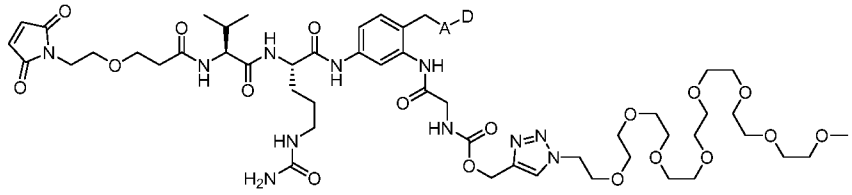
A is a bond, -OC(=O)-*,  ,  ,  ,

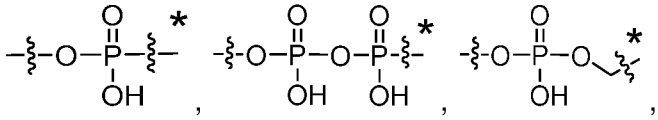
 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

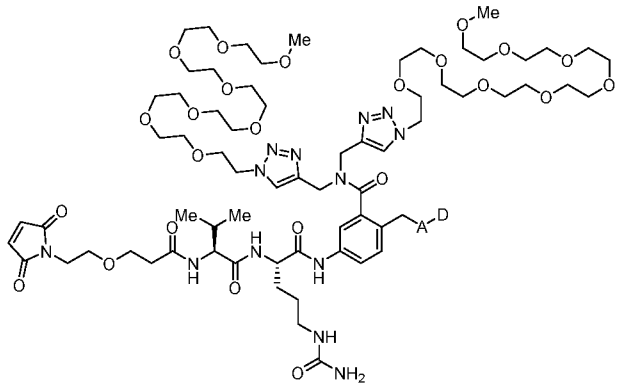
D is a Bcl-xL inhibitor;

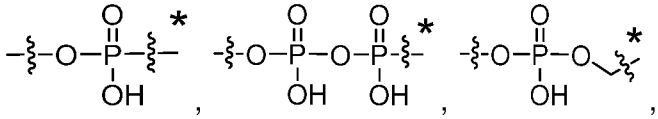
(12)  , wherein:

A is a bond, -OC(=O)-*,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

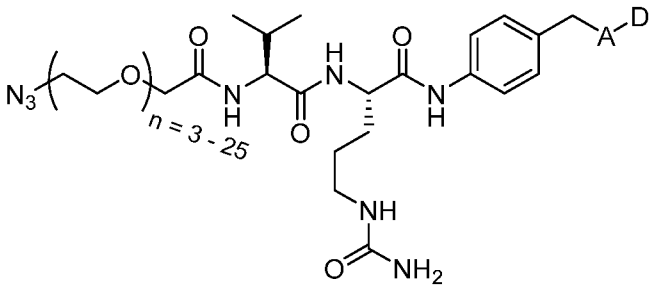
D is a Bcl-xL inhibitor;

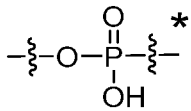
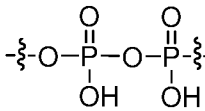
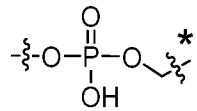
(13)  , wherein:

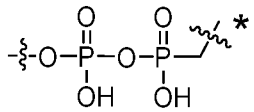
A is a bond, -OC(=O)-*,  , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or -OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(14)  , wherein:

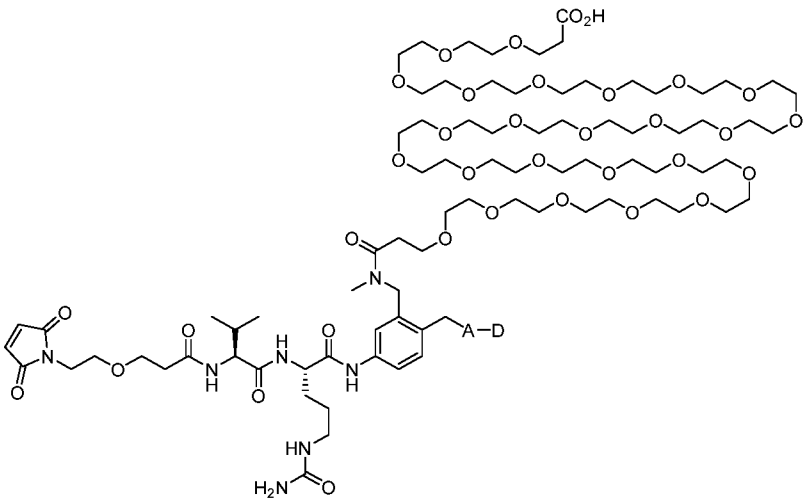
A is a bond, -OC(=O)-*,  ,  ,  ,

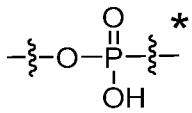
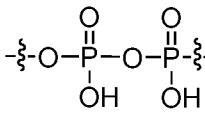
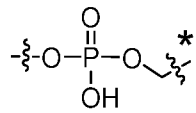
 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

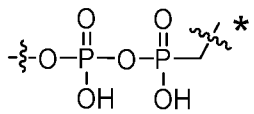
-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor;

(15)  , wherein:

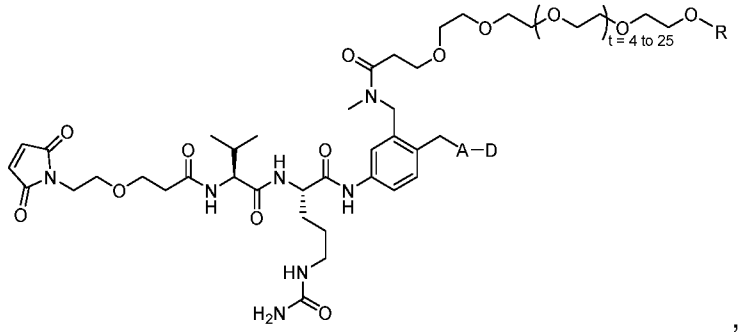
A is a bond, -OC(=O)-*,  ,  ,  ,

 , -OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)-* or

-OC(=O)N(CH_3)C(R^a)$_2$C(R^a)$_2$N(CH_3)C(=O)-*,

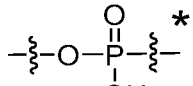
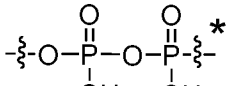
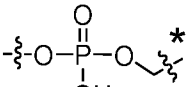
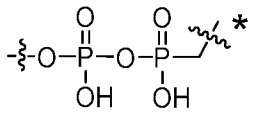
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor; or



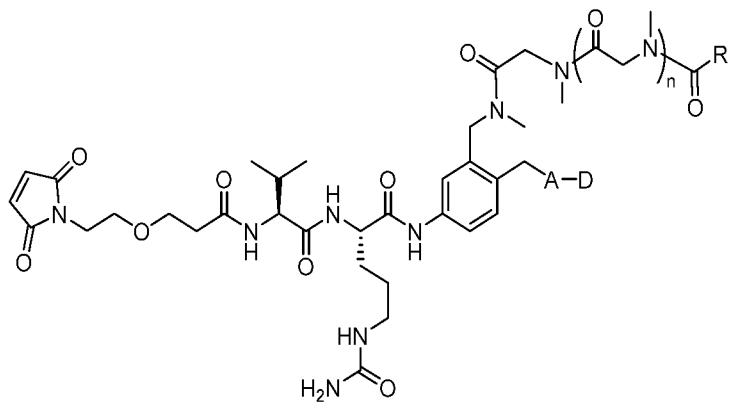
(16) , wherein:

each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

A is a bond, -OC(=O)-*,  ,  ,  ,  , $-OC(=O)N(CH_3)CH_2CH_2N(CH_3)C(=O)$-* or $-OC(=O)N(CH_3)C(R^a)_2C(R^a)_2N(CH_3)C(=O)$-*,

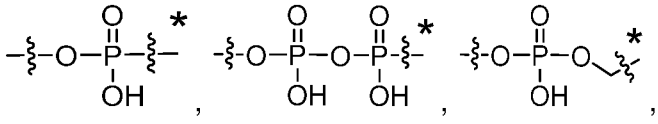
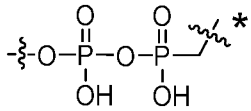
wherein each R^a is independently selected from H, C_1-C_6 alkyl, and C_3-C_8 cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor, or



(17) ,wherein:

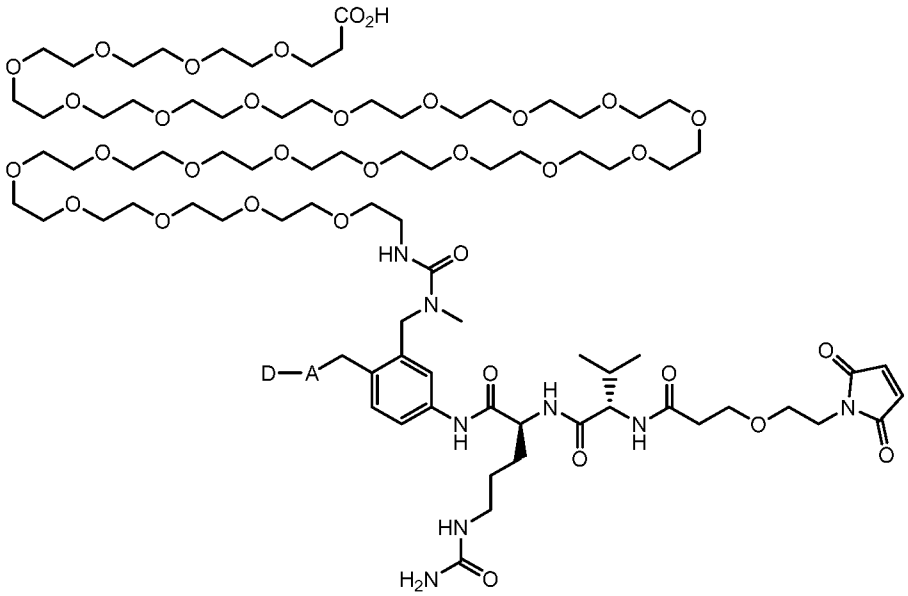
each R independently is H, $-CH_3$ or $-CH_2CH_2C(=O)OH$;

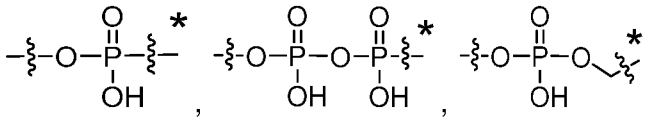
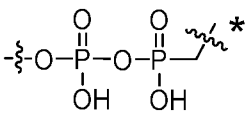
A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*,

wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D;

n is an integer between 2 and 24; and

D is a Bcl-xL inhibitor, or

(18)  , wherein:

A is a bond, -OC(=O)-*,  ,  , -OC(=O)N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)C(=O)-* or -OC(=O)N(CH$_3$)C(R^a)$_2$C(R^a)$_2$N(CH$_3$)C(=O)-*,

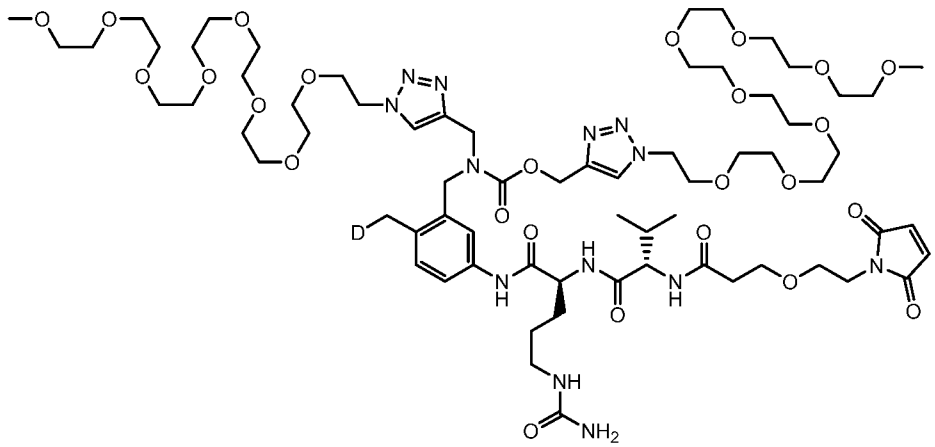
wherein each R^a is independently selected from H, C$_1$-C$_6$ alkyl, and C$_3$-C$_8$ cycloalkyl and the * of A indicates the point of attachment to D; and

D is a Bcl-xL inhibitor.

38. The antibody-drug conjugate of any one of claims 23 to 37, wherein A is a bond and/or R is $–CH_3$ or $–CH_2CH_2COOH$.

39. The antibody-drug conjugate of any one of claims 23 to 37, wherein A is -OC(=O)-* and/or R is $–CH_3$ or $–CH_2CH_2COOH$.

40. The antibod-drug conjugat of any one of claims 23-39, wherein –(L-D) is formed from a compound selected from:



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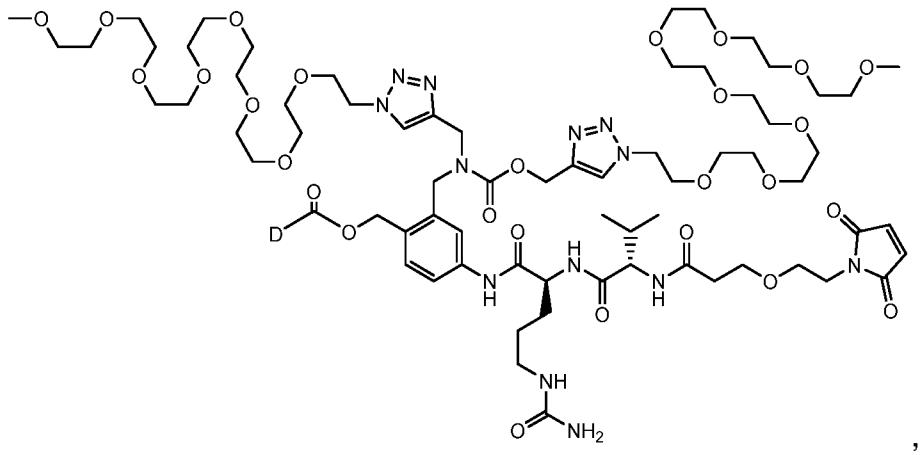

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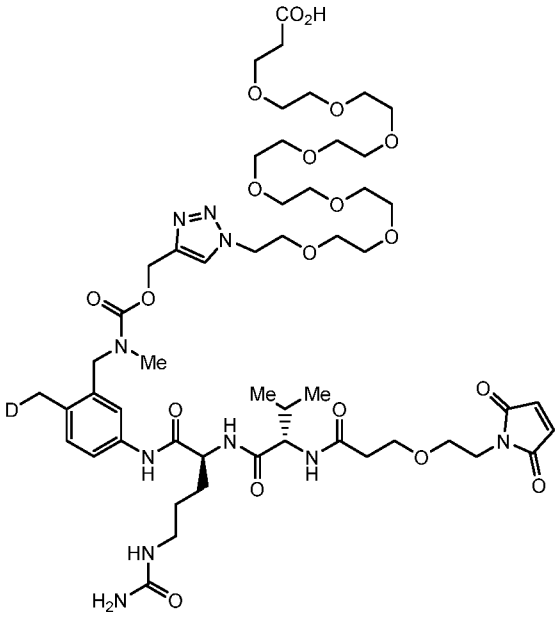

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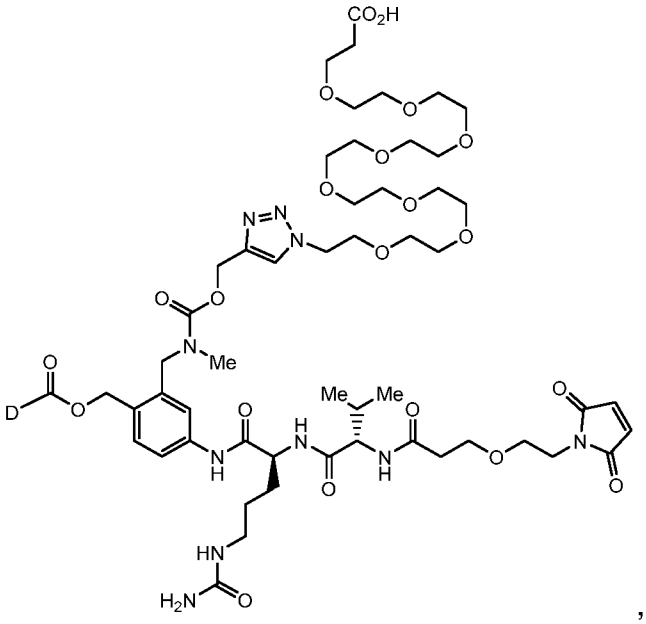

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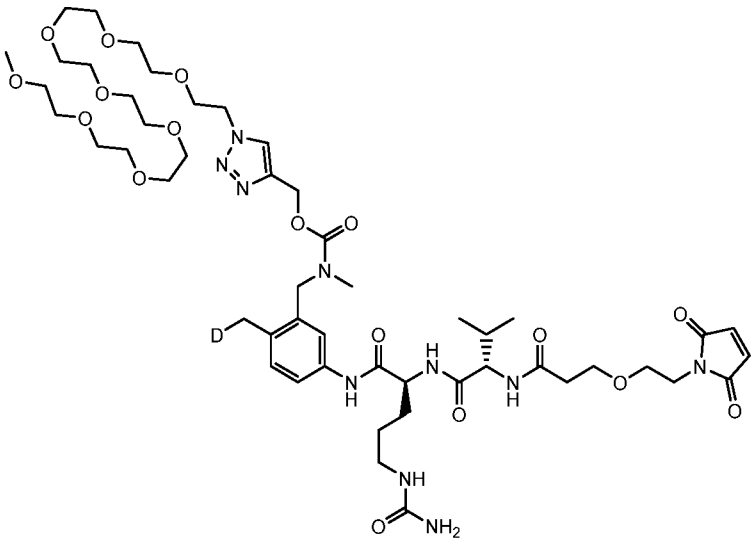

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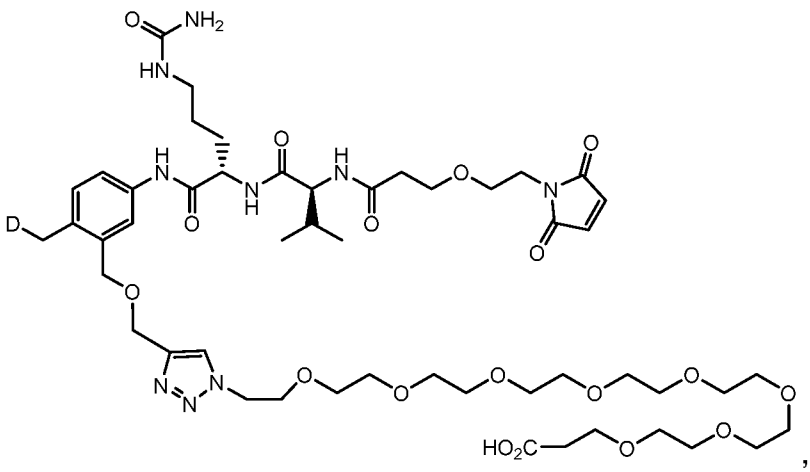

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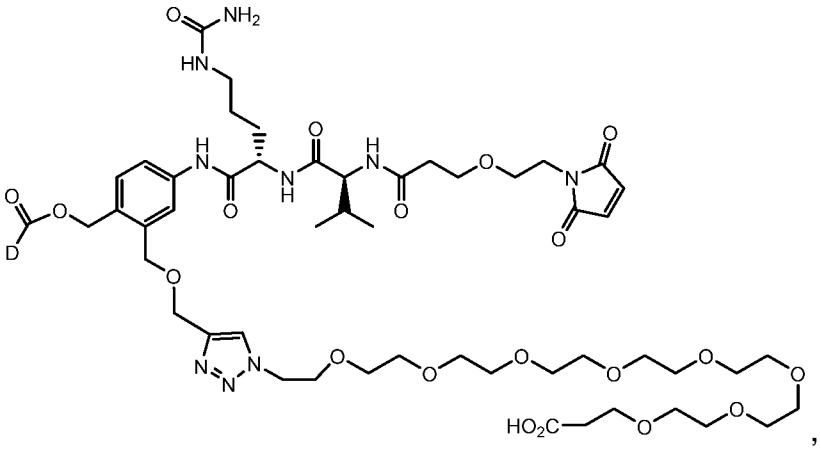

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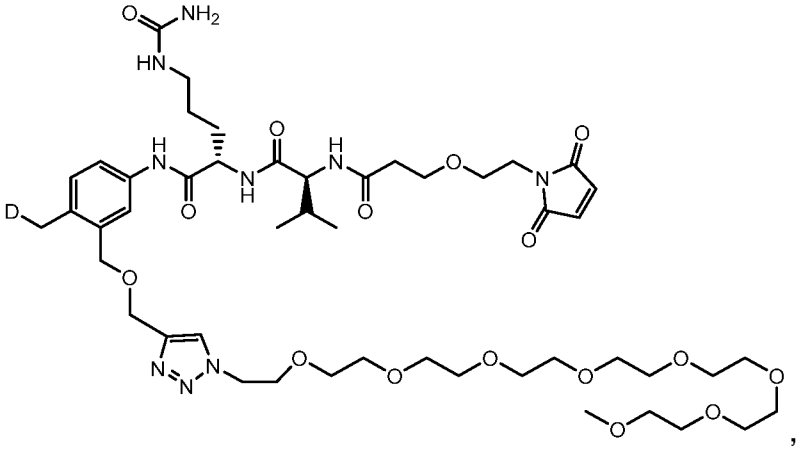

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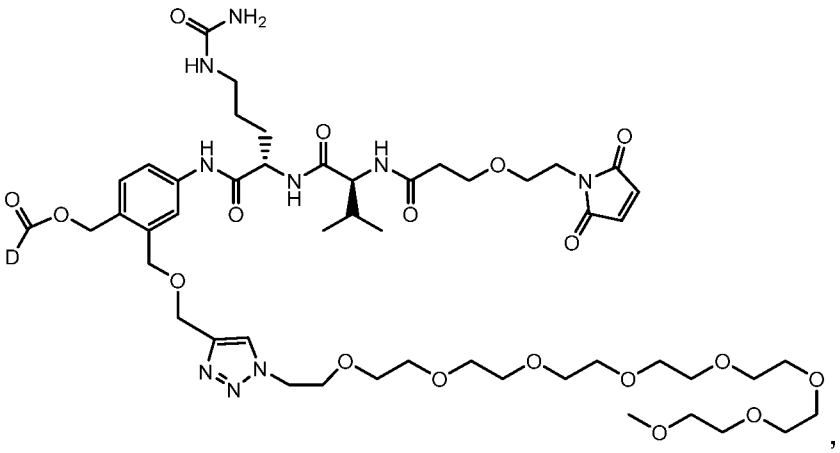

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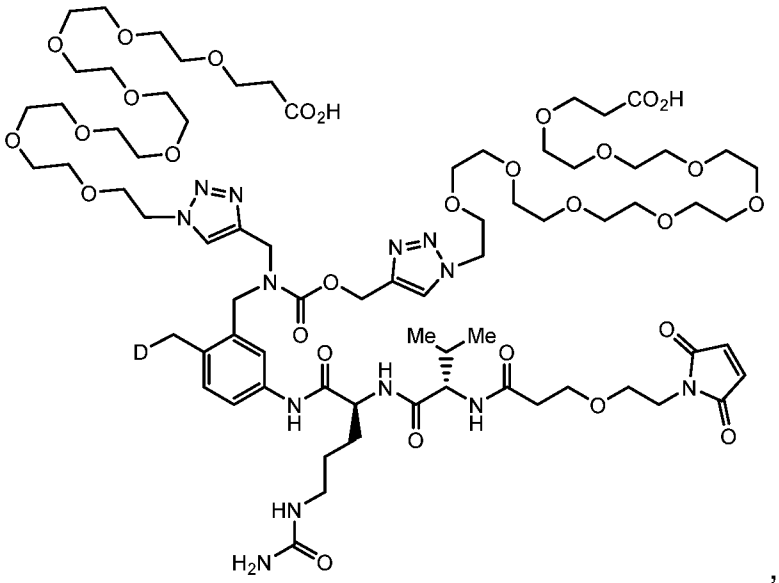

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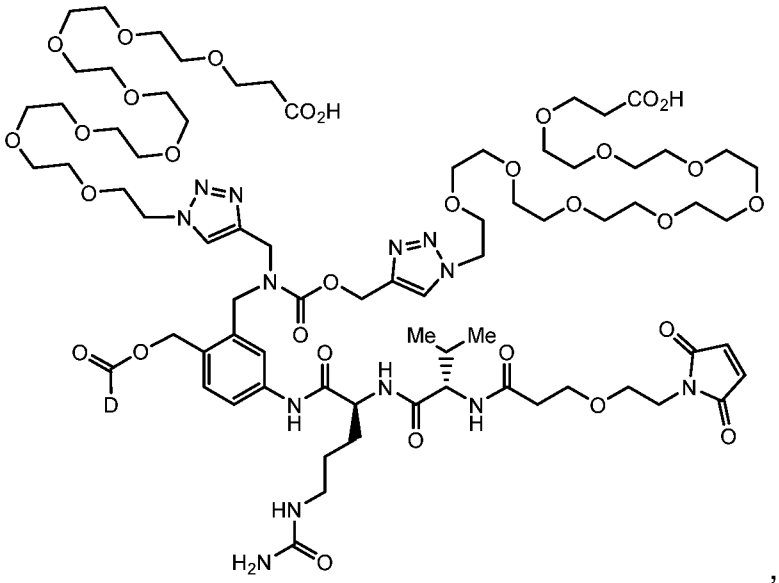

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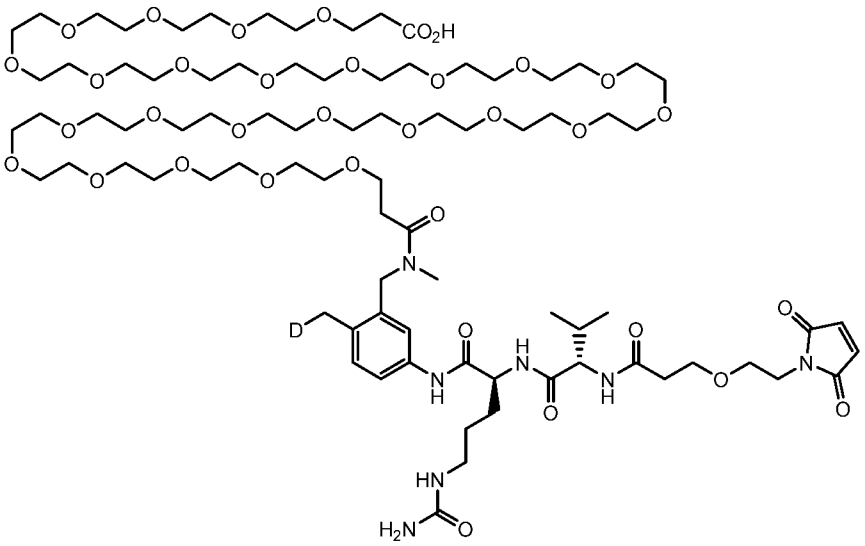

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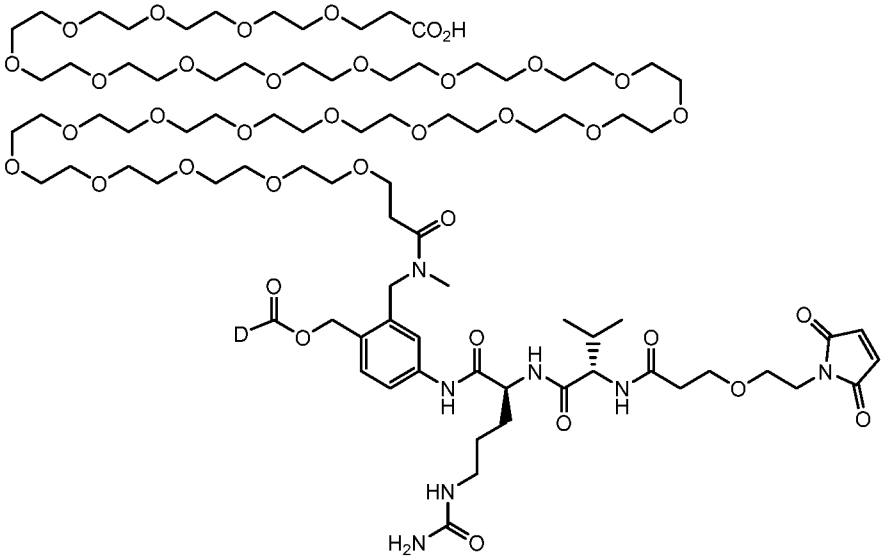

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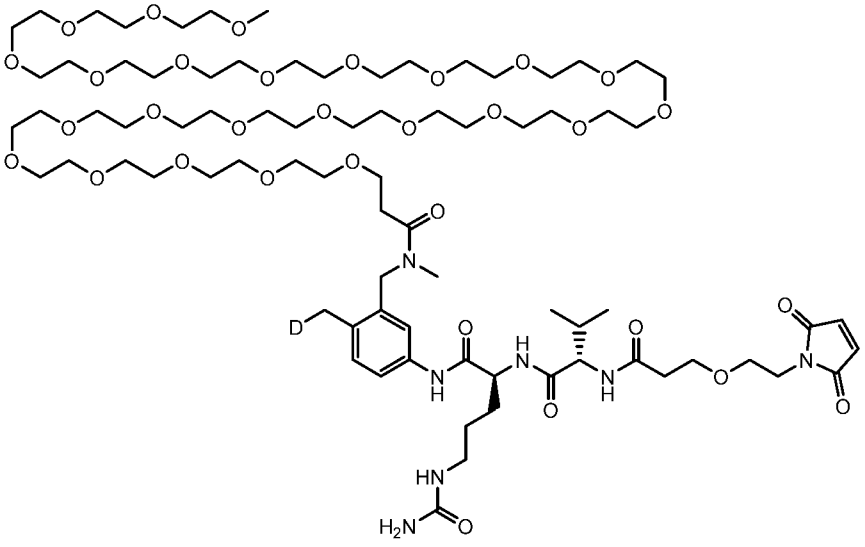

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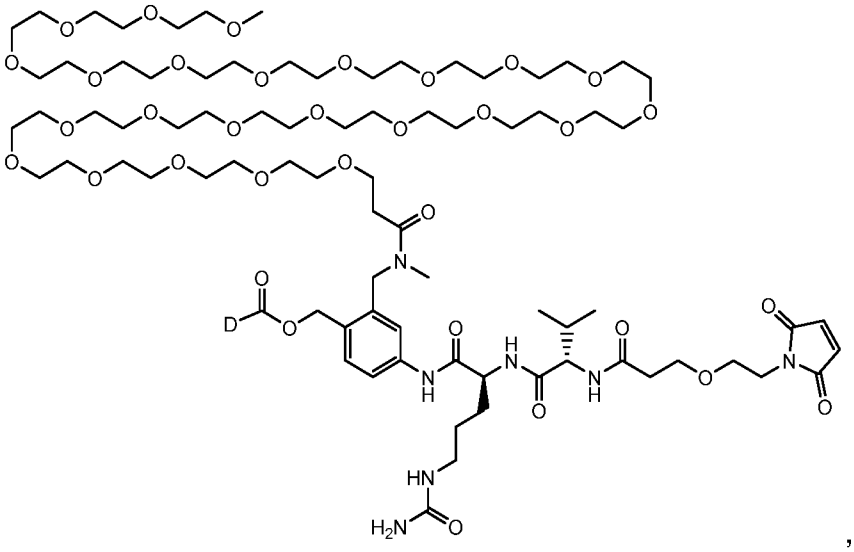

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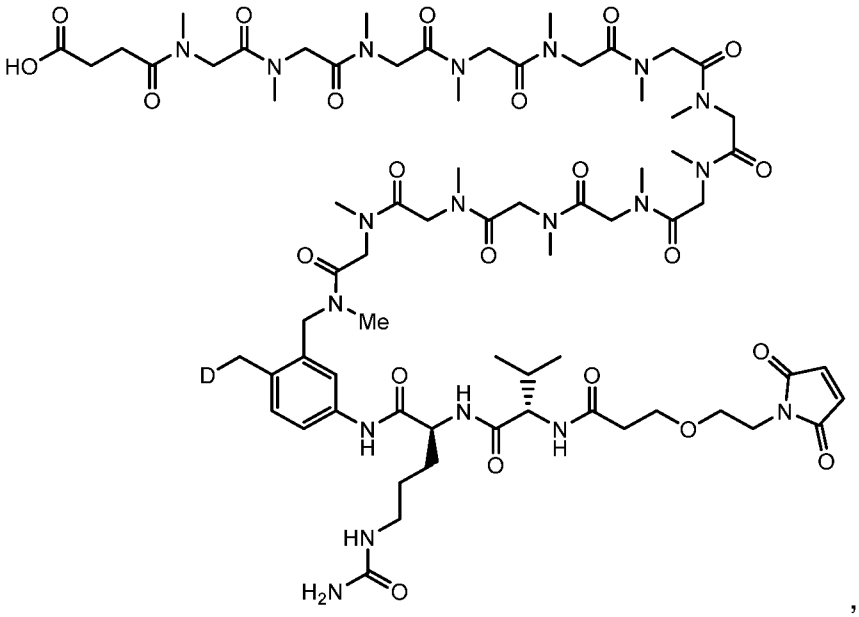

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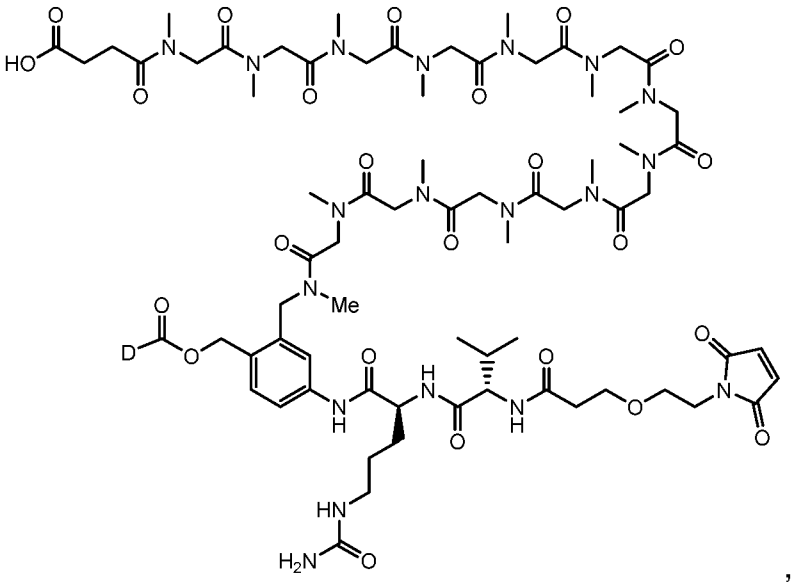

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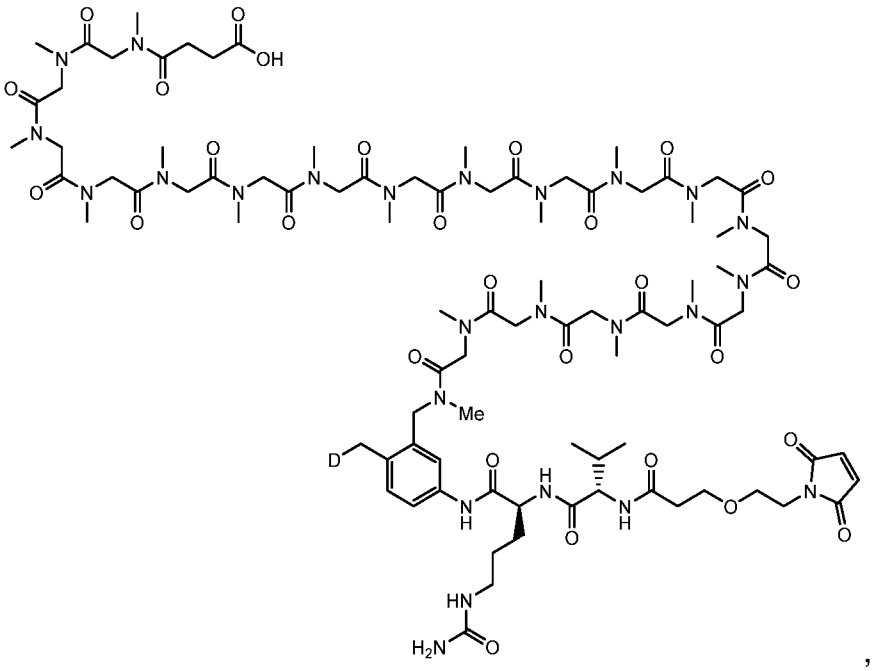

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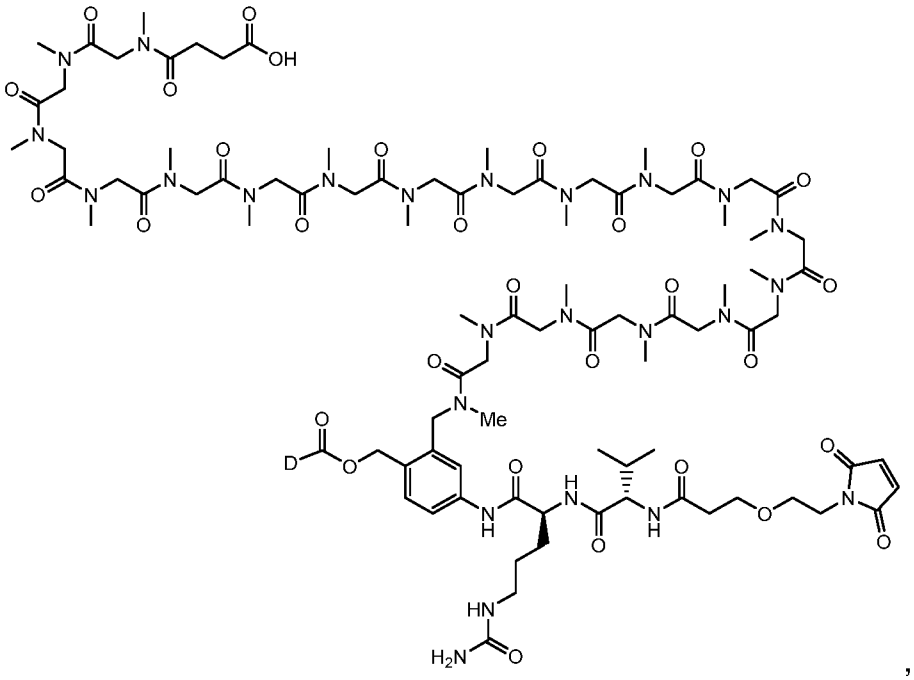

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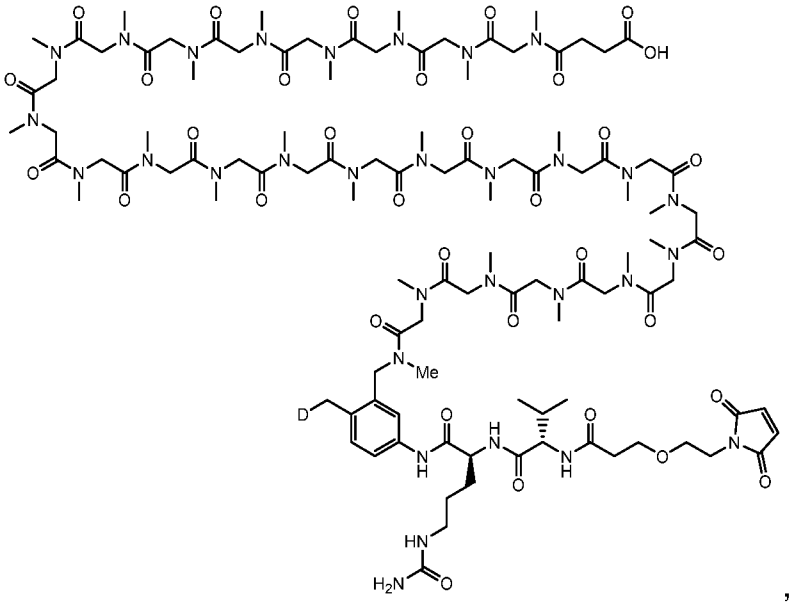

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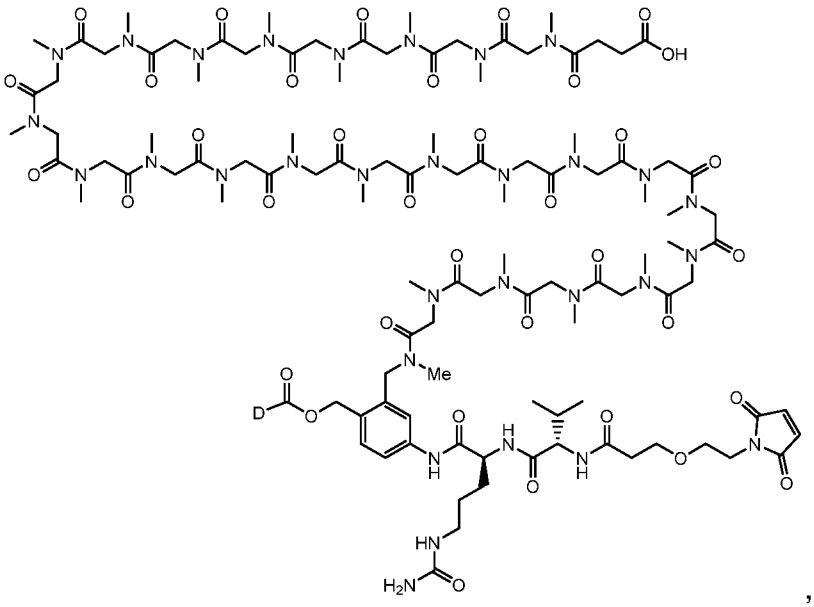

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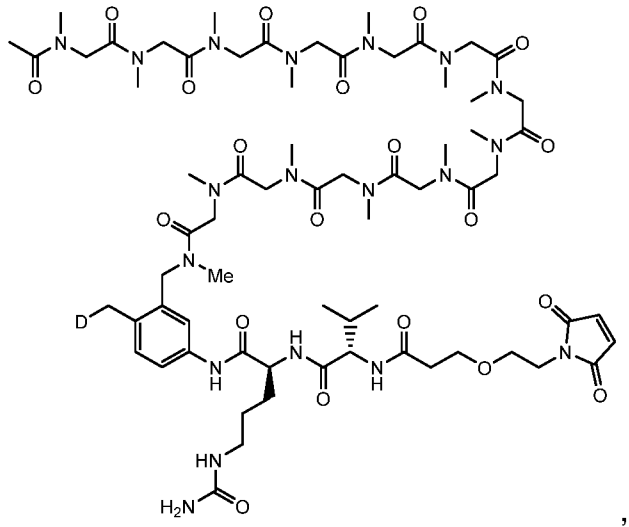

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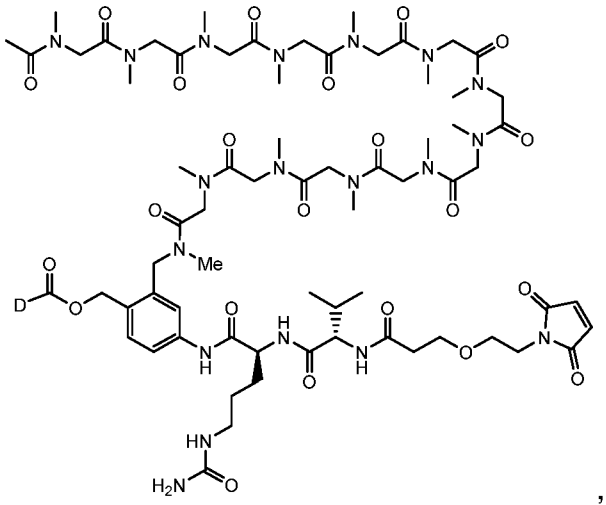

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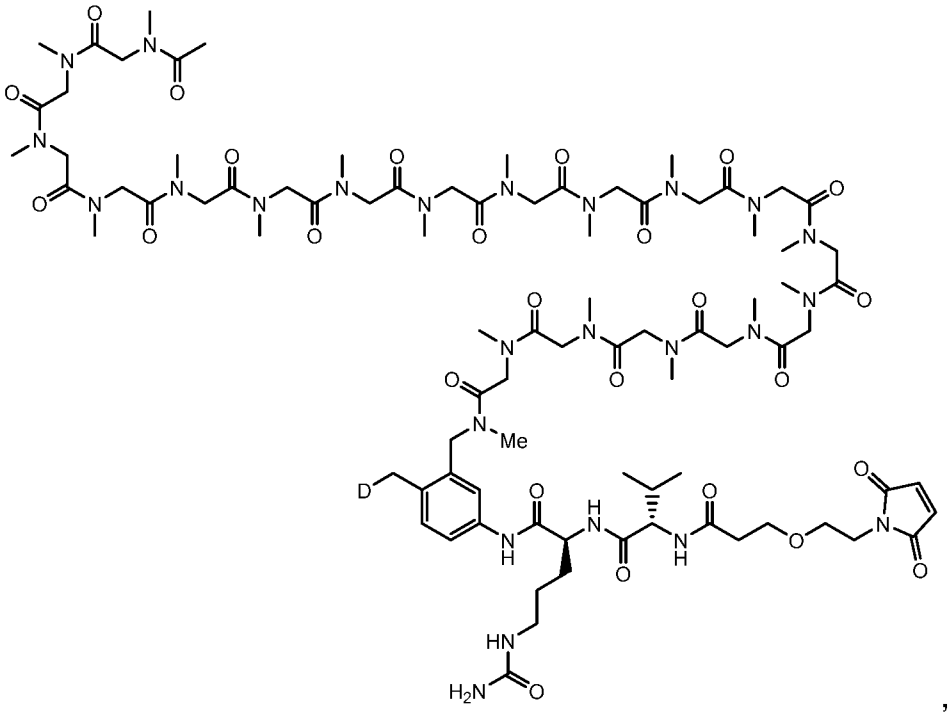

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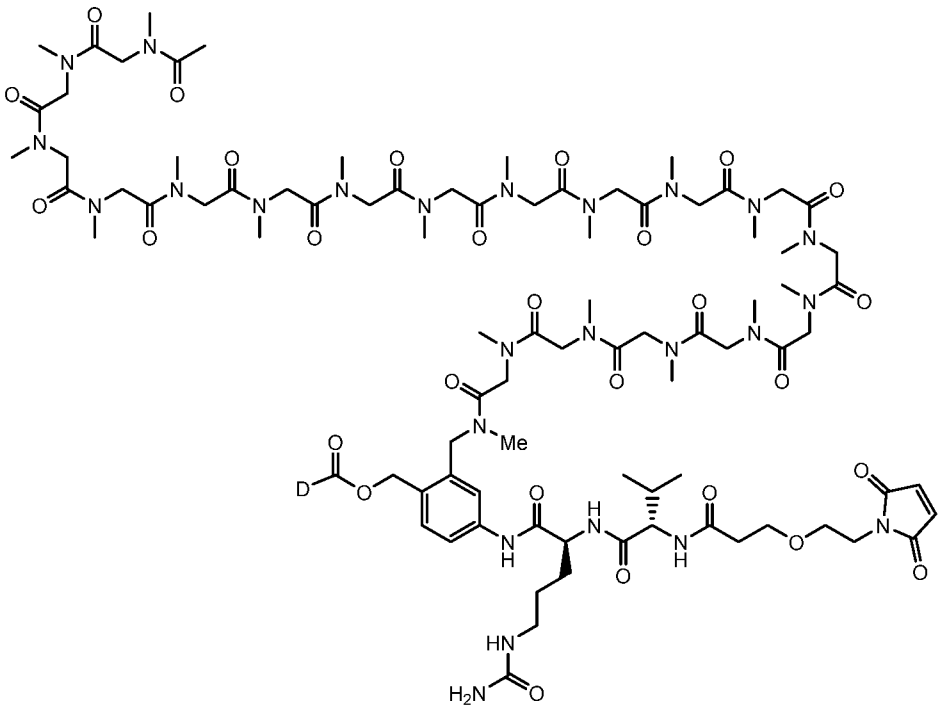

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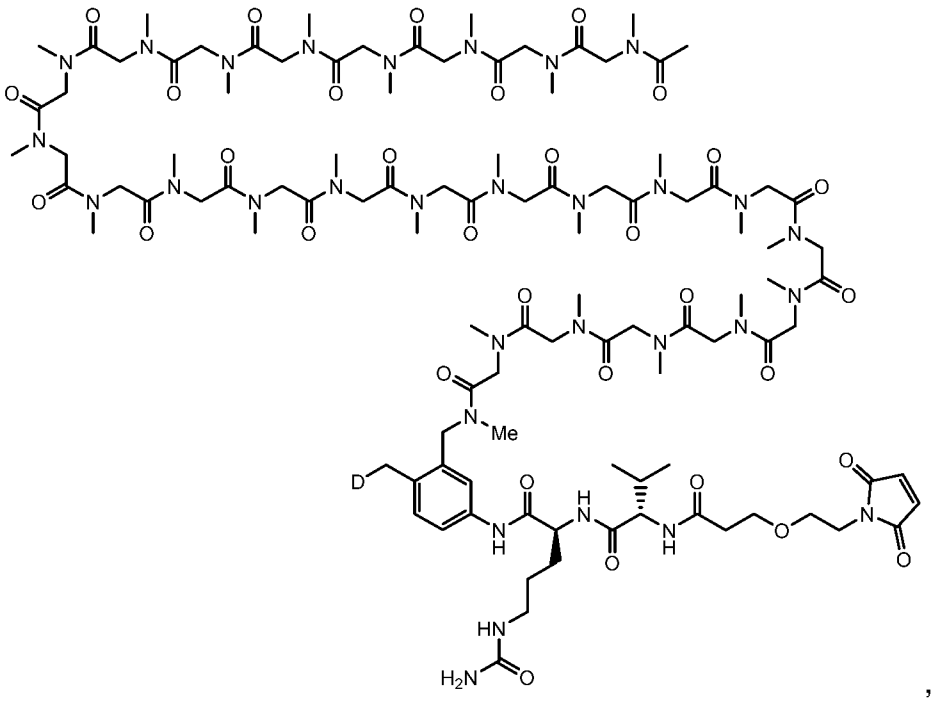

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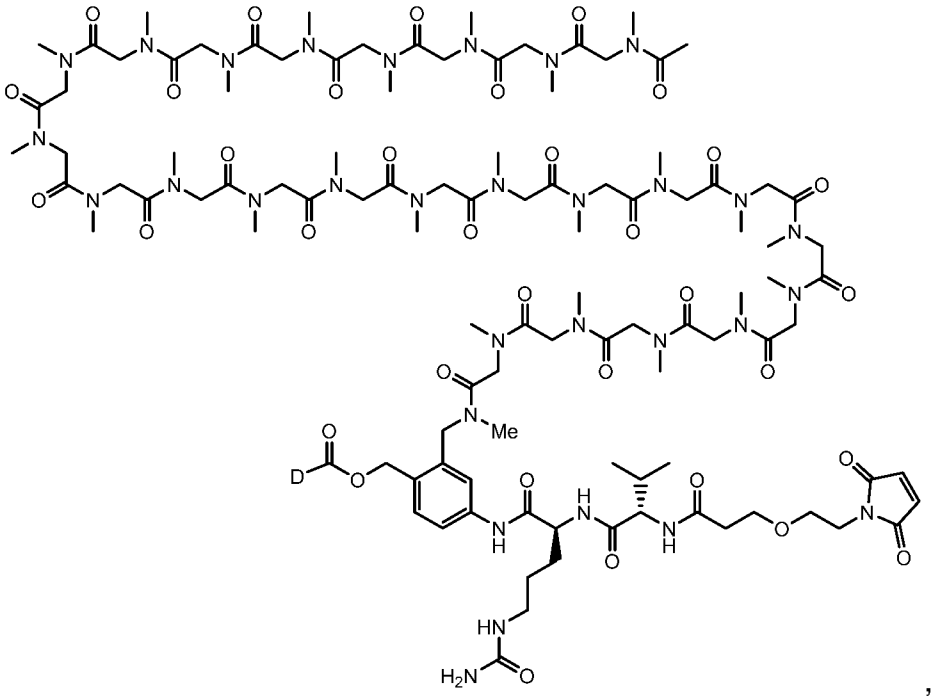

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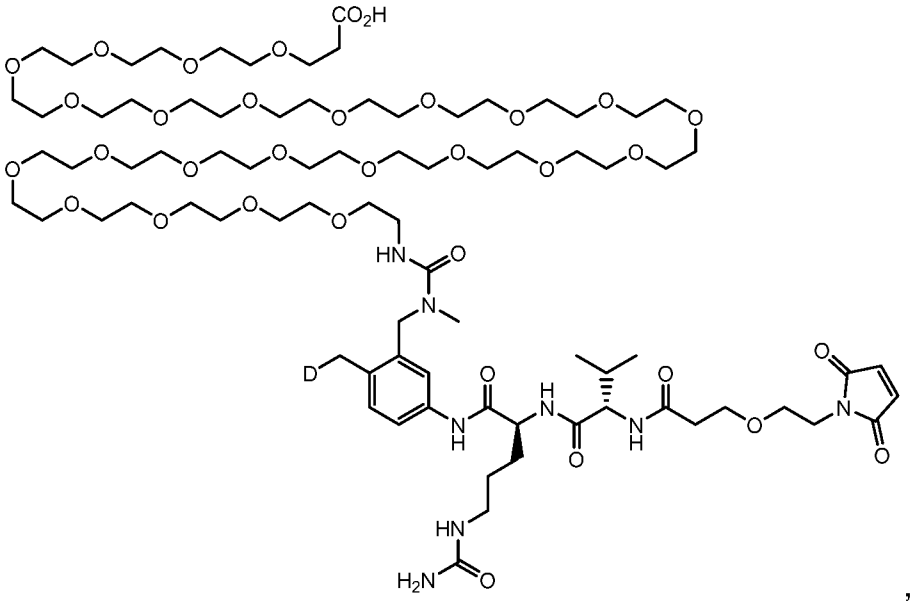

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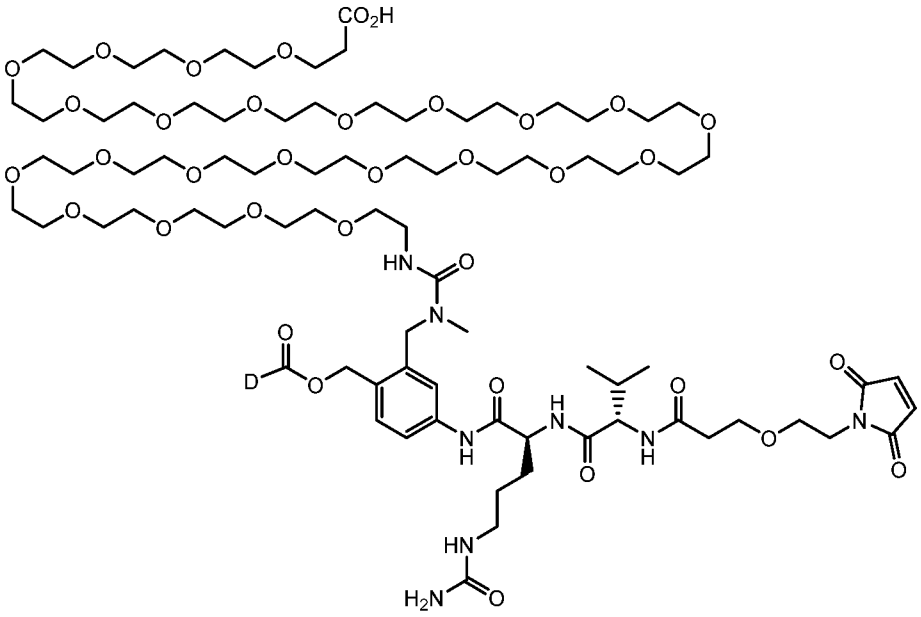

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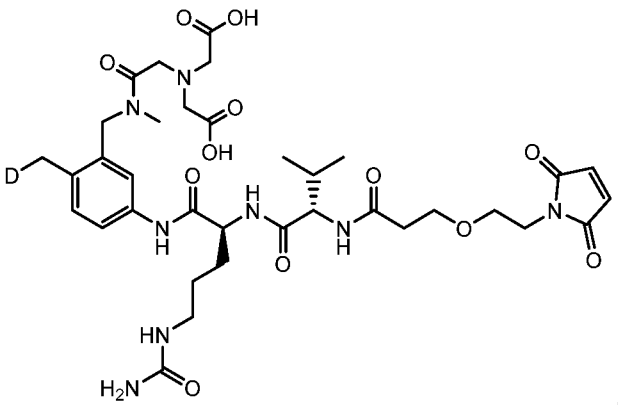

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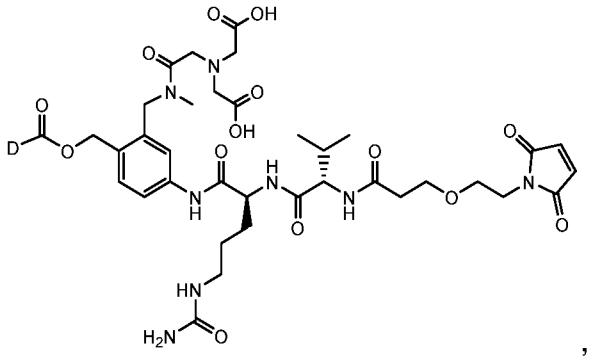

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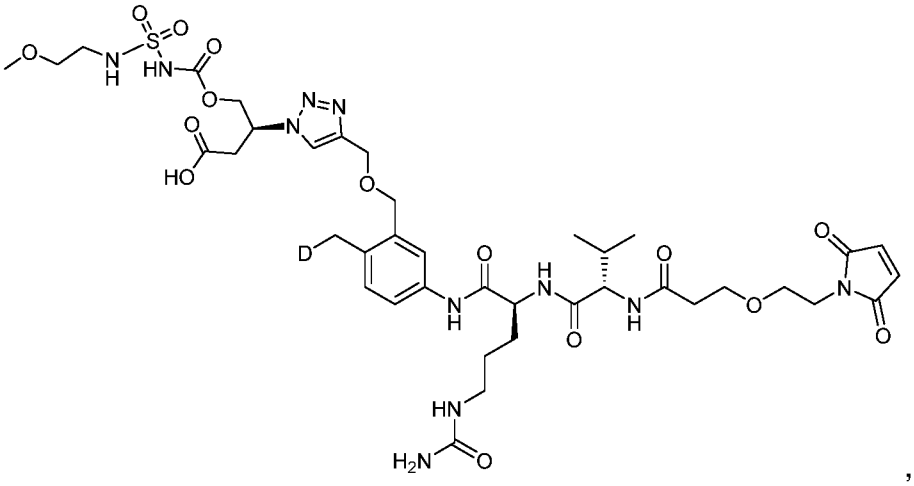

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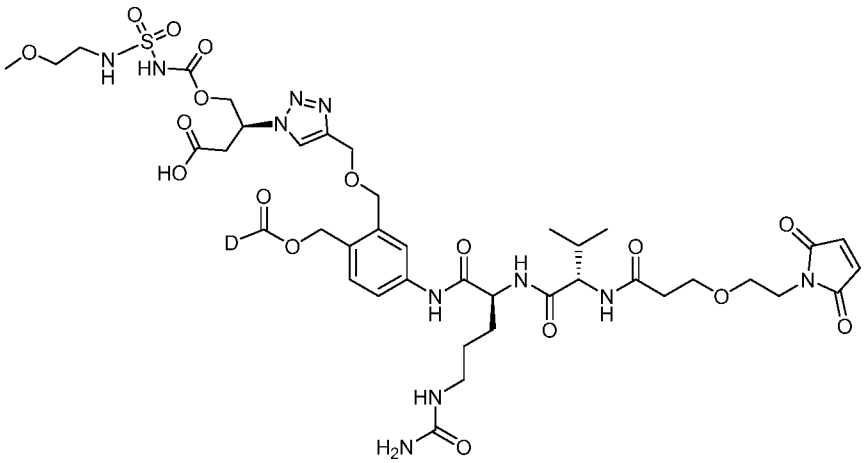

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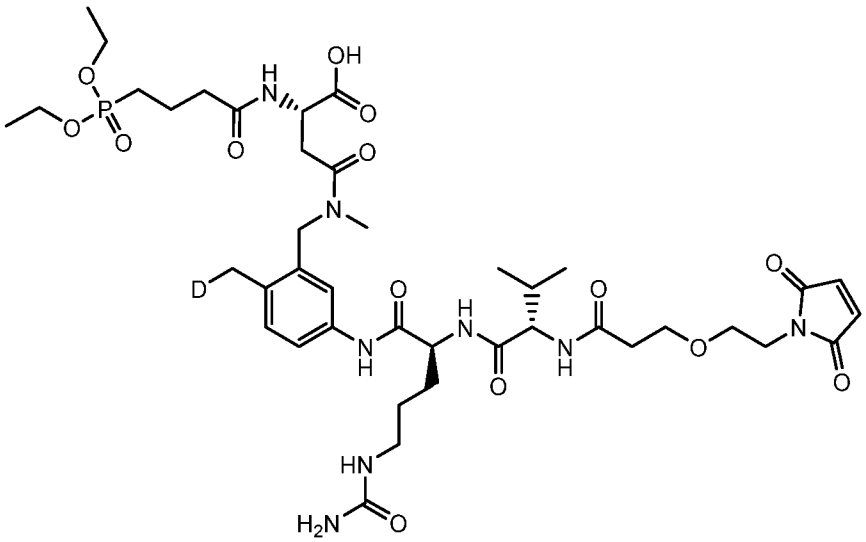

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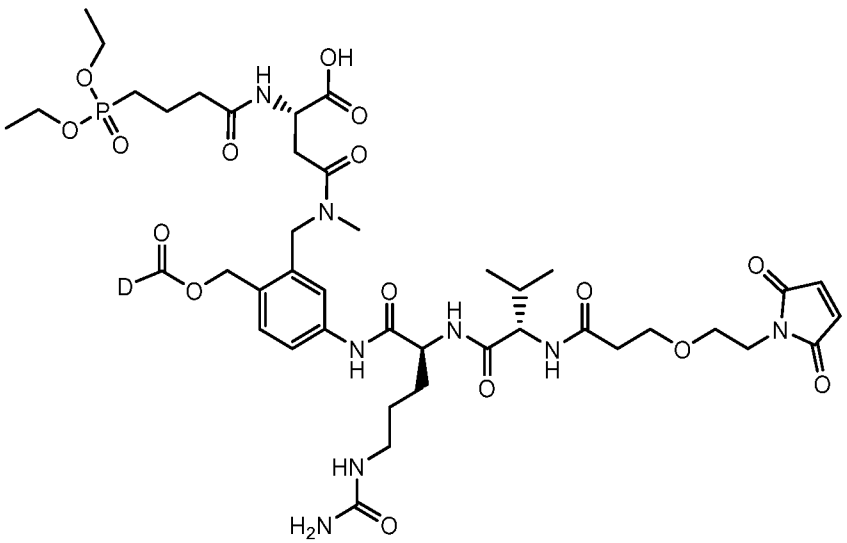

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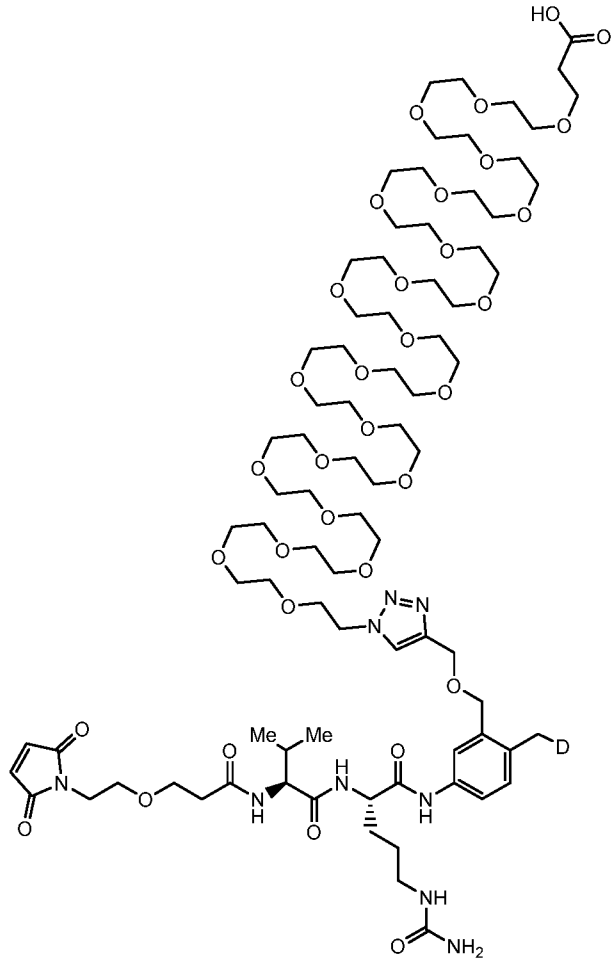

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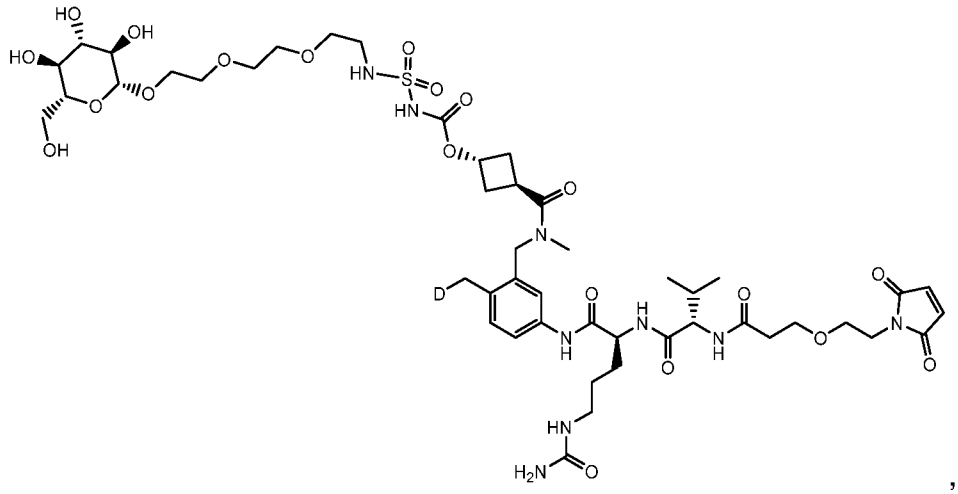

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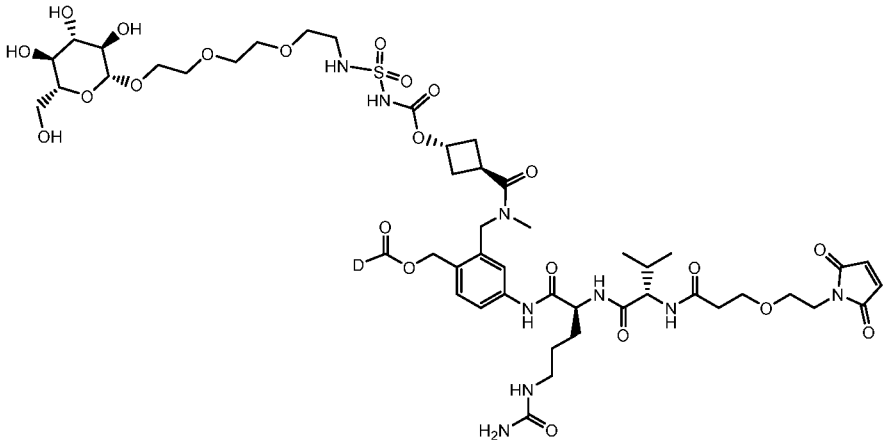

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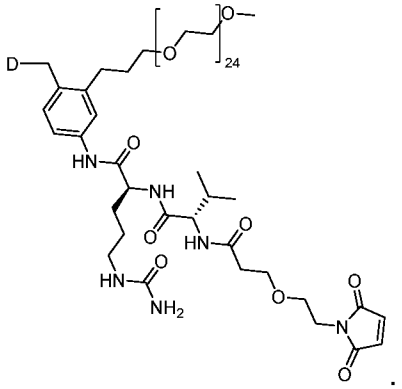

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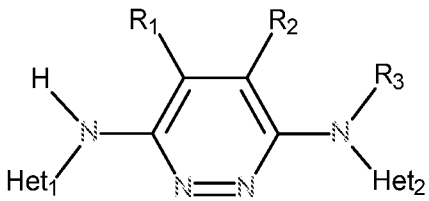
41. The antibody-drug conjugate of any one of claims 1 to 40, wherein D comprises a compound of Formula (I):



(I)

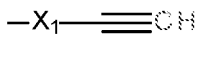
, or

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- R_1 and R_2 independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally substituted by a a linear or branched C_1-C_6alkyl group;

or R_1 and R_2 form with the carbon atoms carrying them a C_3-C_6cycloalkylene group,

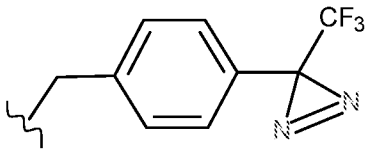
- R_3 represents a group selected from: hydrogen; C_3-C_6cycloalkyl; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; -X_1-O-R_c; -X_1-$COOR_c$; -X_1-$PO(OH)_2$; -X_1-$SO_2(OH)$; -X_1-N_3 and :



,

- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

  the group:



  or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;

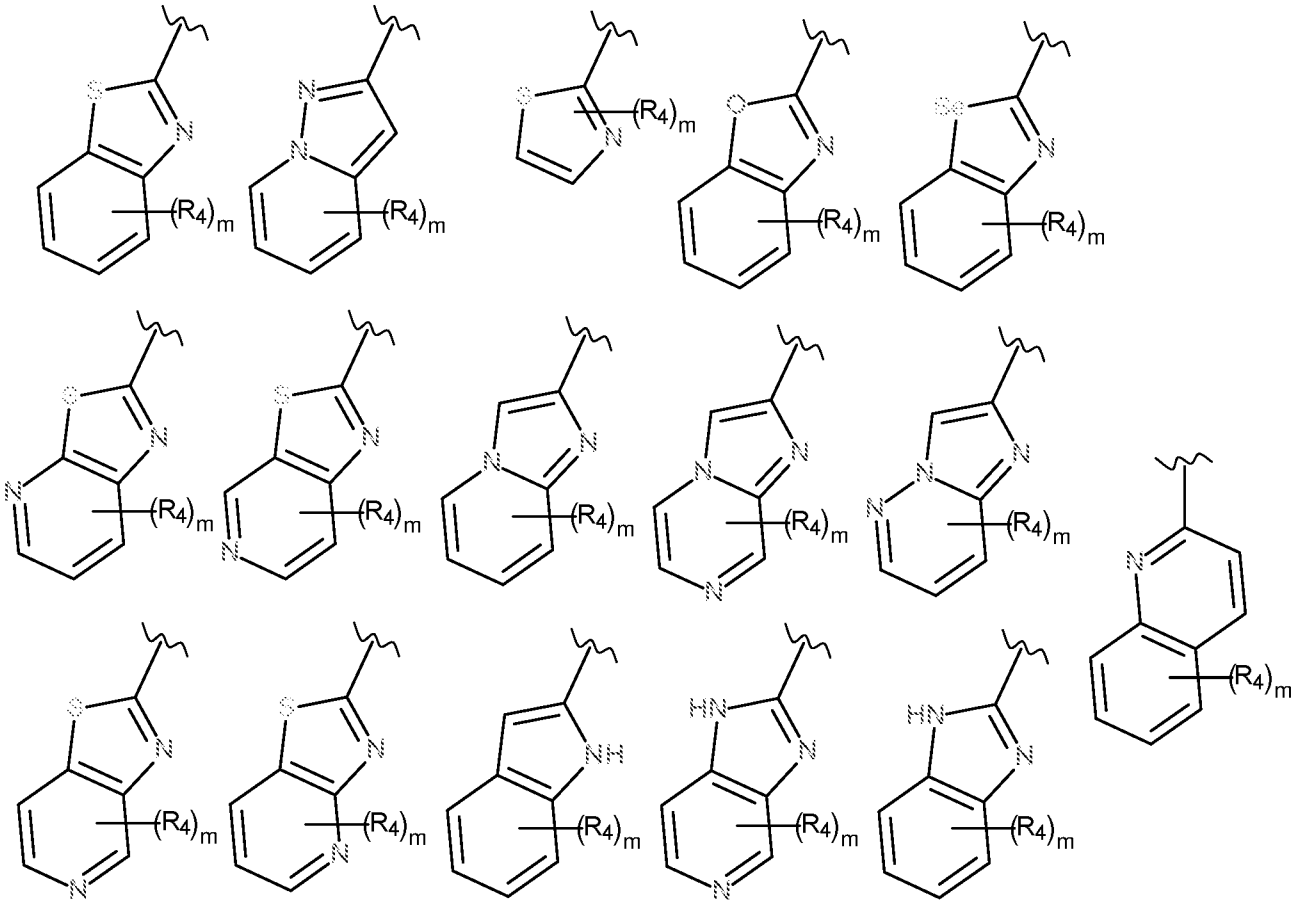
  or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,

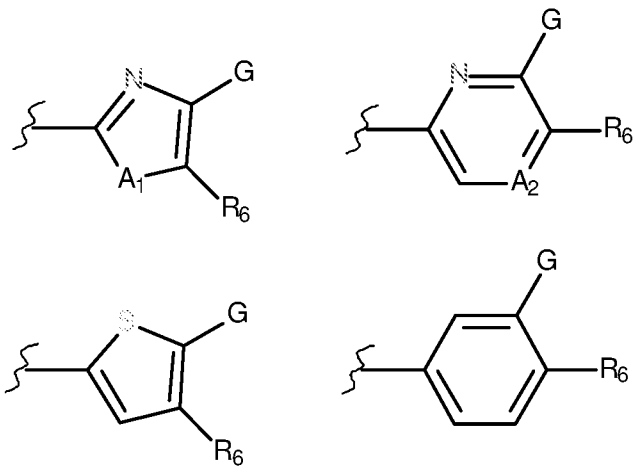
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,

  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



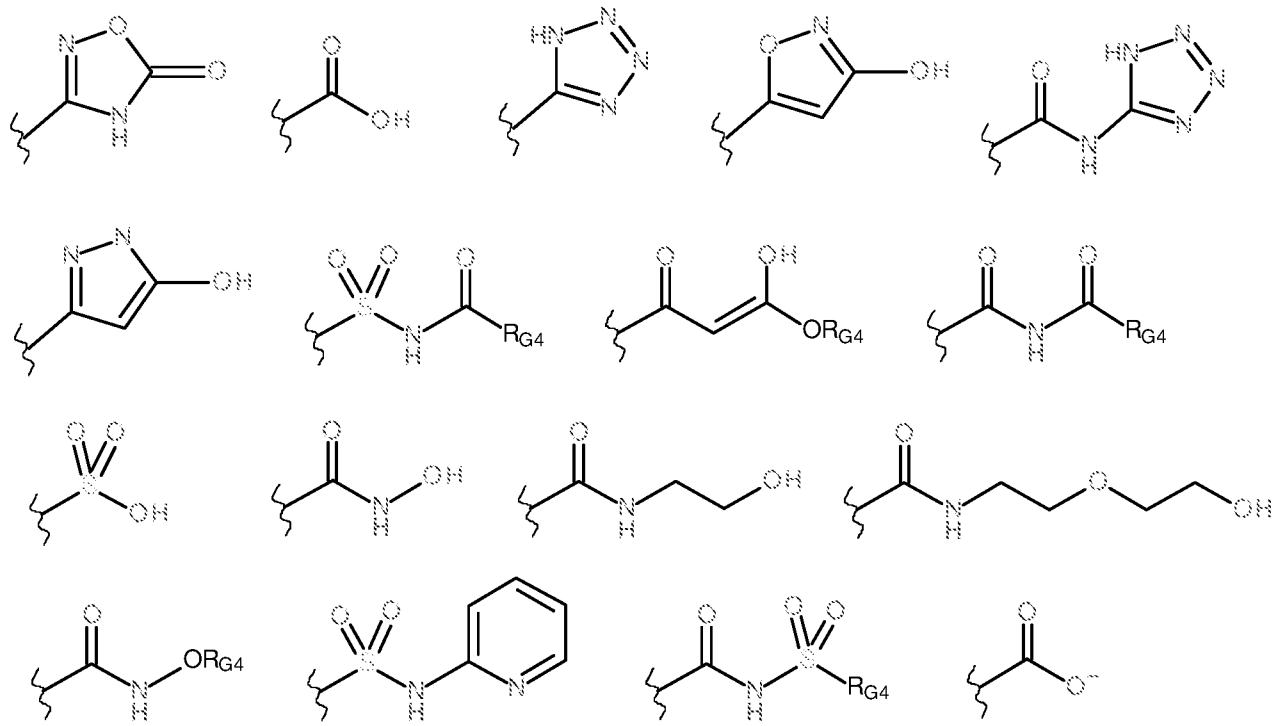
- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

$-C(O)OR_{G3}$, $-C(O)NR_{G1}R_{G2}$, $-C(O)R_{G2}$, $-NR_{G1}C(O)R_{G2}$, $-NR_{G1}C(O)NR_{G1}R_{G2}$, $-OC(O)NR_{G1}R_{G2}$, $-NR_{G1}C(O)OR_{G3}$, $-C(=NOR_{G1})NR_{G1}R_{G2}$, $-NR_{G1}C(=NCN)NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2NR_{G1}R_{G2}$, $-S(O)_2R_{G3}$, $-S(O)_2NR_{G1}R_{G2}$, $-NR_{G1}S(O)_2R_{G2}$, $-NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, $-C(=S)NR_{G1}R_{G2}$, $-C(=NR_{G1})NR_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, $-NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and $-(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen

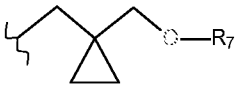
atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,

- R_6 represents a group selected from:

  hydrogen;

  -C_2-C_6alkenyl;

  -X_2-O-R_7;

  ;

  -X_2-NSO_2-R_7;

  -C=C(R_9)-Y_1-O-R_7;

  C_3-C_6cycloalkyl;

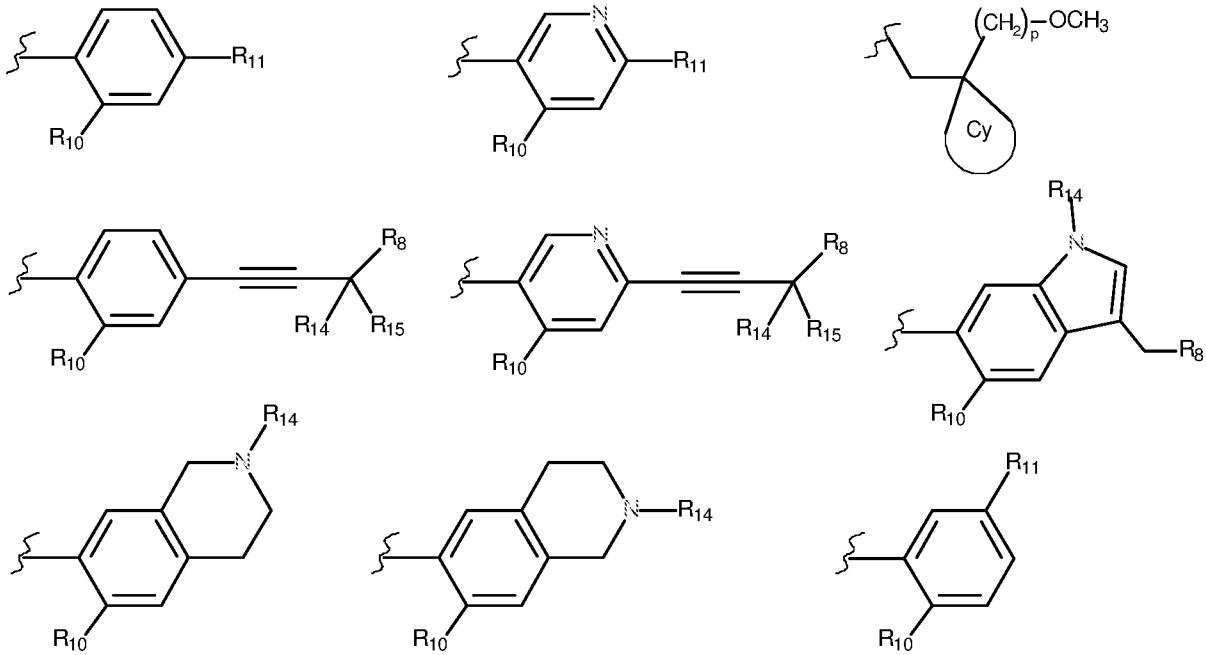
  C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

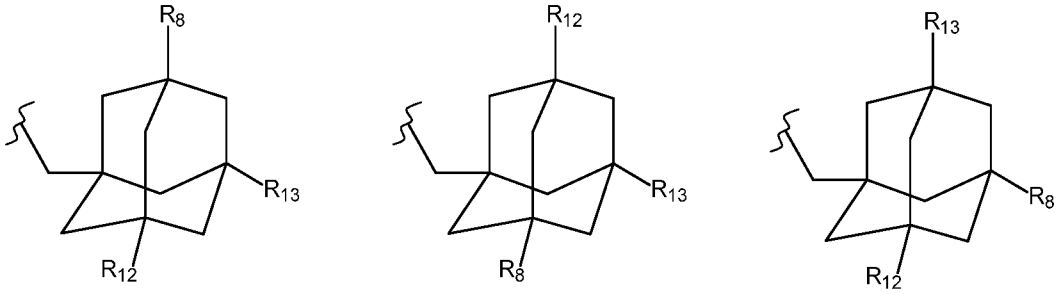
  C_3-C_6cycloalkylene-Y_2-R_7 ;

  C_3-C_6heterocycloalkylene-Y_2-R_7 group,

  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

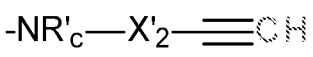
- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:





wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -NR'$_a$R'$_b$; -NR'$_a$-CO-OR'$_c$; -NR'$_a$-CO-R'$_c$; -N$^+$R'$_a$R'$_b$R'$_c$; -O-R'$_c$; -NH-X'$_2$-N$^+$R'$_a$R'$_b$R'$_c$; -O-X'$_2$-NR'$_a$R'$_b$, -X'$_2$-NR'$_a$R'$_b$, -NR'$_c$-X'$_2$-N_3 and :



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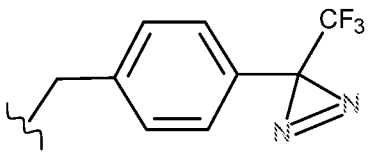
- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'$_2$ represents a linear or branched C_1-C_6alkylene,

- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH;
  C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,

- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,

- m=0, 1 or 2,

- p=1, 2, 3 or 4,

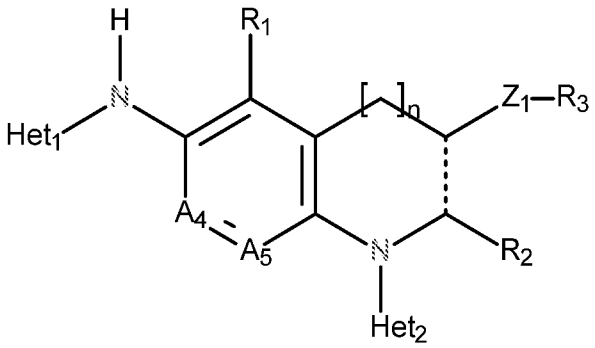
- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl

group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl,

wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceed by virtue of one or more substituents bonded thereto.

42. The antibody-drug conjugate of claim 41, wherein R_1 is linear or branched C_{1-6}alkyl and R_2 is H.

43. The antibody-drug conjugate of any one of claims 1 to 40, wherein D comprises a compound of Formula (II):



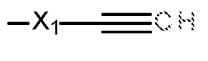
(II),

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- n=0, 1 or 2,
- ------ represents a single or a double bond.
- A_4 and A_5 independently of one another represent a carbon or a nitrogen atom,
- Z_1 represents a bond, -N(R)-, or –O-, wherein R represents a hydrogen or a linear or branched C_1-C_6alkyl,
- R_1 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by a hydroxyl or a

C_1-C_6alkoxy group; C_3-C_6cycloalkyl; trifluoromethyl; linear or branched C_1-C_6alkylene-heterocycloalkyl wherein the heterocycloalkyl group is optionally substituted by a a linear or branched C_1-C_6alkyl group;

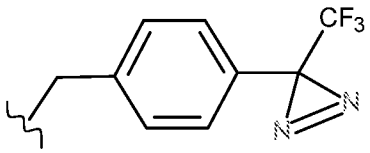
- R_2 represents a hydrogen or a methyl;
- R_3 represents a group selected from: hydrogen; linear or branched C_1-C_4alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; -X_1-O-R_c; -X_1-$COOR_c$; -X_1-$PO(OH)_2$; -X_1-$SO_2(OH)$; -X_1-N_3 and :



,

- R_a and R_b independently of one another represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-NR_dR_e; C_1-C_6alkylene-$N^+R_dR_eR_f$; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a C_1-C_6alkoxy group;

  the group:



  or R_a and R_b form with the nitrogen atom carrying them a cycle B_1;

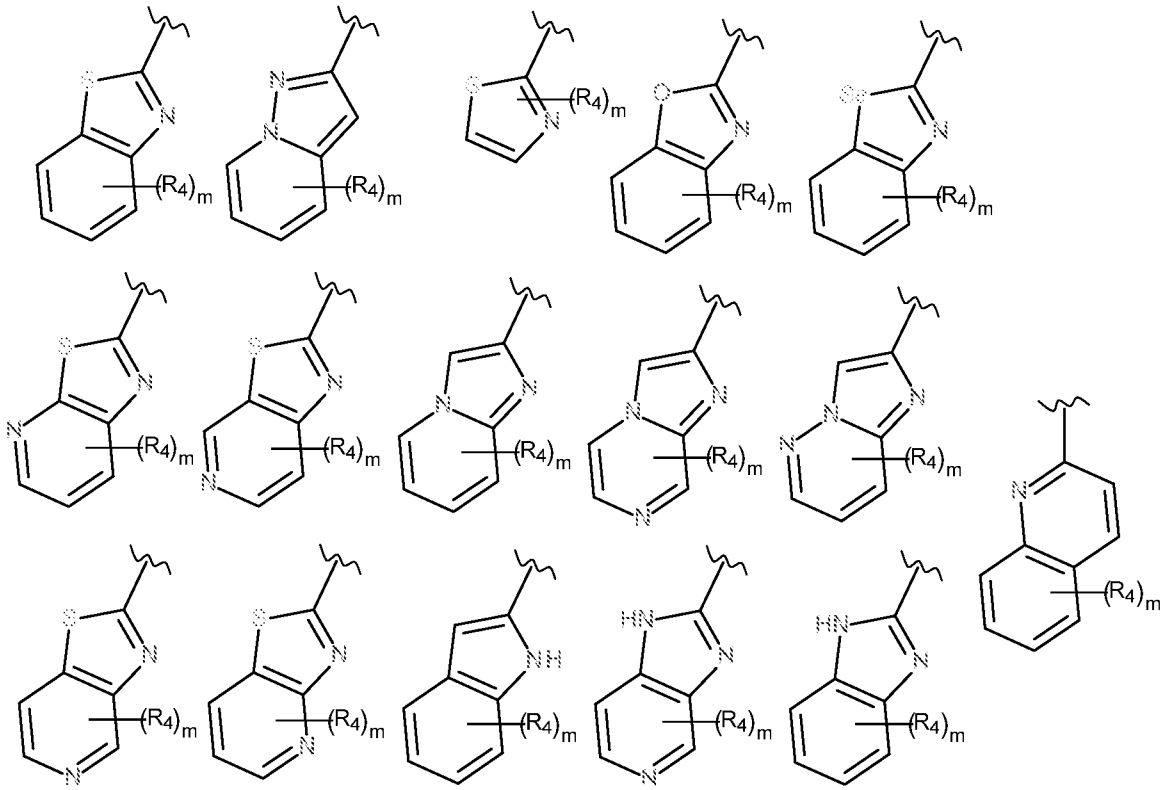
  or R_a, R_b and R_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R_c, R_d, R_e, R_f, independently of one another represents a hydrogen or a linear or branched C_1-C_6alkyl group,

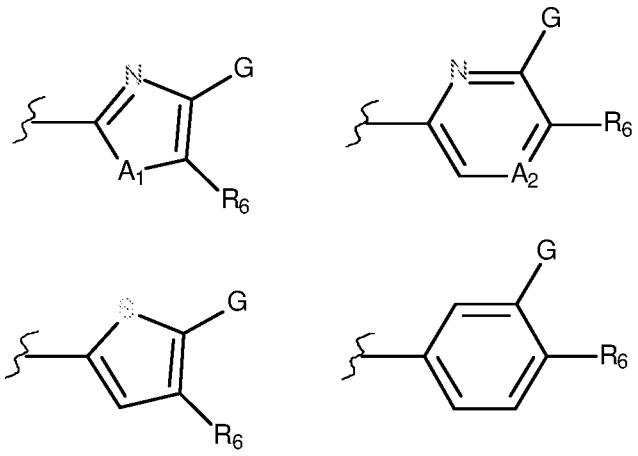
  or R_d and R_e form with the nitrogen atom carrying them a a cycle B_2,

  or R_d, R_e and R_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Het_1 represents a group selected from:



- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

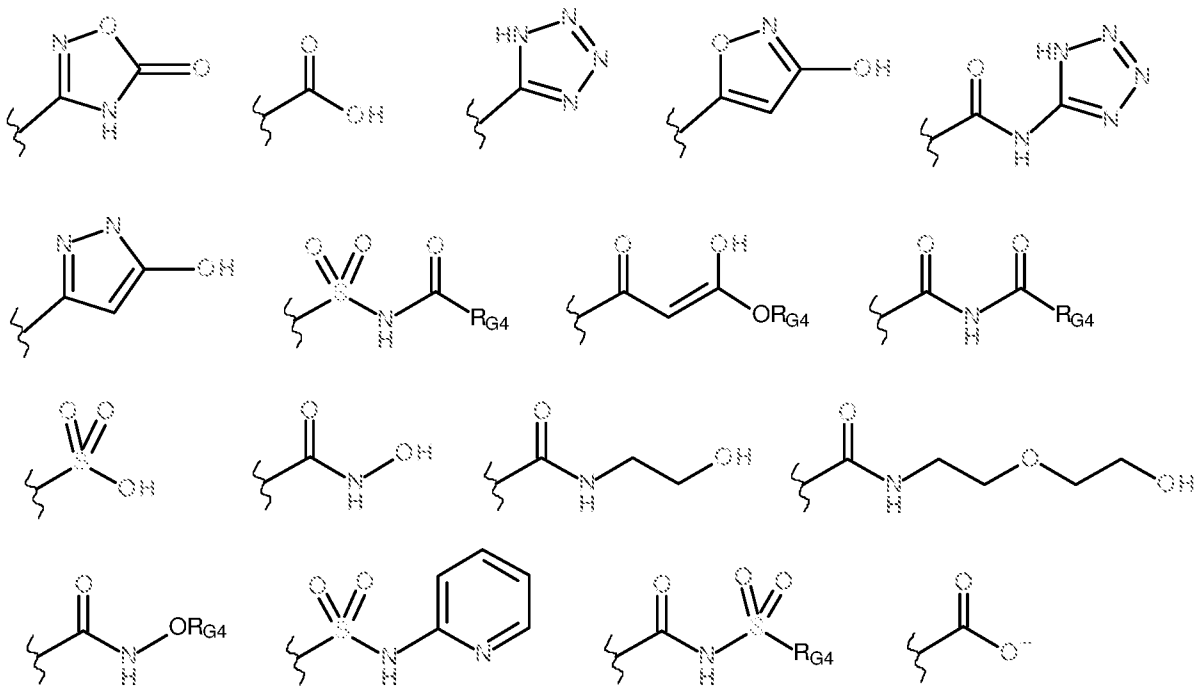
  -C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$,

-$NR_{G1}C(=NCN)NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2NR_{G1}R_{G2}$, -$S(O)_2R_{G3}$, -$S(O)_2NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2R_{G2}$, -$NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, -$C(=S)NR_{G1}R_{G2}$, -$C(=NR_{G1})NR_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, -NO_2, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl;

- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



wherein R_{G4} is selected from hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl,

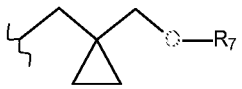
- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; C_2-C_6alkenyl; C_2-C_6alkynyl; halogen or –CN,

- R_6 represents a group selected from:

  hydrogen;

  -C_2-C_6alkenyl;

  -X_2-O-R_7;

  ;

  -X_2-NSO_2-R_7;

  -C=C(R_9)-Y_1-O-R_7;

  C_3-C_6cycloalkyl;

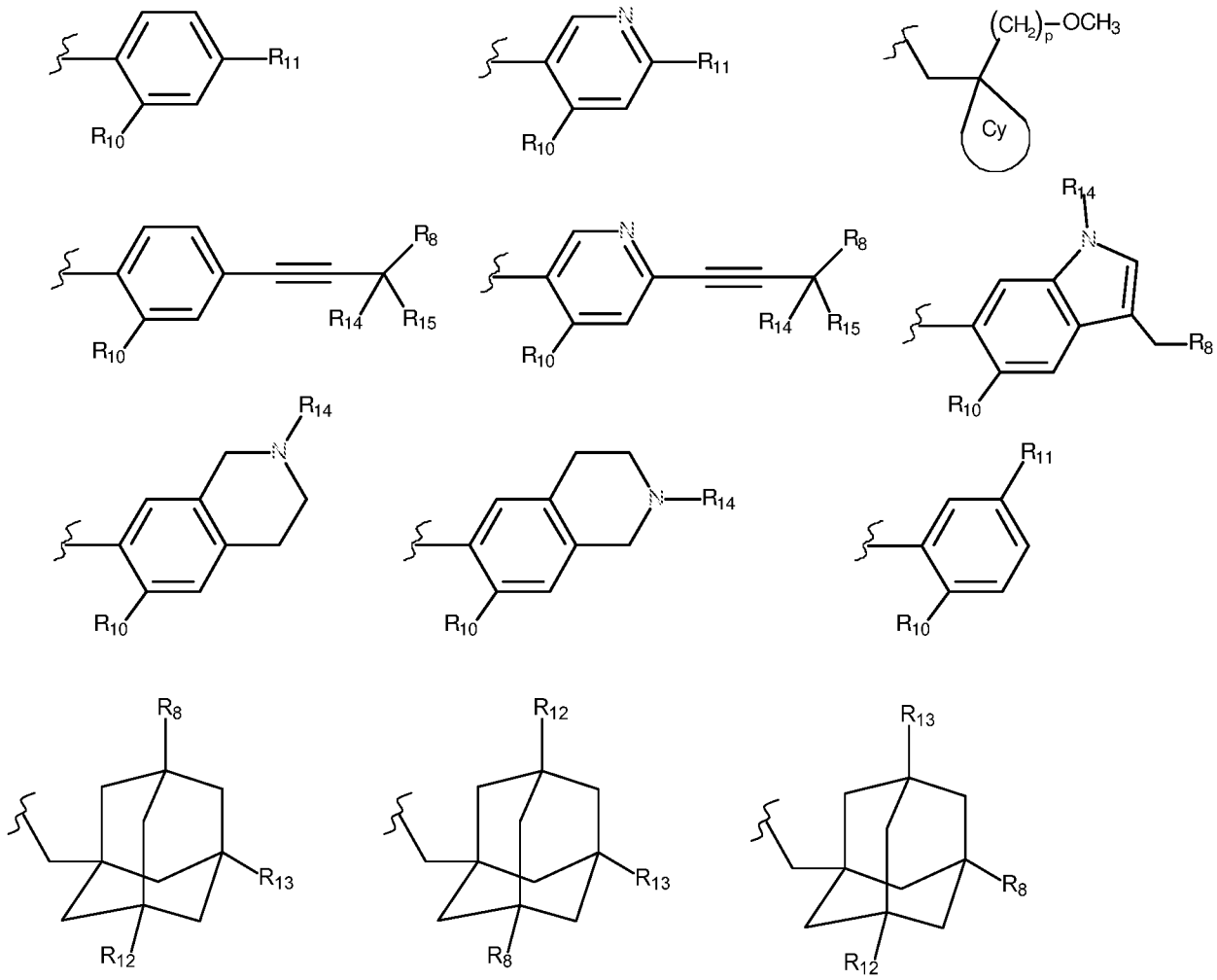
  C_3-C_6heterocycloalkyl optionally substituted by a hydroxyl group;

  C_3-C_6cycloalkylene-Y_2-R_7 ;

  C_3-C_6heterocycloalkylene-Y_2-R_7 group,

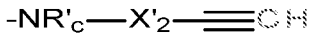
  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:



wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -NR'$_a$R'$_b$; -NR'$_a$-CO-OR'$_c$; -NR'$_a$-CO-R'$_c$; -N$^+$R'$_a$R'$_b$R'$_c$; -O-R'c; -NH-X'$_2$-N$^+$R'$_a$R'$_b$R'$_c$; -O-X'$_2$-NR'$_a$R'$_b$, -X'$_2$-NR'$_a$R'$_b$, -NR'$_c$-X'$_2$-N$_3$ and :



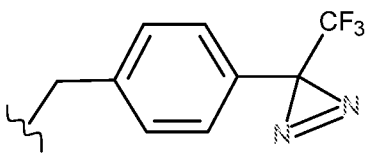
,

- R_9 represents a group selected from linear or branched C_1-C_6alkyl, trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,
- R_{11} represents a group selected from hydrogen, halogen, C_1-C_3alkylene-R_8, -O-C_1-

C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,

- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 represents a linear or branched C_1-C_4alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X_2 represents a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged

C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,

  or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

  or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- Y_1 represents a linear or branched C_1-C_4alkylene,

- Y_2 represents a bond, -O-, -O-CH_2-, -O-CO-, -O-SO_2-, -CH_2-, -CH_2-O, -CH_2-CO-, -CH_2-SO_2-,-C_2H_5-, -CO-, -CO-O-, -CO-CH_2-, -CO-NH-CH_2-, -SO_2-, -SO_2-CH_2-, -NH-CO-, -NH-SO_2-,

- m=0, 1 or 2,

- p=1, 2, 3 or 4,

- B_1, B_2, B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, –NH_2, oxo or piperidinyl,

wherein one of the R_3 and R_8 groups, if present, is covalently attached to the linker, and wherein the valency of an atom is not exceed by virtue of one or more substituents bonded thereto.

44. The antibody-drug conjugate of claim 43, wherein A_1 and A_5 both represent a nitrogen atom, R_1 is linear or branched $C_{1\text{-}6}$alkyl; R_2 is H; n is 1; and ------ represents a single bond.

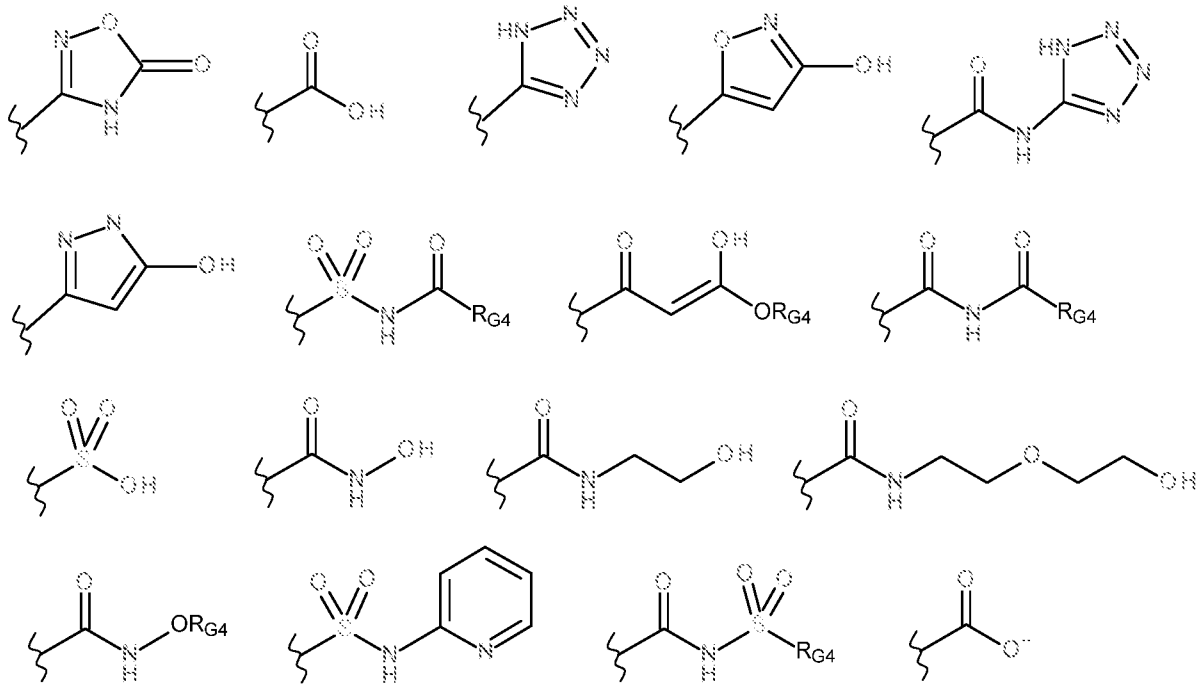
45. The antibody-drug conjugate of any one of claims 1-44, wherein The antibody-drug conjugate of claim 1, wherein G is selected from the group consisting of: -C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$, -NR_{G1}C(=NCN)$NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2NR_{G1}R_{G2}$, -$S(O)_2R_{G3}$, -$S(O)_2NR_{G1}R_{G2}$,

-$NR_{G1}S(O)_2R_{G2}$, -$NR_{G1}C(=NR_{G2})NR_{G1}R_{G2}$, -$C(=S)NR_{G1}R_{G2}$, -$C(=NR_{G1})NR_{G1}R_{G2}$, halogen, -$NO_2$, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl;

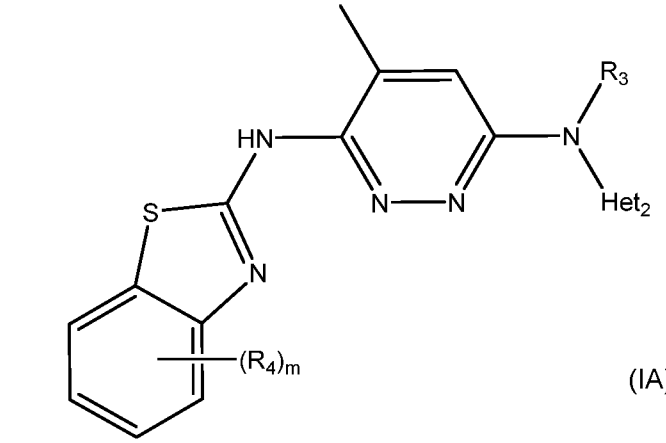
- R_{G3} is selected from the group consisting of C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl, C_3-C_6cycloalkyl, phenyl and -$(CH_2)_{1-4}$-phenyl; or

R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl ; or in the alternative, G is selected from the group consisting of:



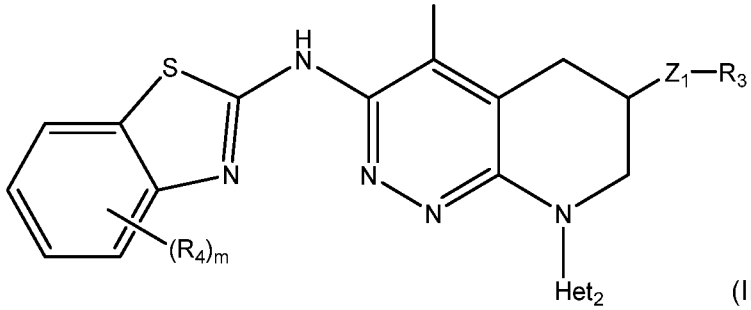
wherein R_{G4} is selected from C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms, C_2-C_6alkenyl, C_2-C_6alkynyl and C_3-C_6cycloalkyl.

46. The antibody-drug conjugate of any one of claims 1-40, wherein D comprises a compound of formula (IA) or (IIA):



(IA)

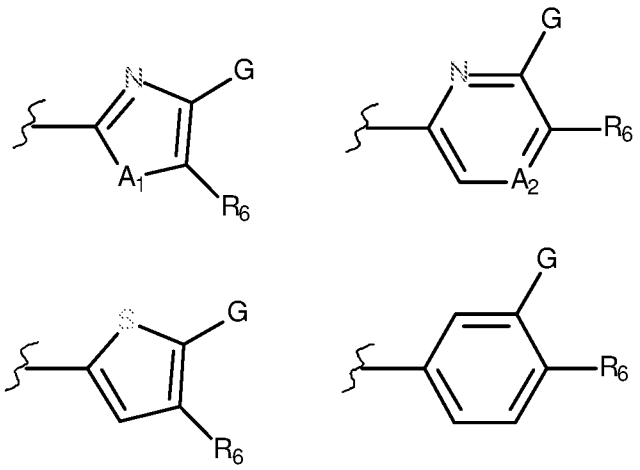
, or



(IIA)

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- Z_1 represents a bond or –O-,
- R_3 represents a group selected from: hydrogen; C_3-C_6cycloalkyl; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; and -X_1-O-R_c,
- R_a and R_b independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; and C_1-C_6alkylene-SO_2O^-,
- R_c represents a hydrogen or a linear or branched C_1-C_6alkyl group,
- Het_2 represents a group selected from:



- A_1 is –NH-, -N(C_1-C_3alkyl), O, S or Se,
- A_2 is N, CH or C(R_5),
- G is selected from the group consisting of:

-C(O)OH, -C(O)OR_{G3}, -C(O)N$R_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)N$R_{G1}R_{G2}$, -OC(O)N$R_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})N$R_{G1}R_{G2}$, -NR_{G1}C(=NCN)N$R_{G1}R_{G2}$, -NR_{G1}S(O)$_2$N$R_{G1}R_{G2}$, -S(O)$_2$$R_{G3}$, -S(O)$_2NR_{G1}R_{G2}$, -N$R_{G1}$S(O)$_2$$R_{G2}$, -N$R_{G1}$C(=N$R_{G2}$)N$R_{G1}R_{G2}$, -C(=S)N$R_{G1}R_{G2}$, -C(=N$R_{G1}$)N$R_{G1}R_{G2}$, C_1-C_6alkyl optionally substituted by a hydroxyl group, halogen, -NO_2, and -CN, in which:

- R_{G1} and R_{G2} at each occurrence are each independently selected from the group consisting of hydrogen, and C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms;

- R_{G3} is C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; or

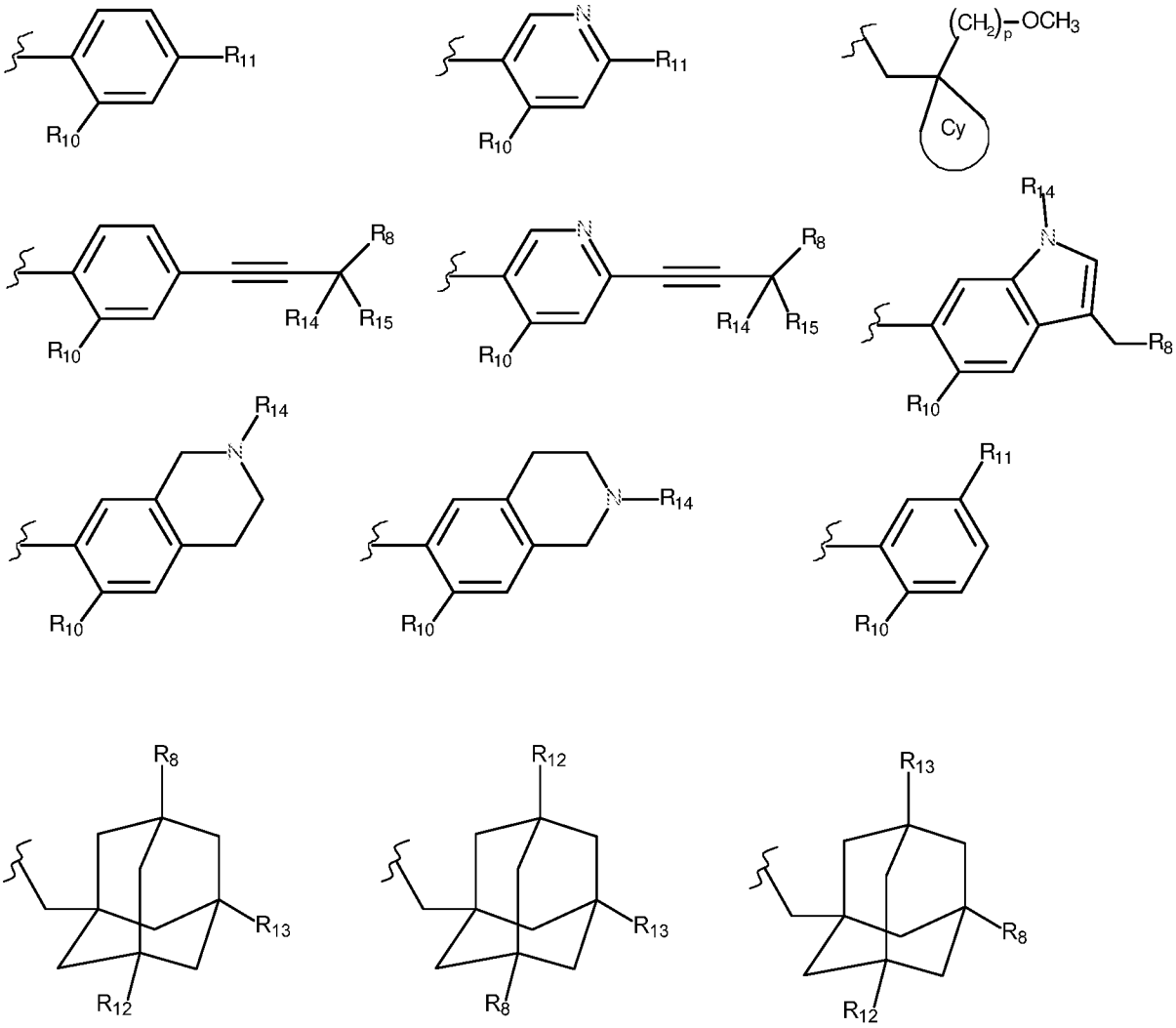
R_{G1} and R_{G2}, together with the atom to which each is attached are combined to form a C_3-C_8heterocycloalkyl;

- R_4 represents a hydrogen, fluorine, chlorine or bromine atom, a methyl, a hydroxyl or a methoxy group,
- R_5 represents a group selected from: C_1-C_6alkyl optionally substituted by 1 to 3 halogen atoms; halogen or –CN,
- R_6 represents a group selected from:

  -X_2-O-R_7; and

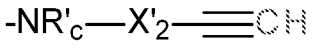
  an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,

- R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:



wherein Cy represents a C_3-C_8cycloalkyl,

- R_8 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl, -NR'$_a$R'$_b$; -NR'$_a$-CO-OR'$_c$; -NR'$_a$-CO-R'$_c$; -N$^+$R'$_a$R'$_b$R'$_c$; -O-R'$_c$; -NH-X'$_2$-N$^+$R'$_a$R'$_b$R'$_c$; -O-X'$_2$-NR'$_a$R'$_b$; -X'$_2$-NR'$_a$R'$_b$: -NR'$_c$-X'$_2$-N$_3$ and :

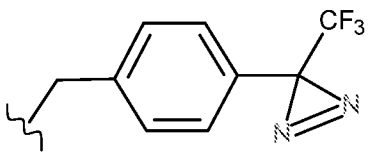


,

- R_{10} represents a group selected from hydrogen, fluorine, chlorine, bromine, -CF_3 and methyl,

- R_{11} represents a group selected from hydrogen, C_1-C_3alkylene-R_8, -O-C_1-C_3alkylene-R_8, -CO-NR_hR_i and -CH=CH-C_1-C_4alkylene-NR_hR_i, -CH=CH-CHO, C_3-C_8cycloalkylene-CH_2-R_8, C_3-C_8heterocycloalkylene-CH_2-R_8,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group, or R_{14} and R_{15} form with the carbon atom carrying them a a cyclohexyl,
- R_h and R_i, independently of one another, represent a hydrogen or a linear or branched C_1-C_6alkyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; heterocycloalkyl; -SO_2-phenyl wherein the phenyl may be substituted by a linear or branched C_1-C_6alkyl; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-SO_2OH; C_1-C_6alkylene-SO_2O^-; C_1-C_6alkylene-COOH; C_1-C_6alkylene-$PO(OH)_2$; C_1-C_6alkylene-$NR'_dR'_e$; C_1-C_6alkylene-$N^+R'_dR'_eR'_f$; C_1-C_6alkylene-O-C_1-C_6alkylene-OH; C_1-C_6alkylene-phenyl wherein the phenyl may be substituted by a hydroxyl or a C_1-C_6alkoxy group;

  the group:



  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,

  or R'_a, R'_b and R'_c form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- R'_c, R'_d, R'_e, R'_f, independently of one another, represents a hydrogen or a linear or

branched C_1-C_6alkyl group,

or R'_d and R'_e form with the nitrogen atom carrying them a cycle B_4,

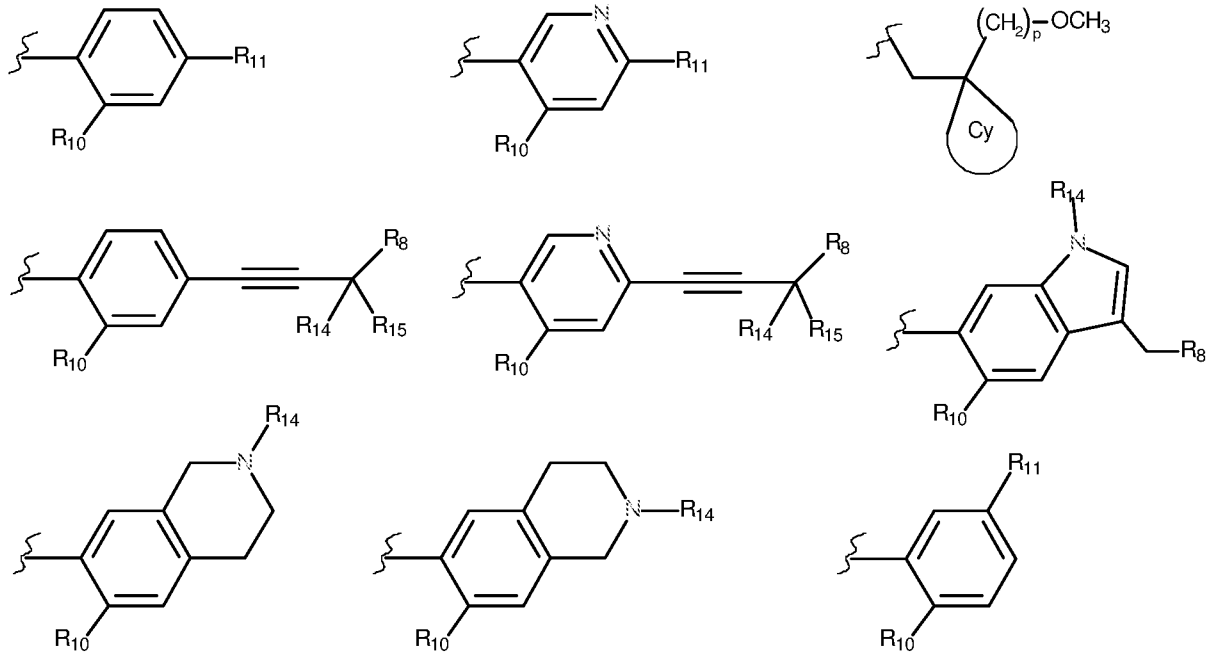
or R'_d, R'_e and R'_f form with the nitrogen atom carrying them a bridged C_3-C_8heterocycloalkyl,

- m=0, 1 or 2,
- p=1, 2, 3 or 4,
- B_3 and B_4, independently of one another, represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen, sulphur and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, $–NH_2$, oxo or piperidinyl.

47. The antibody-drug conjugate of claim 46, wherein G is selected from the group consisting of: -C(O)OH, -C(O)OR_{G3}, -C(O)$NR_{G1}R_{G2}$, -C(O)R_{G2}, -NR_{G1}C(O)R_{G2}, -NR_{G1}C(O)$NR_{G1}R_{G2}$, -OC(O)$NR_{G1}R_{G2}$, -NR_{G1}C(O)OR_{G3}, -C(=NOR_{G1})$NR_{G1}R_{G2}$, -NR_{G1}C(=NCN)$NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2NR_{G1}R_{G2}$, -$S(O)_2R_{G3}$, -$S(O)_2NR_{G1}R_{G2}$, -$NR_{G1}S(O)_2R_{G2}$, -$NR_{G1}$C(=$NR_{G2}$)$NR_{G1}R_{G2}$, -C(=S)$NR_{G1}R_{G2}$, -C(=$NR_{G1}$)$NR_{G1}R_{G2}$, halogen, $-NO_2$, and -CN.

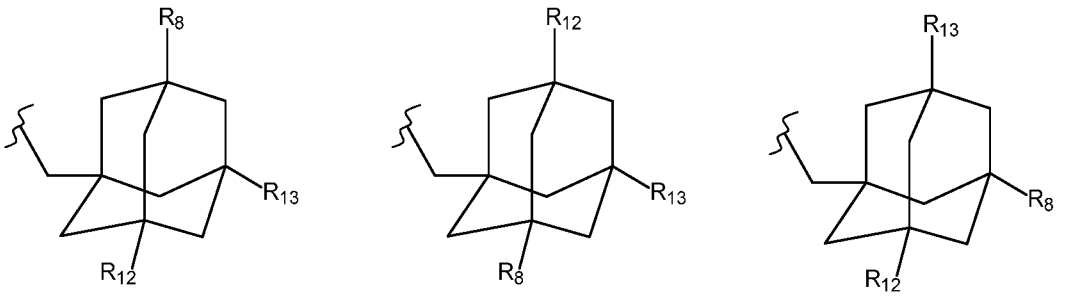
48. The antibody-drug conjugate of any one of claims 1-47, wherein

R_7 represents a group selected from: linear or branched C_1-C_6alkyl group; (C_3-C_6)cycloalkylene-R_8; or:

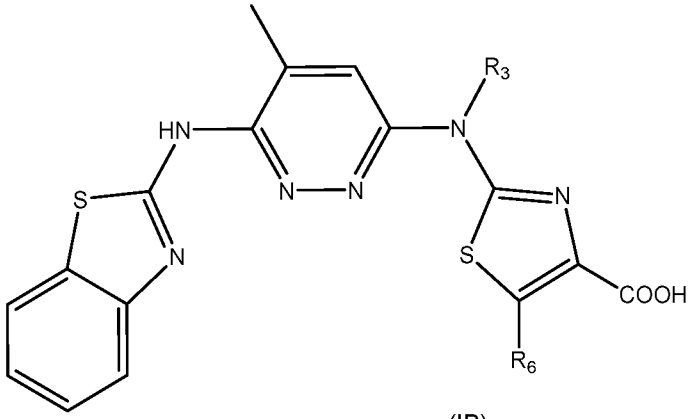


wherein Cy represents a C_3-C_8cycloalkyl.

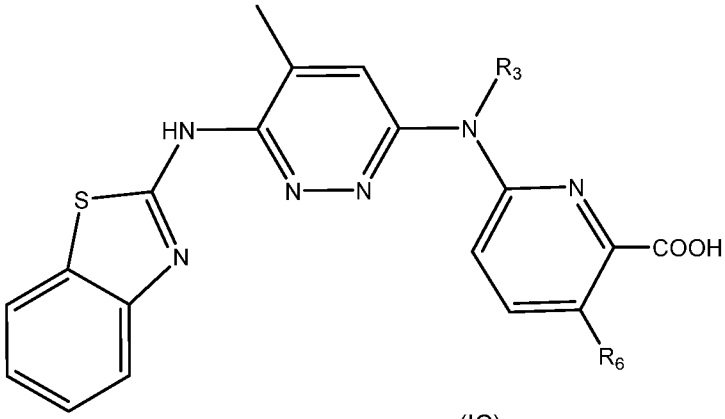
49. The antibody-drug conjugate of any one of claims 1-47, wherein R_7 represents a group selected from:



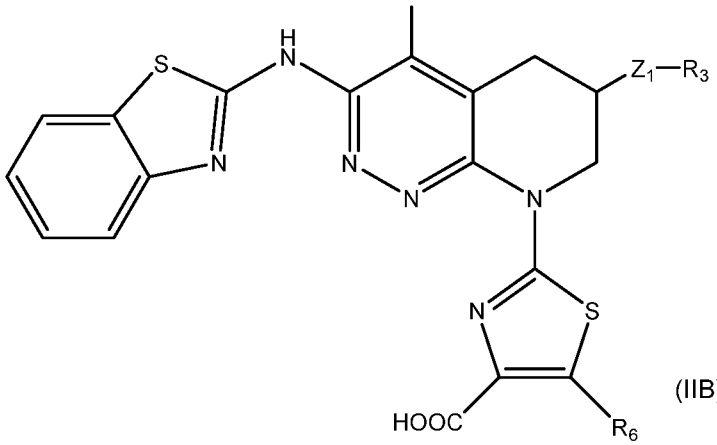
50. The antibody-drug conjugate of any one of claims 1-40, wherein D comprises a compound of formula (IB), (IC), (IIB) or (IIC):



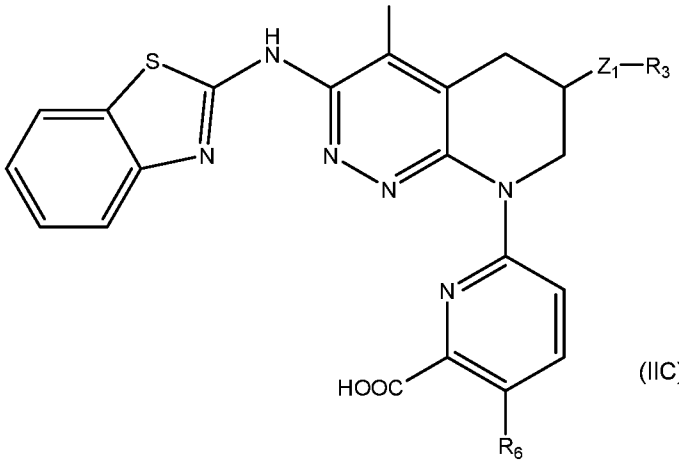
(IB) ,



(IC) ,



(IIB) , or

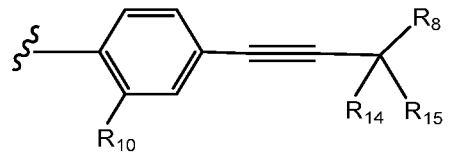


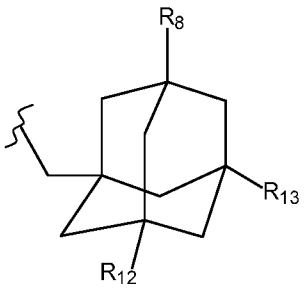
(IIC)

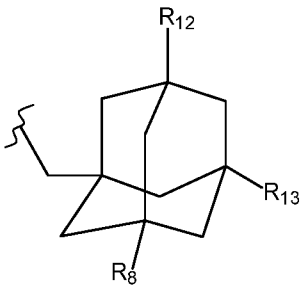
,

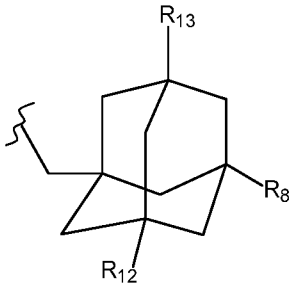
or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing, wherein:

- for formula (IB) or (IC), R_3 represents a group selected from: hydrogen; linear or branched C_1-C_6alkyl; -X_1-NR_aR_b; -X_1-$N^+R_aR_bR_c$; and -X_1-O-R_c;
  for formula (IIB) or (IIC), Z_1 represents a bond, and R_3 represents hydrogen; or Z_1 represents –O-, and R_3 represents –X_1-NR_aR_b,
- R_a and R_b independently of one another represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl groups; and C_1-C_6alkylene-SO_2O^-,
- R_c represents a hydrogen or a linear or branched C_1-C_6alkyl group
- R_6 represents –X_2-O-R_7 or an heteroarylene-R_7 group optionally substituted by a linear or branched C_1-C_6alkyl group,
- R_7 represents a group selected from:







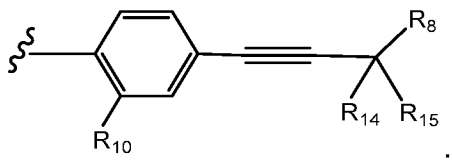


,

- R_8 represents a group selected from: $-NR'_aR'_b$; $-O-X'_2-NR'_aR'_b$; and $-X'_2-NR'_aR'_b$,
- R_{10} represents fluorine,
- R_{12} and R_{13}, independently of one another, represent a hydrogen atom or a methyl group,
- R_{14} and R_{15}, independently of one another, represent a hydrogen or a methyl group,
- X_1 and X_2 independently of one another, represent a linear or branched C_1-C_6alkylene group optionally substituted by one or two groups selected from trifluoromethyl, hydroxyl, halogen, C_1-C_6alkoxy,
- X'_2 represents a linear or branched C_1-C_6alkylene,
- R'_a and R'_b independently of one another, represent a group selected from: hydrogen; linear or branched C_1-C_6alkyl optionally substituted by one or two hydroxyl or C_1-C_6alkoxy groups; C_1-C_6alkylene-$NR'_dR'_e$;

  or R'_a and R'_b form with the nitrogen atom carrying them a cycle B_3,
- R'_d, R'_e independently of one another, represents a hydrogen or a linear or branched C_1-C_6alkyl group,
- B_3 represents a C_3-C_8heterocycloalkyl group, which group can: (i) be a mono- or bi-cyclic group, wherein bicyclic group includes fused, briged or spiro ring system, (ii) can contain, in addition to the nitrogen atom, one or two hetero atoms selected independently from oxygen and nitrogen, (iii) be substituted by one or two groups selected from: fluorine, bromine, chlorine, linear or branched C_1-C_6alkyl, hydroxyl, and oxo.

51. The antibody-drug conjugate of any one of claims 1-50, wherein R_7 represents the following group:



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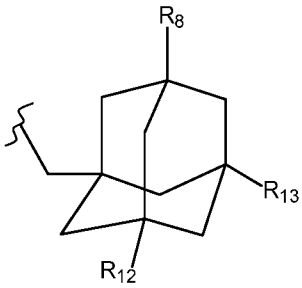
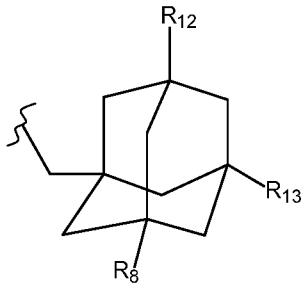
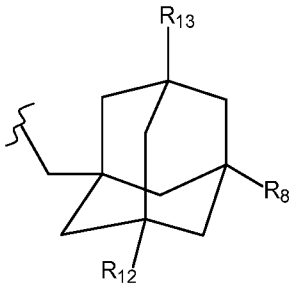
52. The antibody-drug conjugate of any one of claims 1-50, wherein R_7 represents a group selected from:

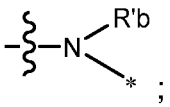


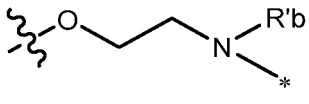


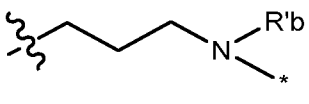


.

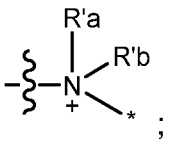
53. The antibody-drug conjugate of any one of claims 41 to 52, wherein R_8 represents a group selected from:

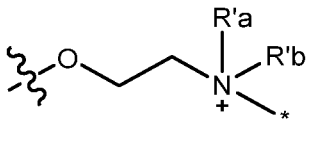


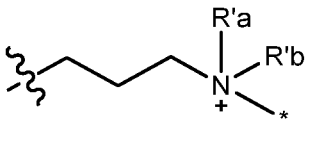
;


; and


,



;


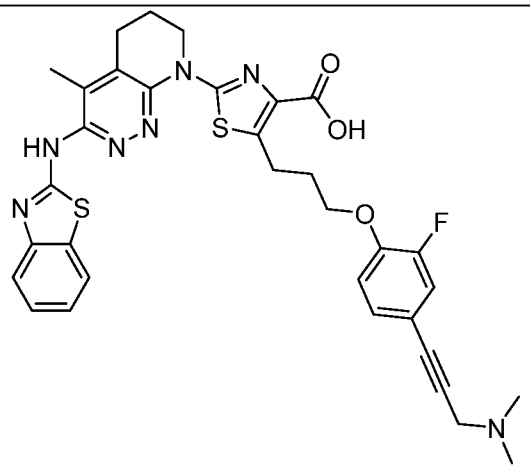
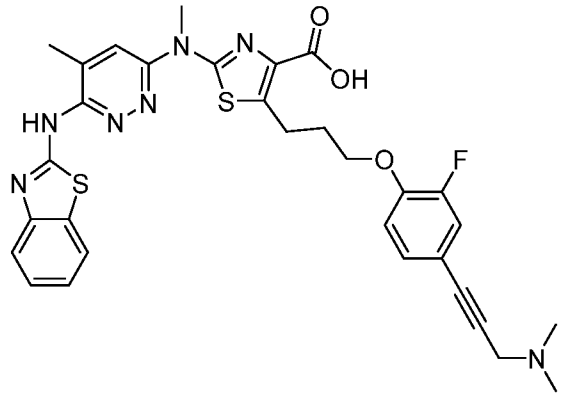
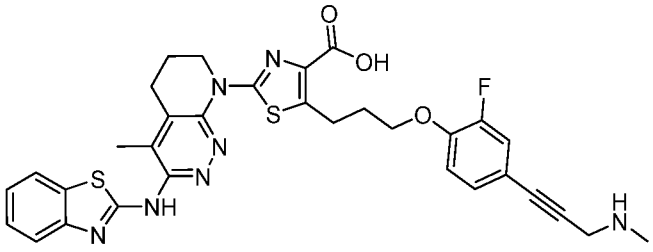
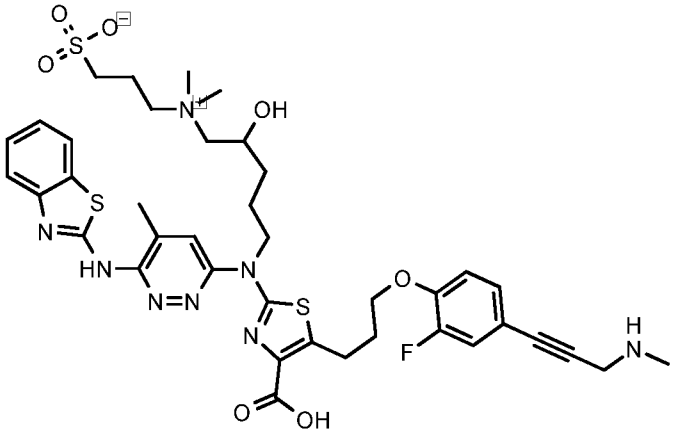
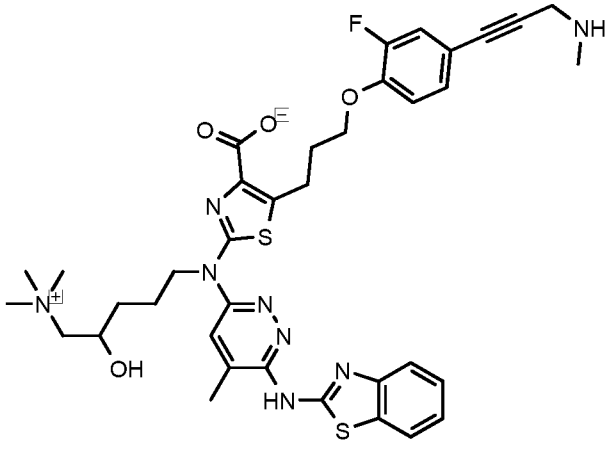
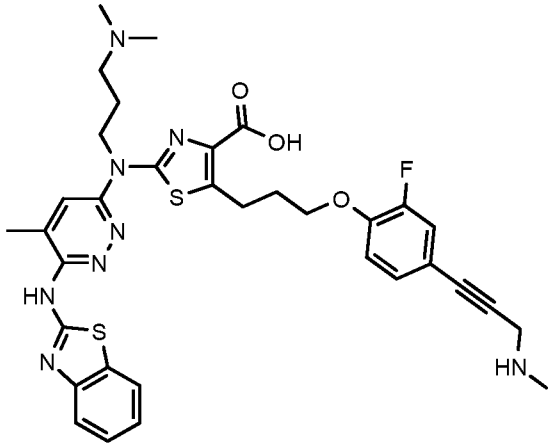
; and


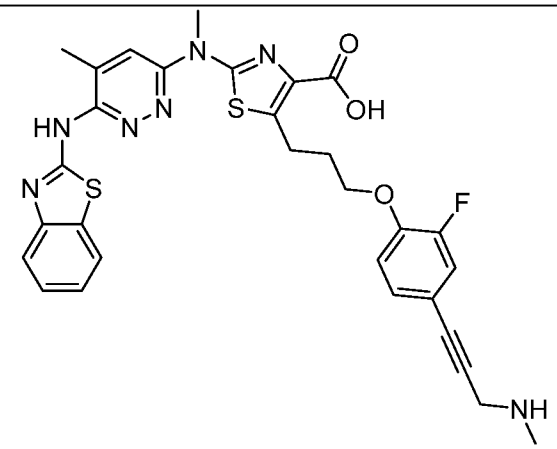
,

wherein —— * represents a bond to the linker.

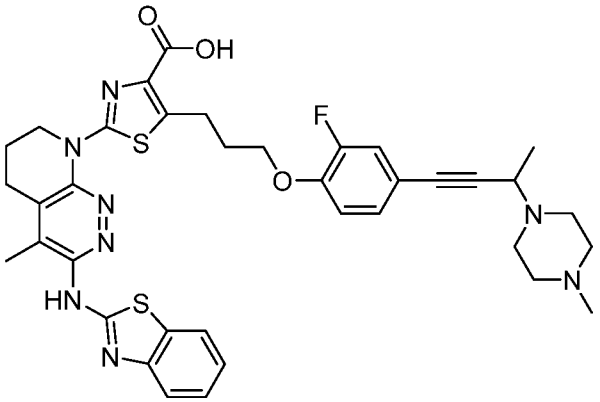
54. The antibody-drug conjugate of any one of claims 41 to 53, wherein B_3 represents a C_3-C_8heterocycloalkyl group selected from a pyrrolidinyl group, a piperidinyl group, a piperazinyl group, a morpholinyl group, an azepanyl group, and a 4,4-difluoropiperidin-1-yl group.

55. The antibody-drug conjugate of any one of claims 1 to 40, wherein D represents any one of the following attached to L:

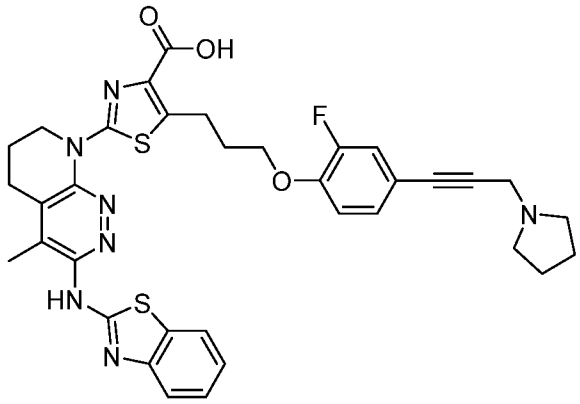
 P1	 P2
 P3	 P4
 P5	 P6



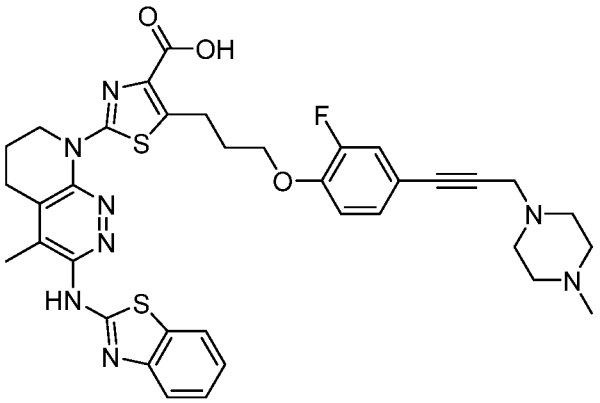
P7



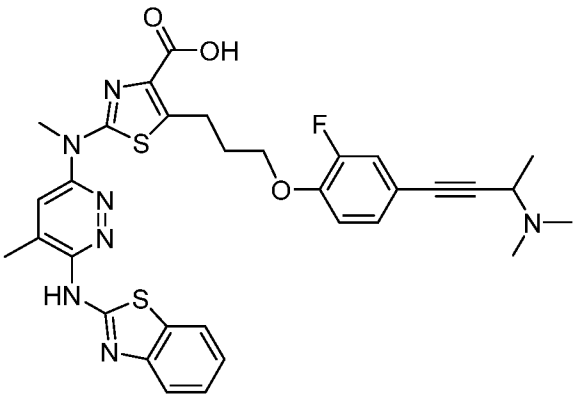
P8



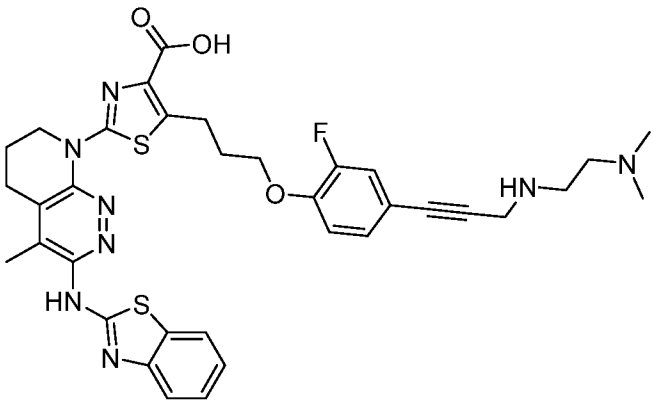
P9



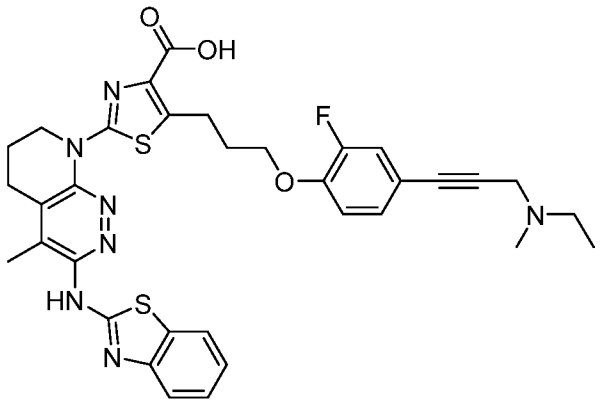
P10



P11



P12



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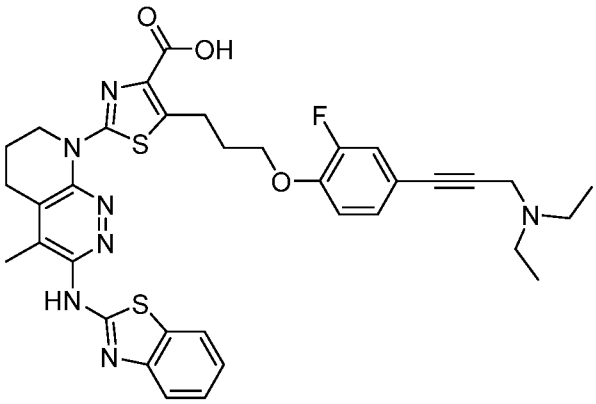

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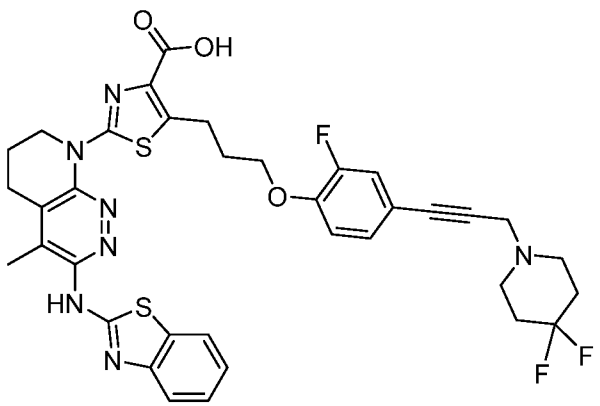

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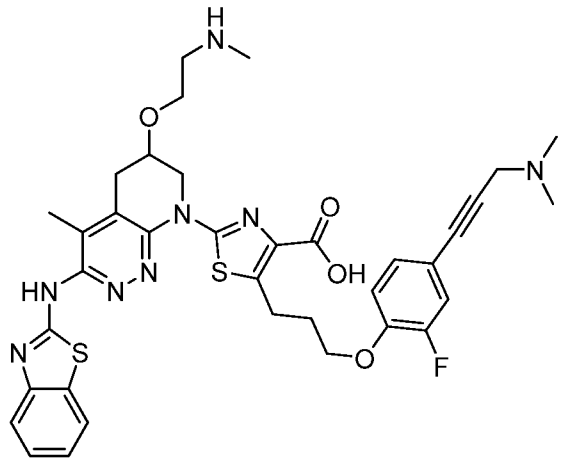

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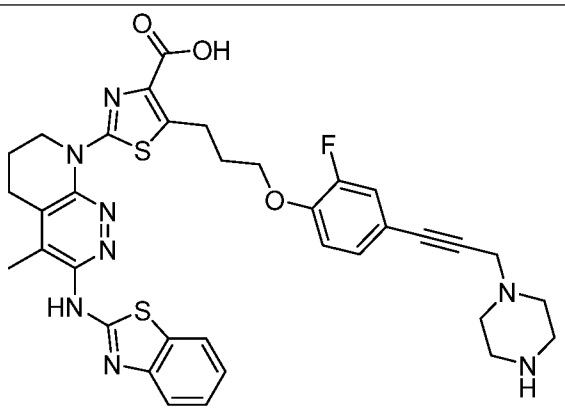

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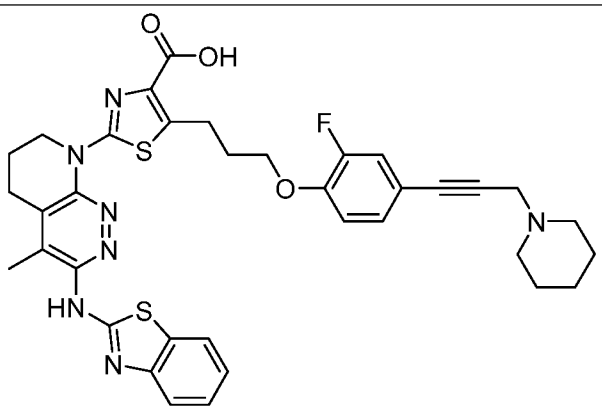

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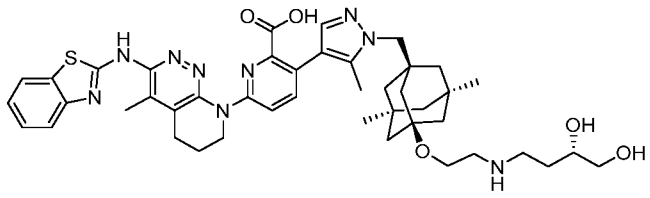
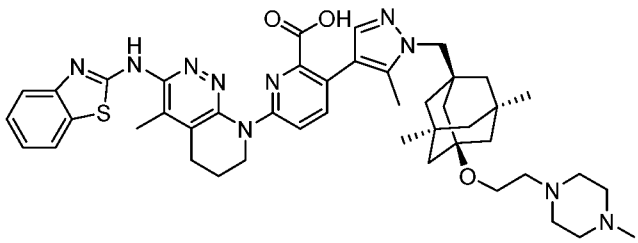
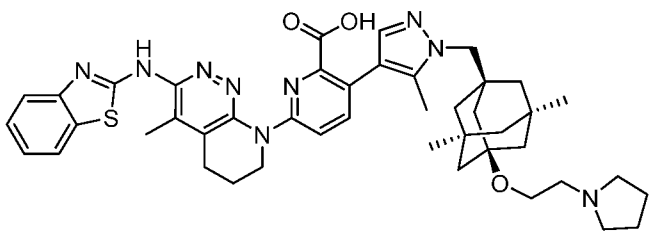
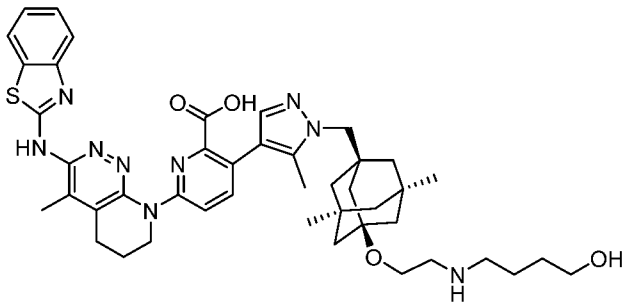
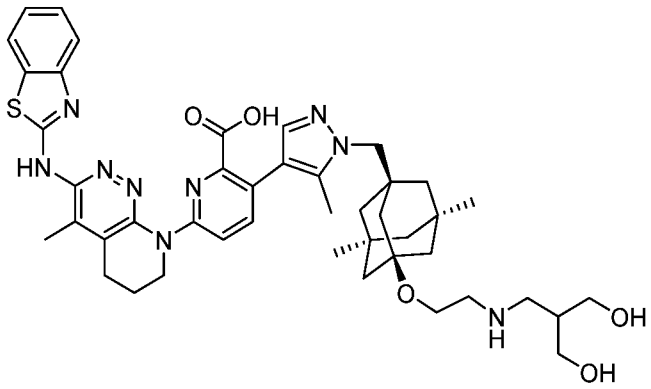
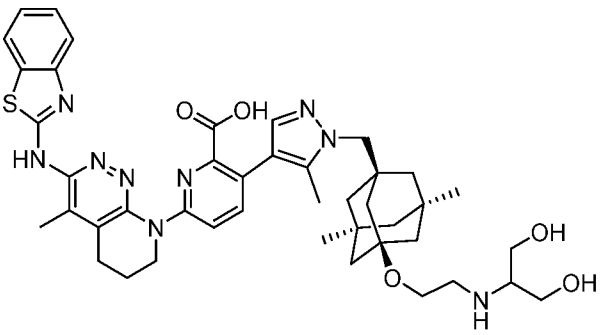
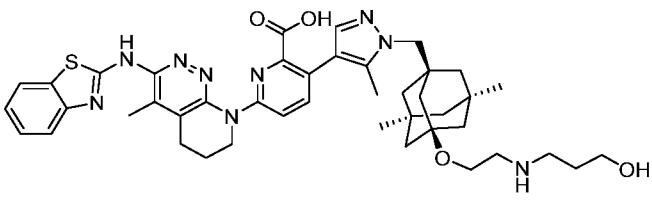
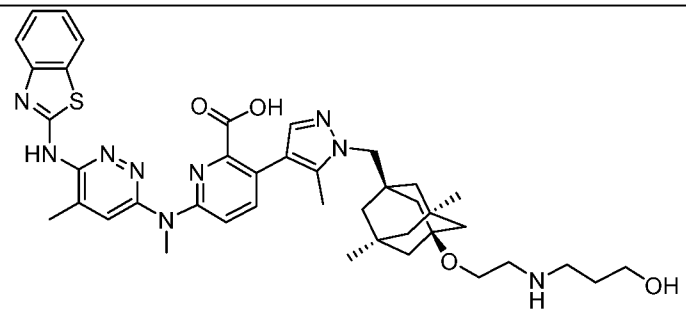
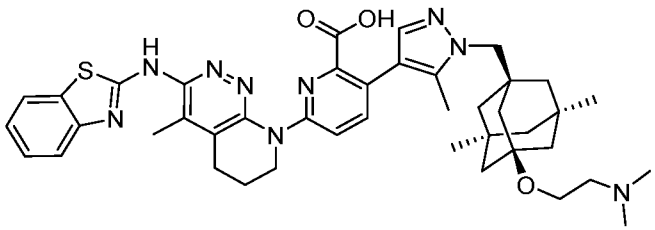
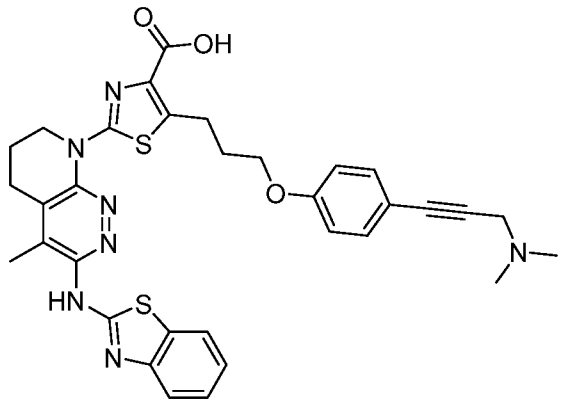
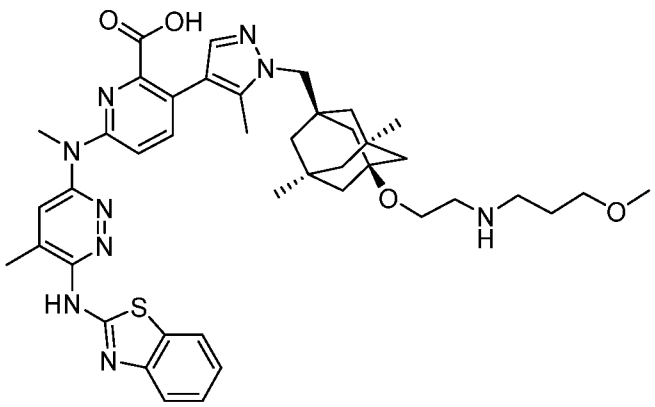
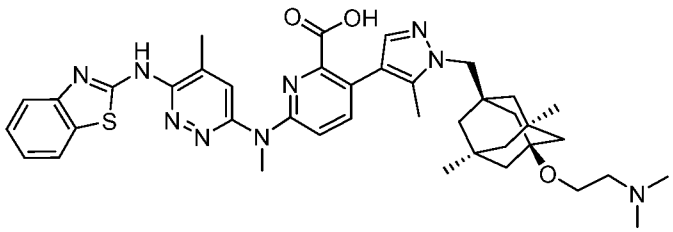
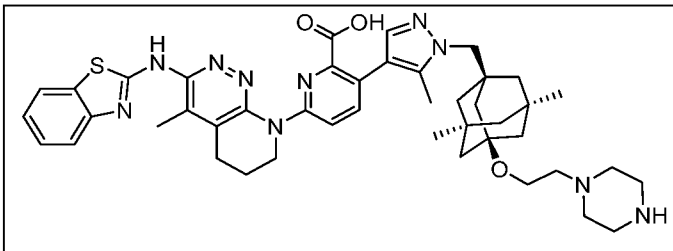
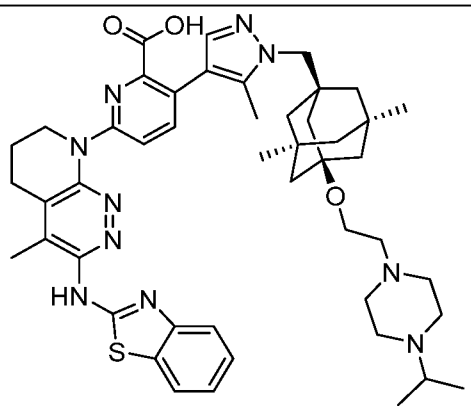
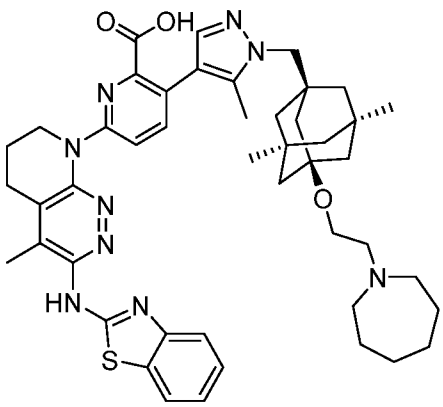
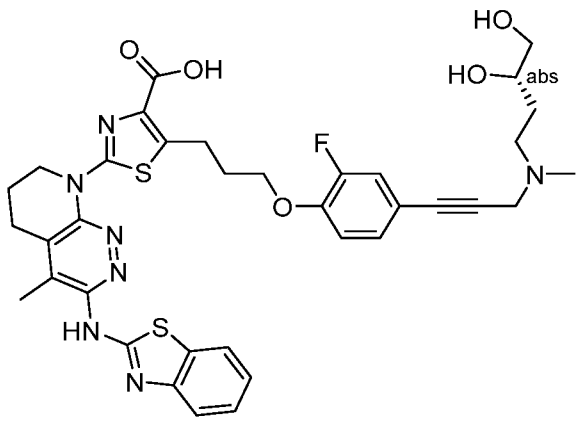
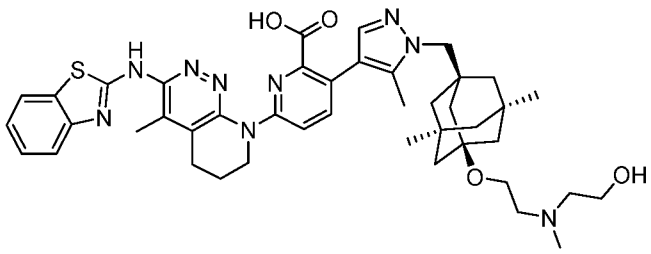
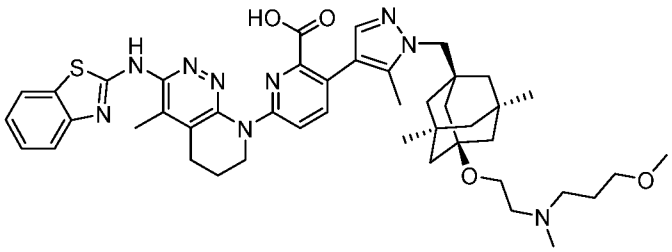
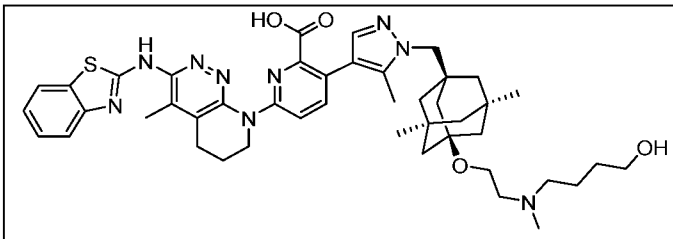
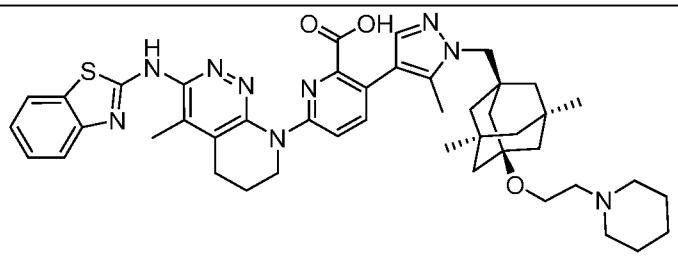
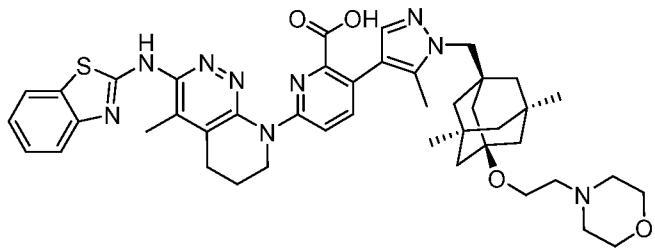
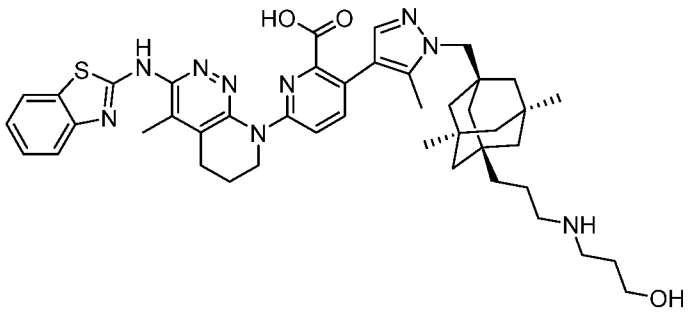
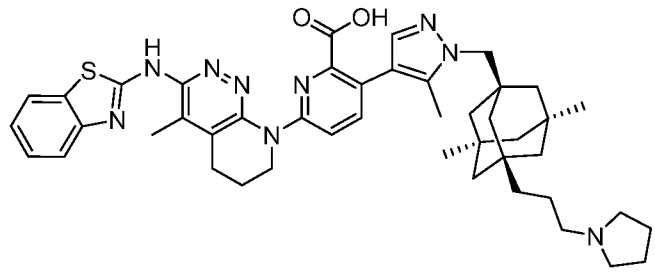
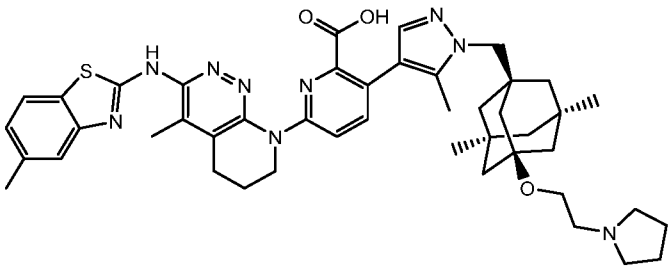
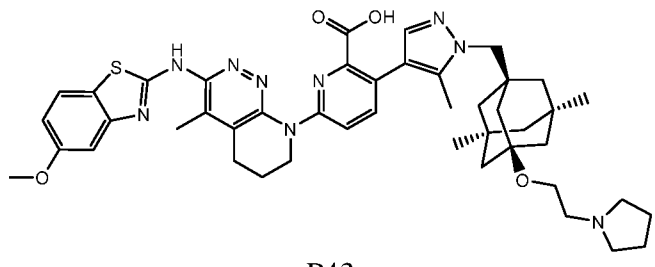
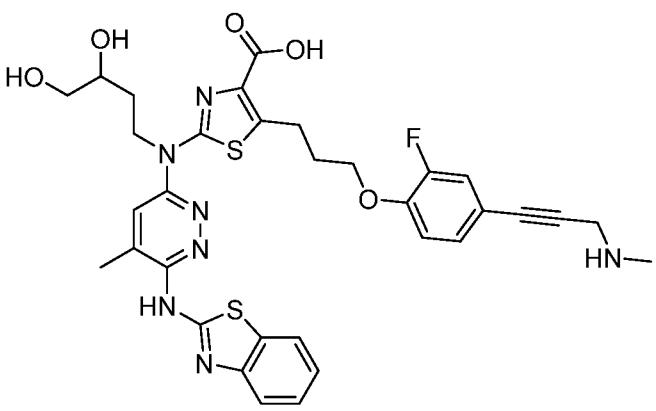
 P19	 P20
 P21	 P22
 P23	 P24
	

P25	 P26
 P27	 P28
 P29	 P30

 P31	 P32
 P33	 P34
 P35	 P36

 P37	 P38
 P39	 P40
 P41	 P42
 P43	

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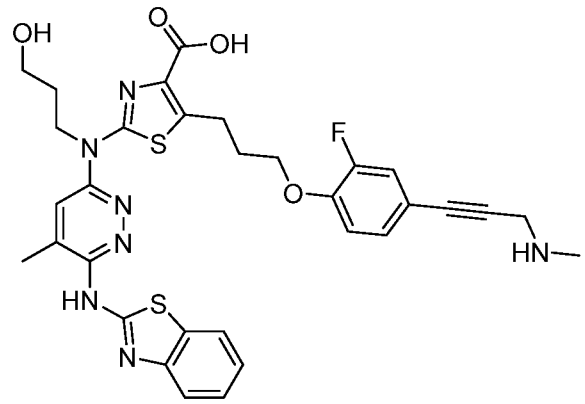
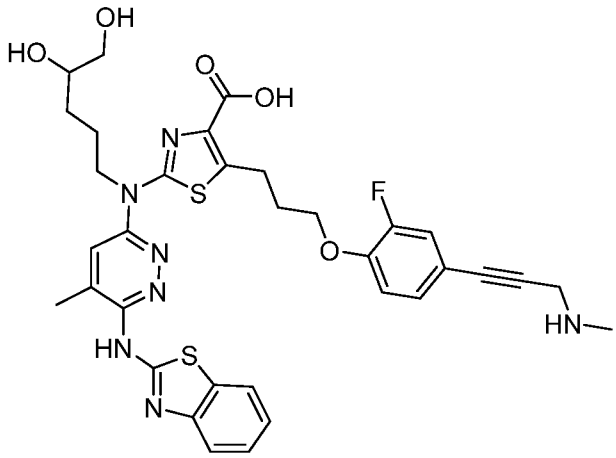
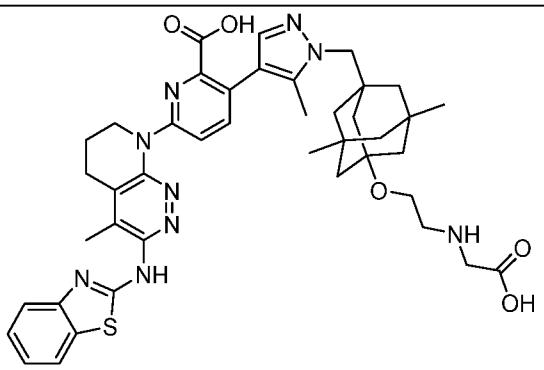
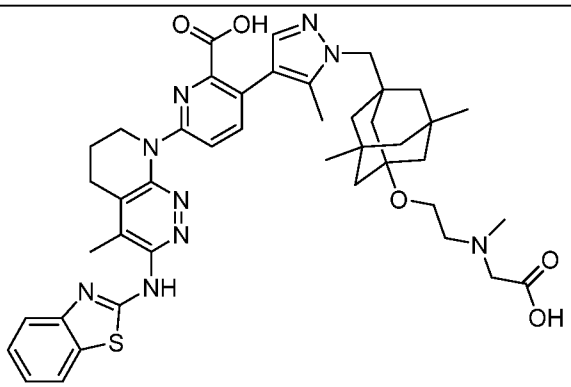
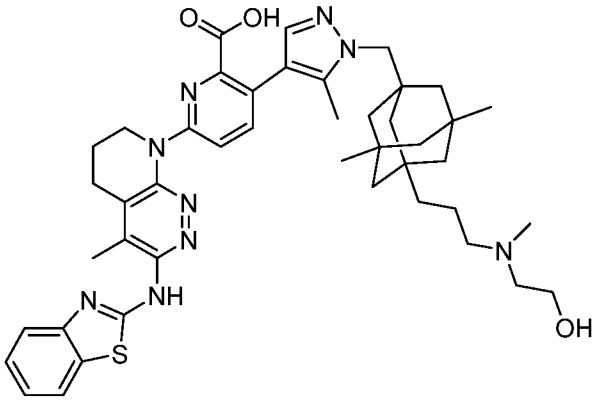
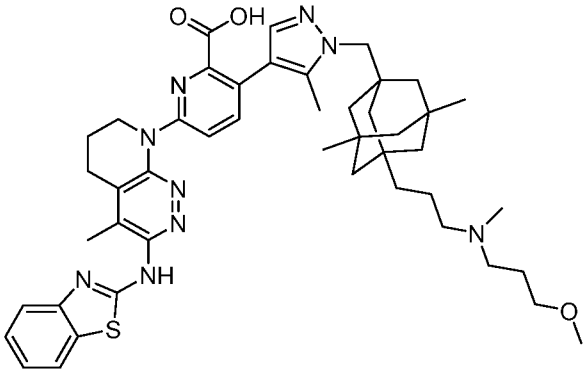
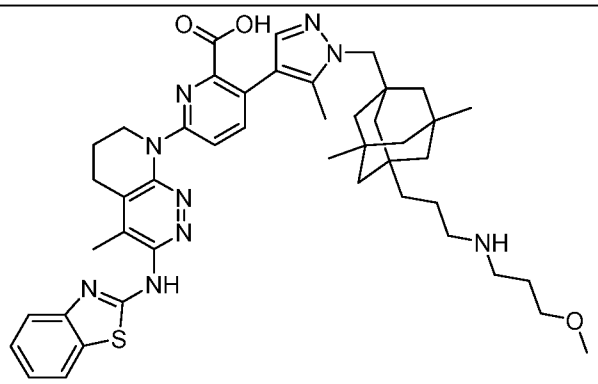
 P45	 P46
 P47	 P48
 P49	 P50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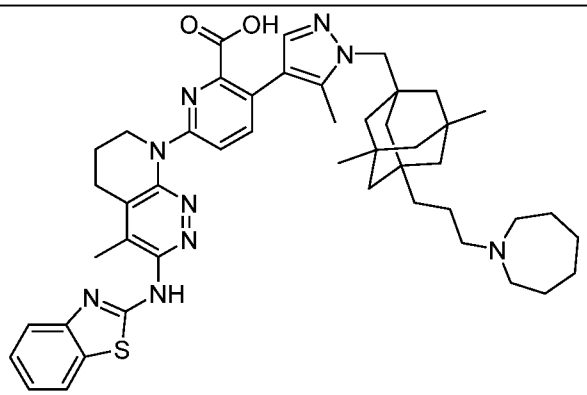
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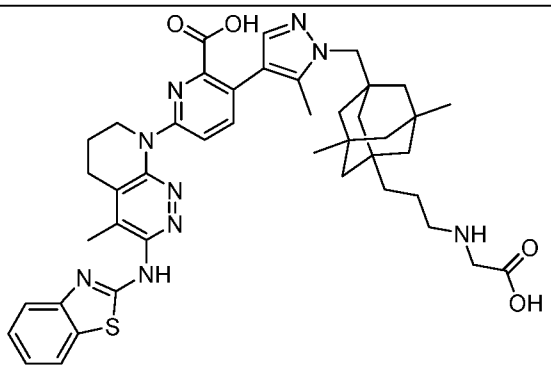
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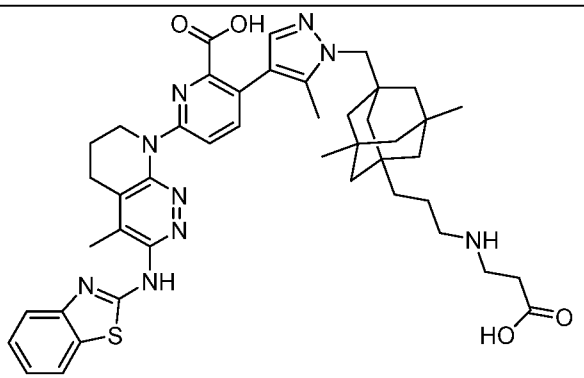
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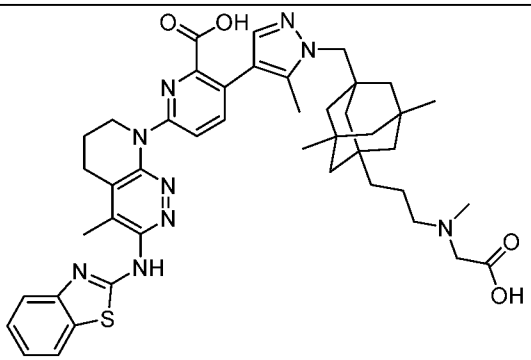
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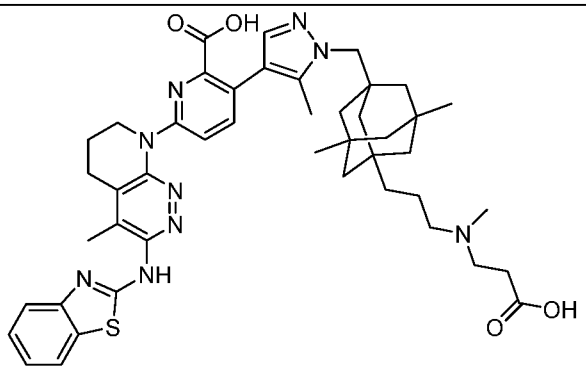
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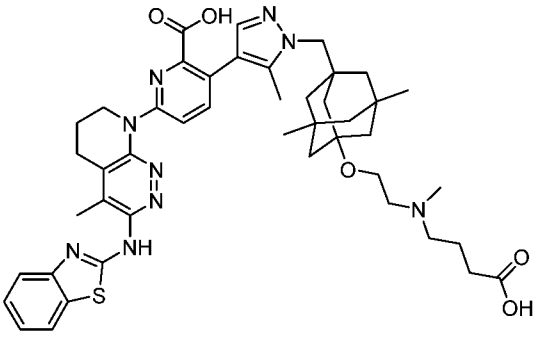
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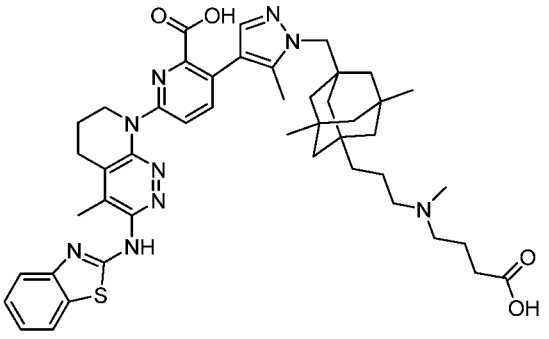
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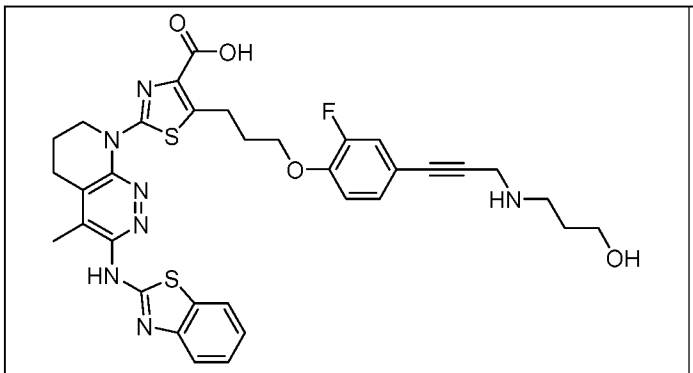
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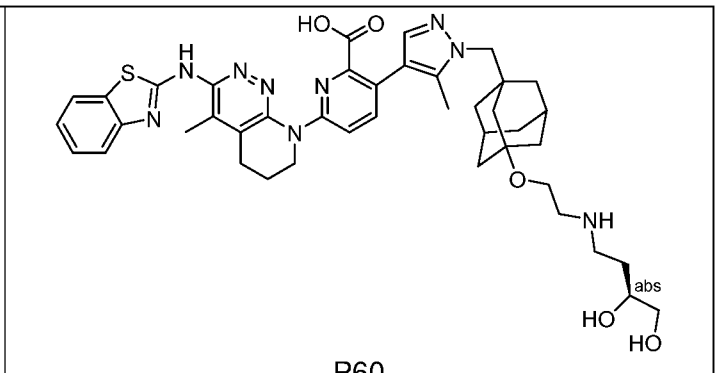
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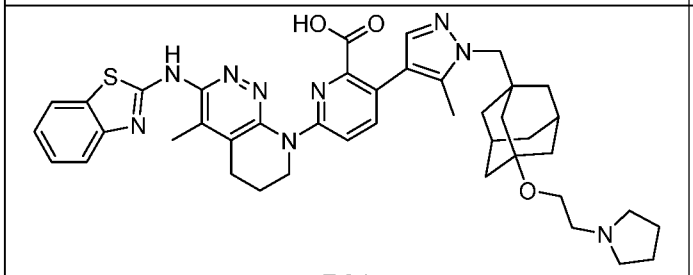
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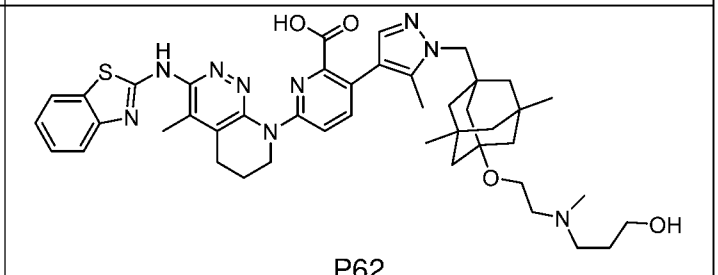
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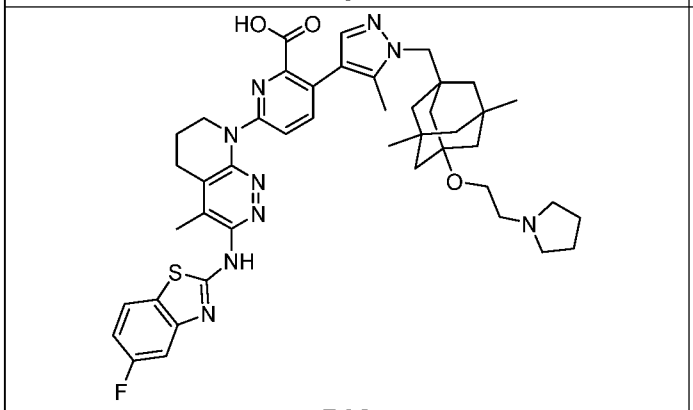
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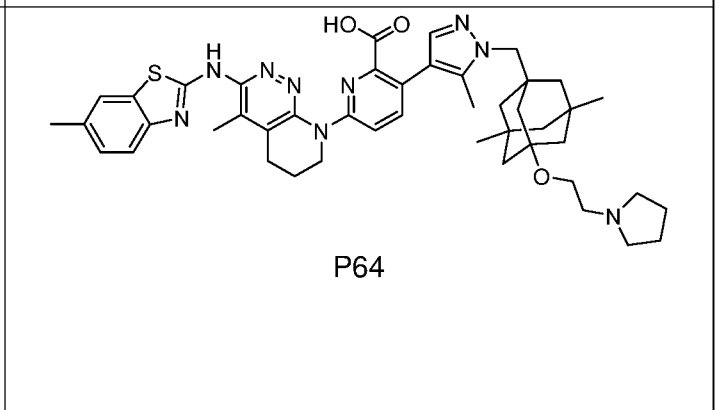
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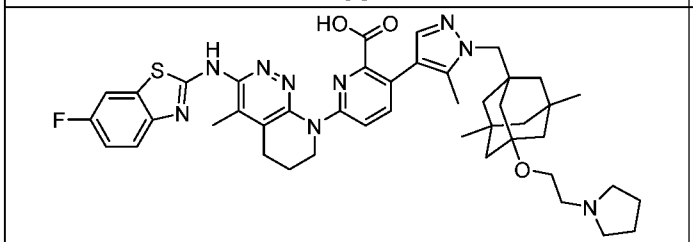
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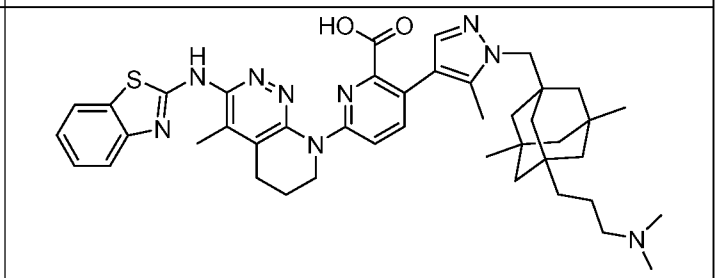
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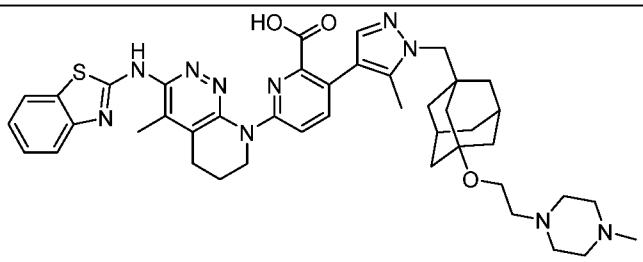
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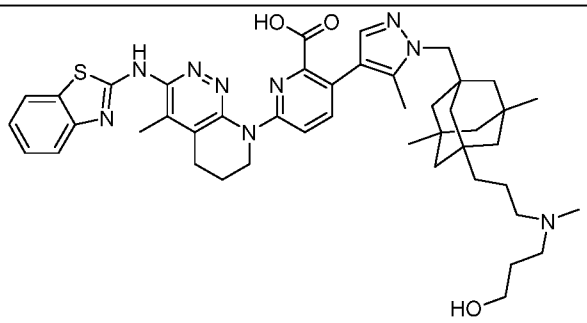
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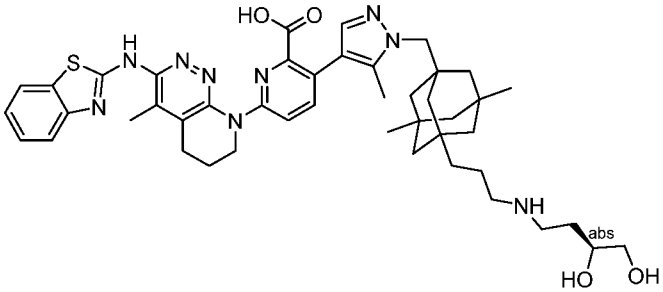
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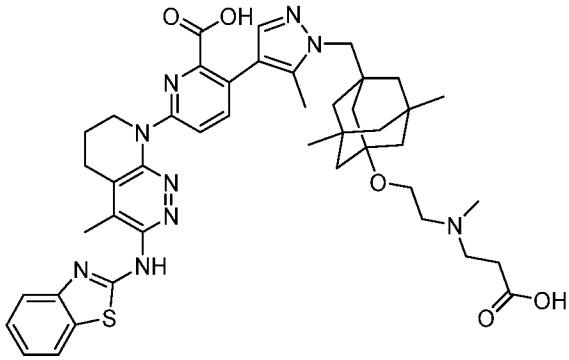
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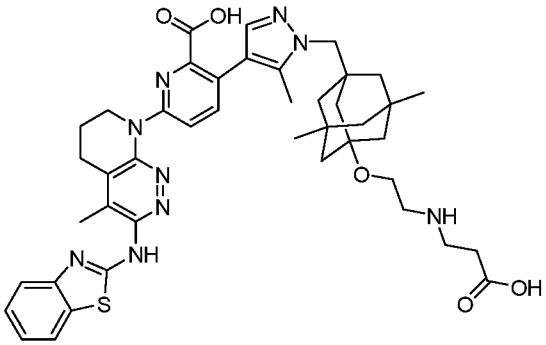
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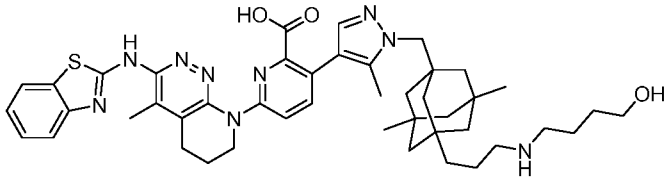
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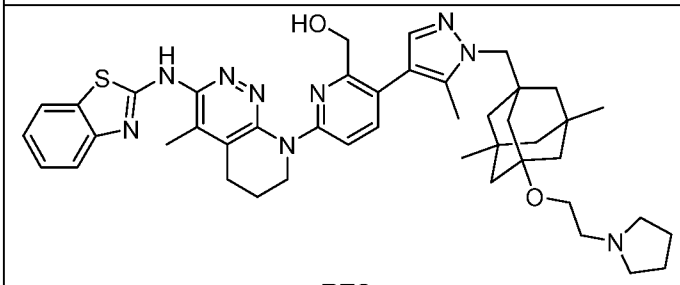
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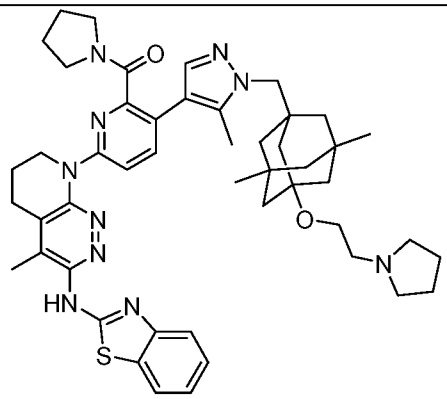
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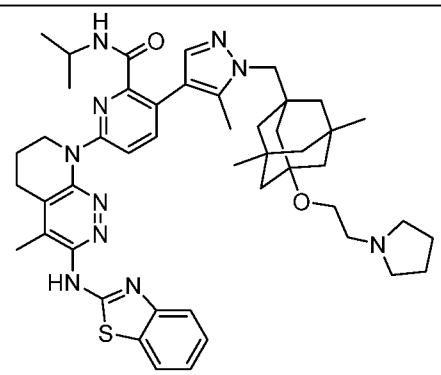
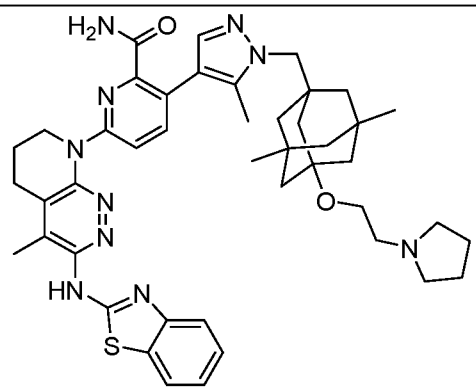
 P75	 P76

or an enantiomer, a diastereoisomer, and/or a pharmaceutically acceptable salt of any one of the foregoing.

56. The antibody-drug conjugate of any one of claims 1 to 40, wherein D comprises a group represented by a formula selected from those in Table A2.

57. The antibody-drug conjugate any one of claims 1 to 40, wherein -(L-D) is formed from a compound in Table B or an enantiomer, diastereoisomer, and/or pharmaceutically acceptable salt of any of the foregoing.

58. The antibody-drug conjugate of any one of claims 1 to 57, wherein the antibody or antigen-binding fragment binds to a target antigen on the cancer cell.

59. The antibody-drug conjugate of claim 58, wherein:
(i) the target antigen is selected from BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, SEZ6, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, and GPNMB;
(ii) the target antigen is selected from EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, and GPNMB; or
(iii) the target antigen is EGFR, CD7, or HER2.

60. The antibody-drug conjugate of any one of claims 1 to 59, wherein the antibody or antigen-binding fragment is an anti-EGFR antibody or antigen-binding fragment;.

61. The antibody-drug conjugate of claim 60, wherein the anti-EGFR antibody or antigen-binding fragment comprises:
(a) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:92, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:93; or
(b) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:124, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:125.

62. The antibody-drug conjugate of any one of claims 1 to 59, wherein the antibody or antigen-binding fragment is an anti-CD7 antibody or antigen-binding fragment.

63. The antibody-drug conjugate of claim 62, wherein the anti-CD7 antibody comprises: the heavy chain amino acid sequence of SEQ ID NO:143, and the light chain amino acid sequence of SEQ ID NO:144.

64. The antibody-drug conjugate of any one of claims 1 to 59, wherein the antibody or antigen-binding fragment is an anti-HER2 antibody or antigen-binding fragment.

65. The antibody-drug conjugate of claim 64, wherein:
(a) the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDRs) comprising amino acid sequences of SEQ ID NO:39 (HCDR1), SEQ ID NO:40 (HCDR2), and SEQ ID NO:41 (HCDR3); and three light chain complementarity determining regions (LCDRs) comprising amino acid sequences of SEQ ID NO:42 (LCDR1), SEQ ID NO:43 (LCDR2), and SEQ ID NO:44 (LCDR3); and/or
(b) the antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO:10.

66. The antibody-drug conjugate of claim 64 or 65, wherein:
(a) the antibody or antigen-binding fragment comprises an IgG1 heavy chain constant domain or a modified IgG1 heavy chain constant domain, optionally the IgG1 heavy chain constant domain comprises a glutamine (Q) at position 297 or the IgG1 heavy chain constant domain comprises a serine (S) at position 297; and/or
(b) the antibody or antigen-binding fragment comprises an Ig kappa light chain constant domain.

67. A composition comprising multiple copies of the antibody-drug conjugate of any one of claims 1 to 66, wherein the average *p* of the antibody-drug conjugates in the composition is from about 2 to about 16, e.g., about 2 to about 8, e.g., about 2 to about 4.

68. A pharmaceutical composition comprising the antibody-drug conjugate of any one of claims 1 to 64 or the composition of claim 67, and a pharmaceutically acceptable carrier.

69. A method of treating a subject having or suspected of having a cancer, comprising administering to the subject a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 66, the composition of claim 67, or the pharmaceutical composition of claim 68.

70. The method of claim 69, wherein the cancer expresses a target antigen.

71. The method of claim 69 or 70, wherein the cancer is a tumor or a hematological cancer, optionally, the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer.

72. A method of reducing or inhibiting the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 66, the composition of claim 65, or the pharmaceutical composition of claim 66.

73. The method of claim 72, wherein the tumor expresses a target antigen.

74. The method of claim 72 or 73, wherein the tumor is a breast cancer, gastric cancer, bladder cancer, brain cancer, cervical cancer, colorectal cancer, esophageal cancer,

hepatocellular cancer, melanoma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, or spleen cancer.

75. A method of reducing or inhibiting a hematological cancer in a subject, comprising administering to the subject a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 66, the composition of claim 67, or the pharmaceutical composition of claim 68.

76. The method of claim 75, wherein the hematological cancer expresses a target antigen.

77. The method of claim 75 or 76, wherein the hematological cancer is chronic lymphocytic leukemia (CLL), follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), acute monocytic leukemia (AMoL), Hodgkin's lymphoma, non-Hodgkin's lymphoma or myelodysplasia syndrome (MDS).

78. The method of any one of claims 72 to 77, wherein administration of the antibody-drug conjugate, composition, or pharmaceutical composition reduces or inhibits the growth of the tumor or hematological cancer by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%.

79. A method of reducing or slowing the expansion of a cancer cell population in a subject, comprising administering to the subject a therapeutically effective amount of the antibody-drug conjugate of any one of claims 1 to 66, the composition of claim 67, or the pharmaceutical composition of claim 68.

80. The method of claim 79, wherein the cancer cell population expresses a target antigen.

81. The method of claim 79 or 80, wherein the cancer cell population is from a tumor or a hematological cancer, optionally wherein the cancer cell population is from a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic

lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer.

82. The method of any one of claims 79 to 81, wherein administration of the antibody-drug conjugate, composition, or pharmaceutical composition reduces the cancer cell population or slows the expansion of the cancer cell population by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99%.

83. The method of any one of claims 69 to 82, wherein the antibody-drug conjugate is administered as monotherapy.

84. The method of any one of claims 69 to 82, wherein the antibody-drug conjugate is administered adjunctive o another therapeutic agent or radiation therapy.

85. The method of claim 84, wherein the antibody-drug conjugate is administered in an amount effective to sensitize the tumor cells to one or more additional therapeutic agents and/or radiation therapy.

86. The method of any one of claims 69 to 82, further comprising administering to the subject in need thereof at least one additional therapeutic agent.

87. The method of claim 86, wherein the one additional therapeutic agent is a Bcl-2 inhibitor, a taxane, a *vinca* alkaloid, a MEK inhibitor, an ERK inhibitor, topoisomerase inhibitor, or a RAF inhibitor.

88. The method of claim 86, wherein the one additional additional therapeutic agent is selected from venetoclax, compound A2, vincristine, topotecan, docetaxel, paclitaxel, LTT463, trametinib, and LXH254.

89. A method of inhibiting Bcl-xL activity in a cell that expresses Bcl-xL, comprising contacting the cell with an antibody-drug conjugate of any one of claims 1 to 66 that is capable of binding the cell, under conditions in which the antibody drug conjugate binds the cell.

90. A method of determining whether a subject having or suspected of having a cancer will be responsive to treatment with the antibody-drug conjugate of any one of claims 1 to 66, the composition of claim 67, or the pharmaceutical composition of claim 68, comprising providing a biological sample from the subject; contacting the sample with the antibody-drug conjugate; and detecting binding of the antibody-drug conjugate to cancer cells in the sample.

91. The method of claim 90, wherein the cancer cells in the sample express a target antigen.

92. The method of claim 90 or claim 91, wherein the cancer expresses a target antigen.

93. The method of any one of claims 90 to 92, wherein the cancer is a tumor or a hematological cancer, optionally the cancer is a breast cancer, multiple myeloma, plasma cell myeloma, leukemia, lymphoma, sarcoma, gastric cancer, acute myeloid leukemia, bladder cancer, brain cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia including acute lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, prostate cancer, small cell lung cancer, spleen cancer, or head and neck cancer.

94. The method of any one of claims 90 to 91, wherein the sample is a tissue biopsy sample, a blood sample, or a bone marrow sample.

95. The method of any one of claims 70 to 94, wherein:
(i) the target antigen is selected from BCMA, CD33, HER2, CD38, CD48, CD79b, PCAD, CD74, CD138, SLAMF7, CD123, CLL1, FLT3, CD7, CKIT, CD56, SEZ6, DLL3, DLK1, B7-H3, EGFR, CD71, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, TROP2, LIV1, CD46, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, and GPNMB;
(ii) the target antigen is selected from EGFR, CD7, HER2, EPCAM, FOLR1, ENPP3, MET, AXL, SLC34A2, Nectin4, MSLN, F3, MUC16, SLC39A6, TFRC, TACSTD2, and GPNMB; or
(iii) the target antigen is selected from EGFR, CD7, and HER2.

96. A method of producing the antibody-drug conjugate of any one of claims 1 to 67, comprising reacting an antibody or antigen-binding fragment with a cleavable linker joined to a Bcl-xL inhibitor under conditions that allow conjugation.

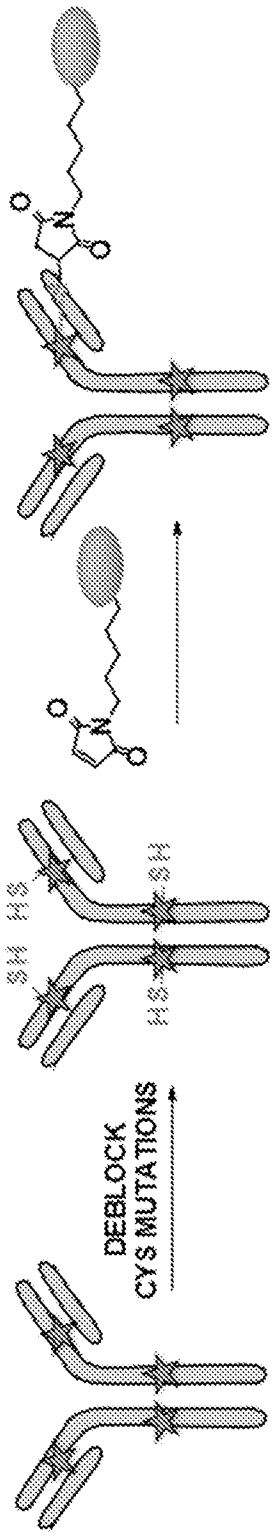

DEBLOCK
CYS MUTATIONS
SH
HS
SH
HS

FIG. 1

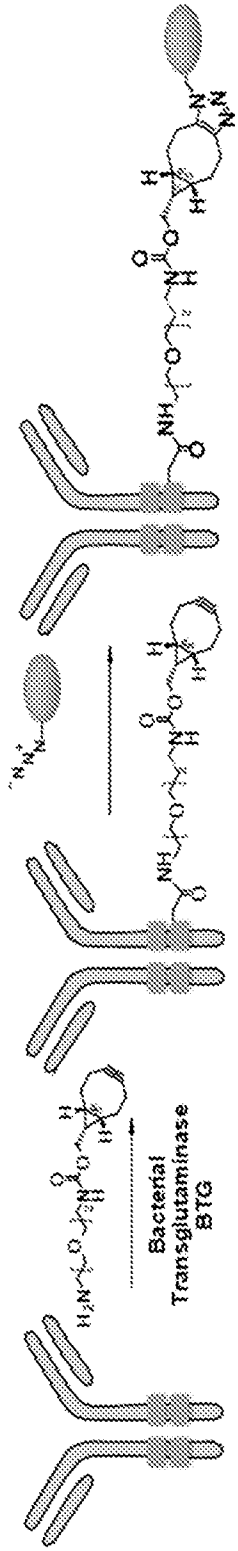

Bacterial
Transglutaminase
BTG

FIG. 2

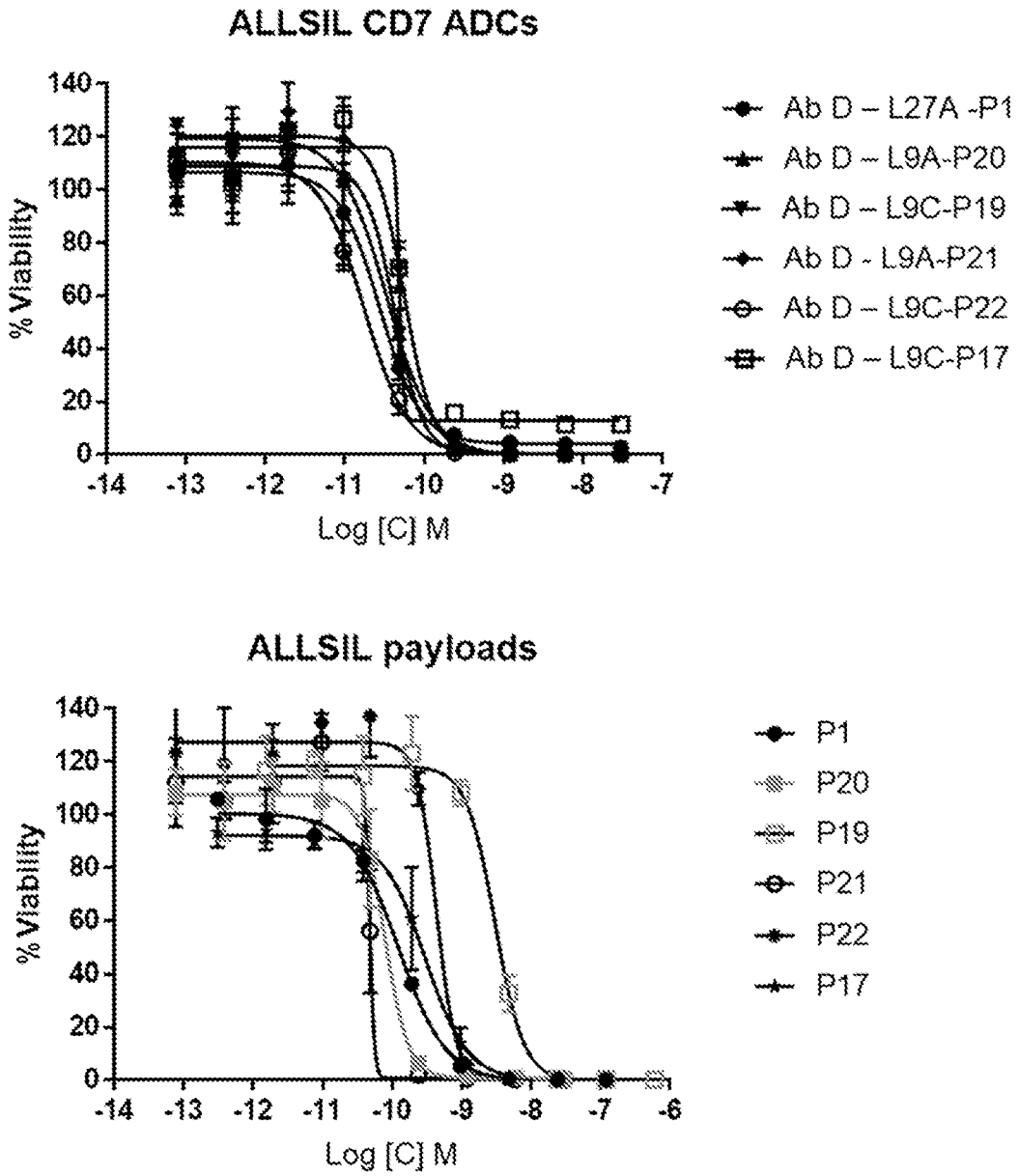

ALLSIL CD7 ADCs
% Viability
Log [C] M
Ab D – L27A -P1
Ab D – L9A-P20
Ab D – L9C-P19
Ab D - L9A-P21
Ab D – L9C-P22
Ab D – L9C-P17
ALLSIL payloads
% Viability
Log [C] M
P1
P20
P19
P21
P22
P17

FIG. 3

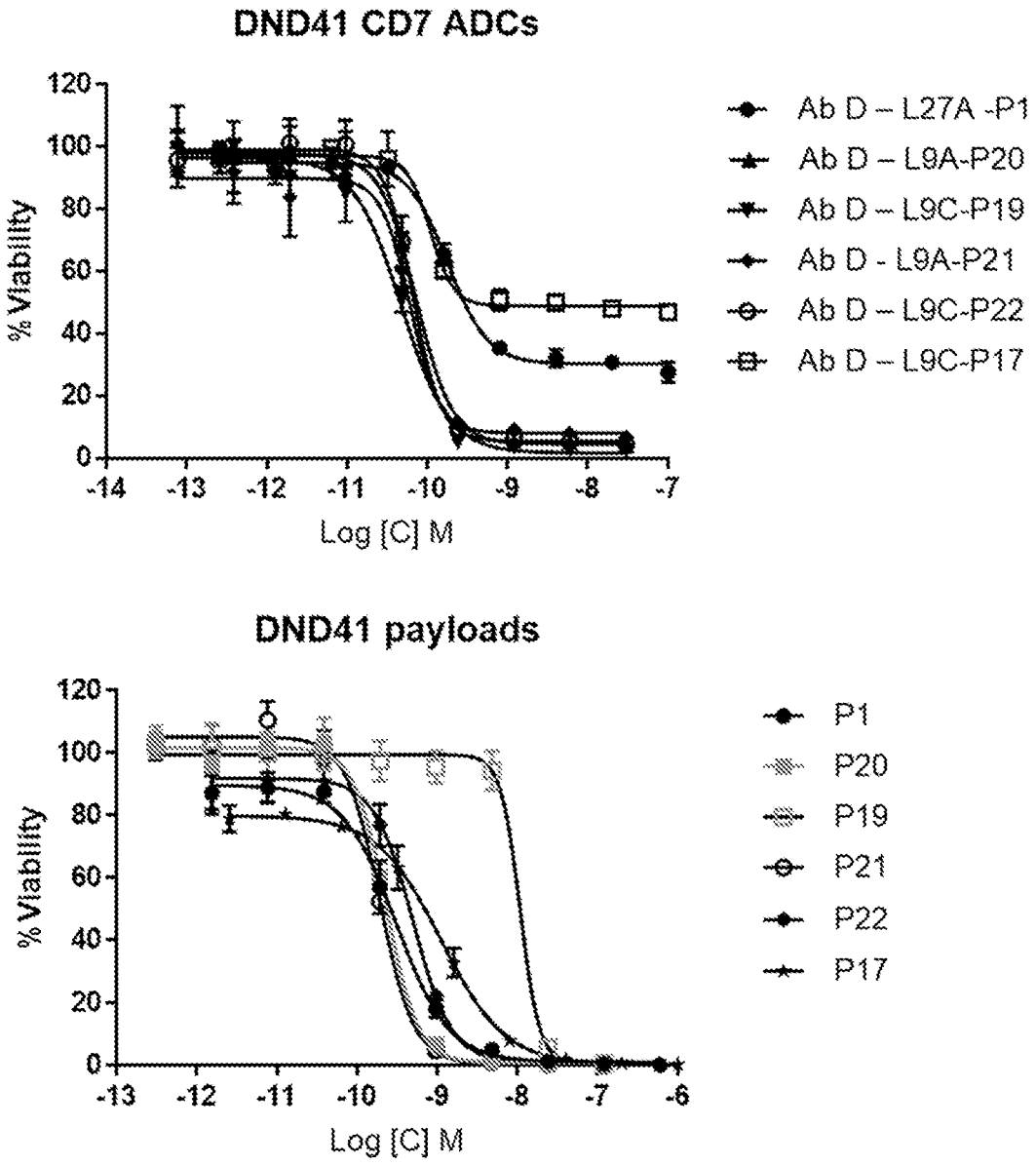

DND41 CD7 ADCs
% Viability
Log [C] M
Ab D – L27A -P1
Ab D – L9A-P20
Ab D – L9C-P19
Ab D - L9A-P21
Ab D – L9C-P22
Ab D – L9C-P17
DND41 payloads
% Viability
Log [C] M
P1
P20
P19
P21
P22
P17

FIG. 4

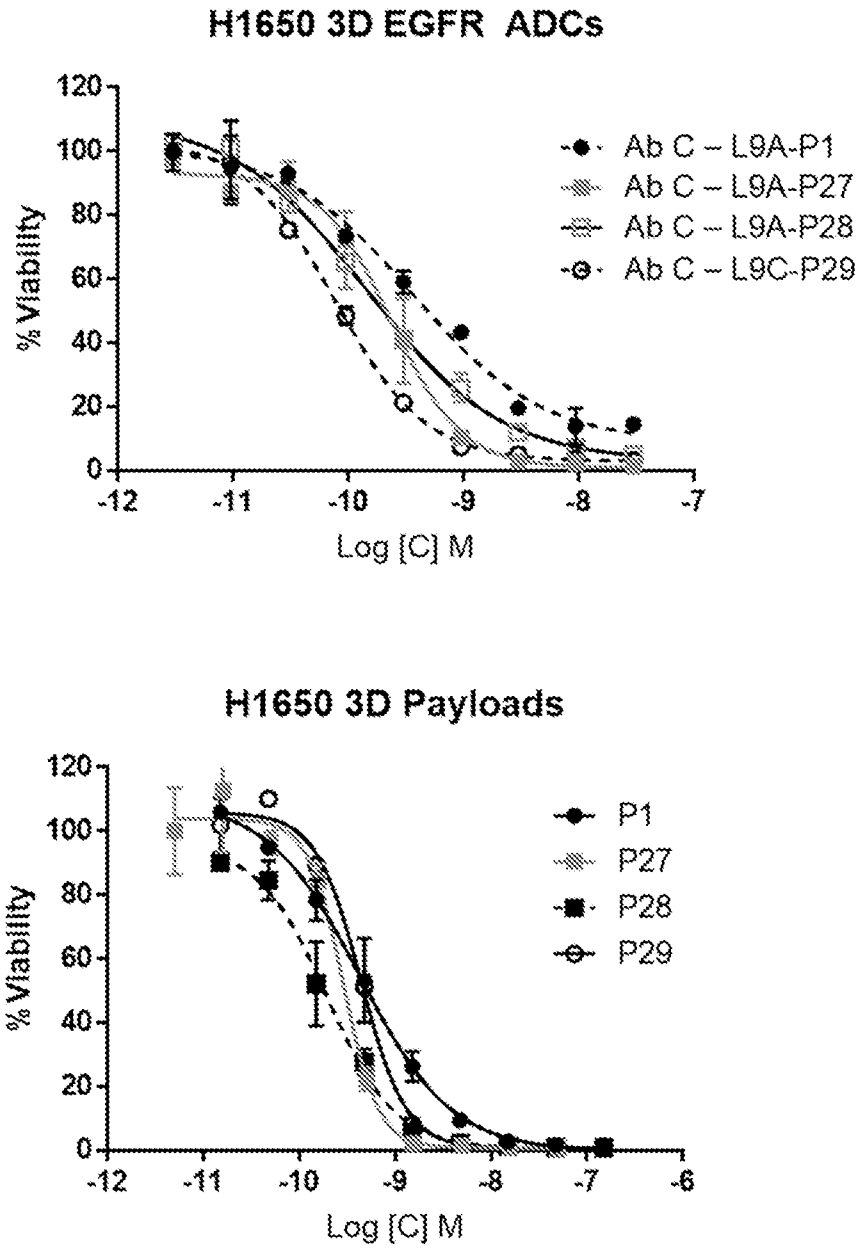

H1650 3D EGFR ADCs
% Viability
Log [C] M
Ab C – L9A-P1
Ab C – L9A-P27
Ab C – L9A-P28
Ab C – L9C-P29
H1650 3D Payloads
% Viability
Log [C] M
P1
P27
P28
P29

FIG. 5A

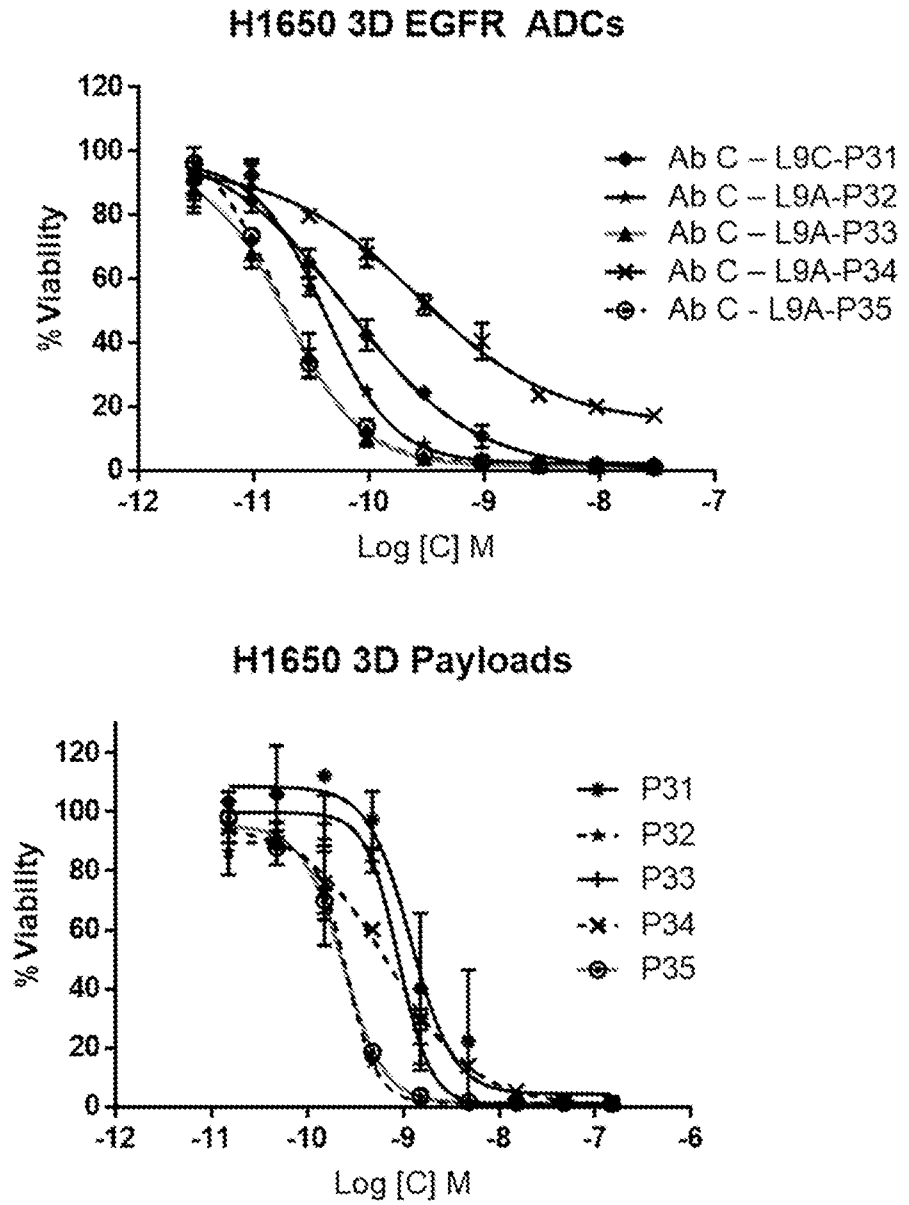
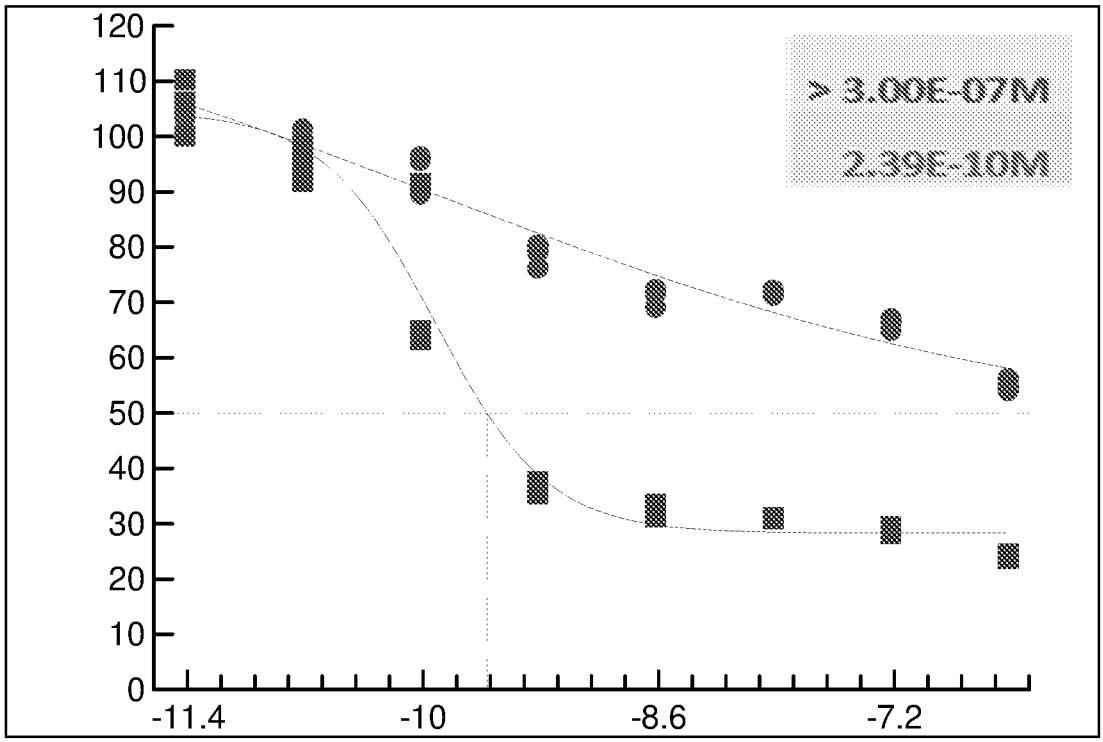

H1650 3D EGFR ADCs
% Viability
Log [C] M
Ab C – L9C-P31
Ab C – L9A-P32
Ab C – L9A-P33
Ab C – L9A-P34
Ab C - L9A-P35
H1650 3D Payloads
% Viability
Log [C] M
P31
P32
P33
P34
P35

FIG. 5B

Ab T – L13A-P2



Payload P2

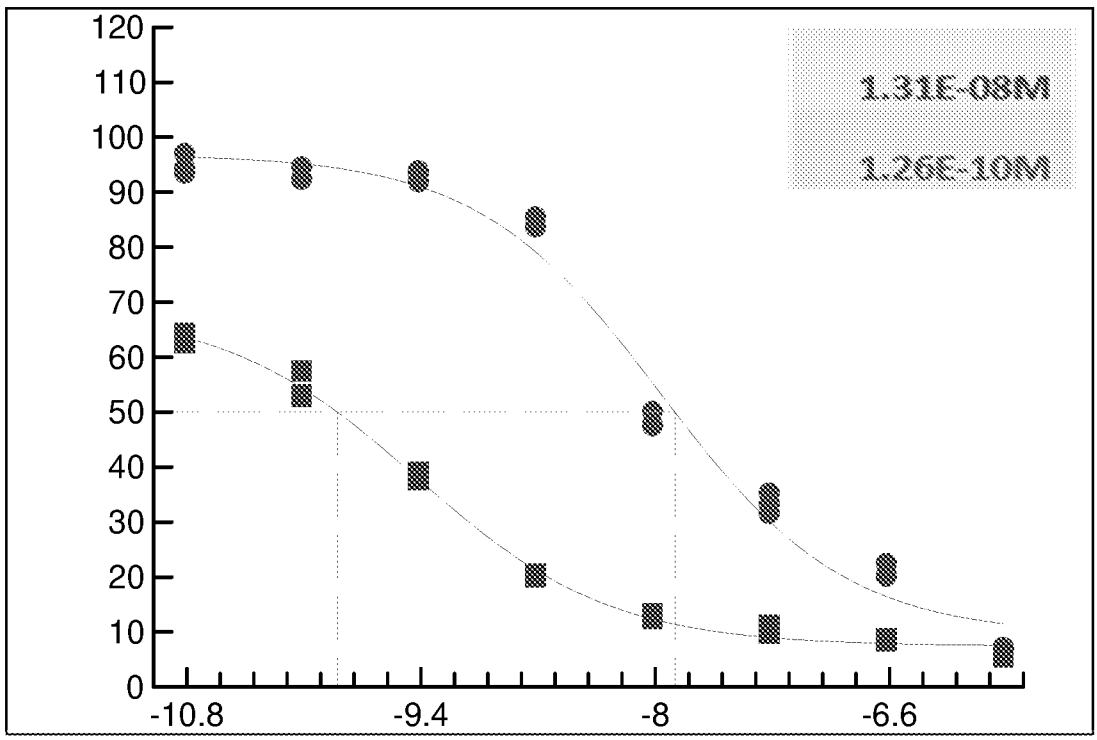
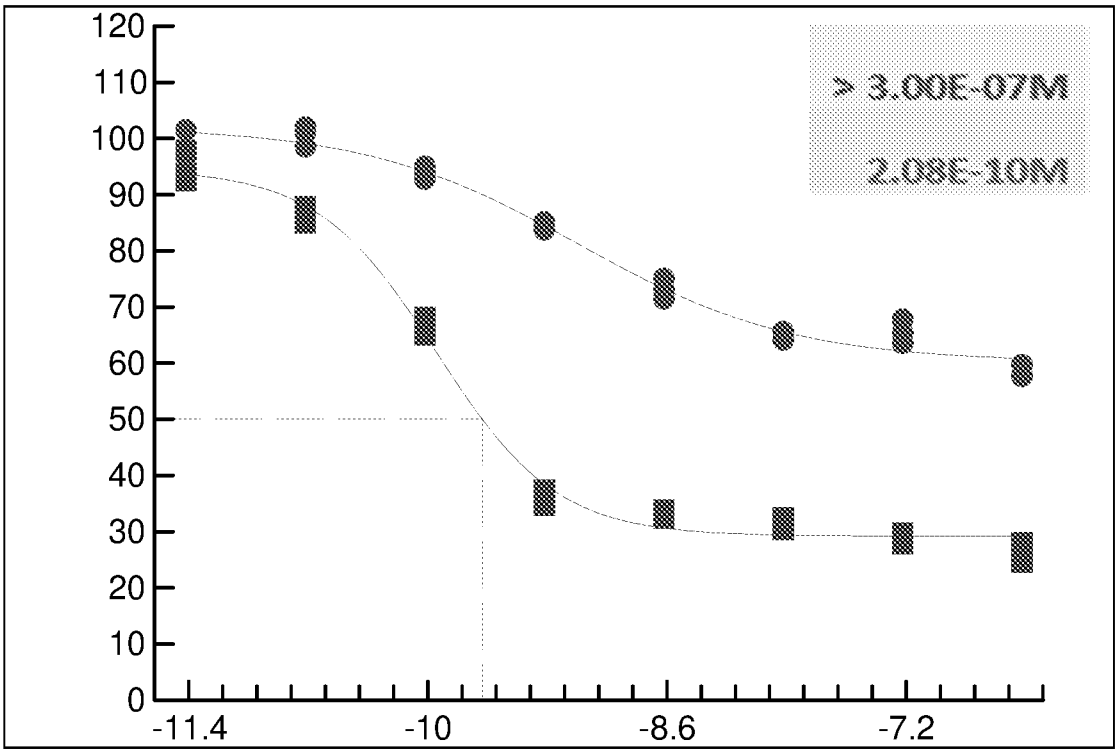


FIG. 6

Ab T – L19C-P7



Payload P7

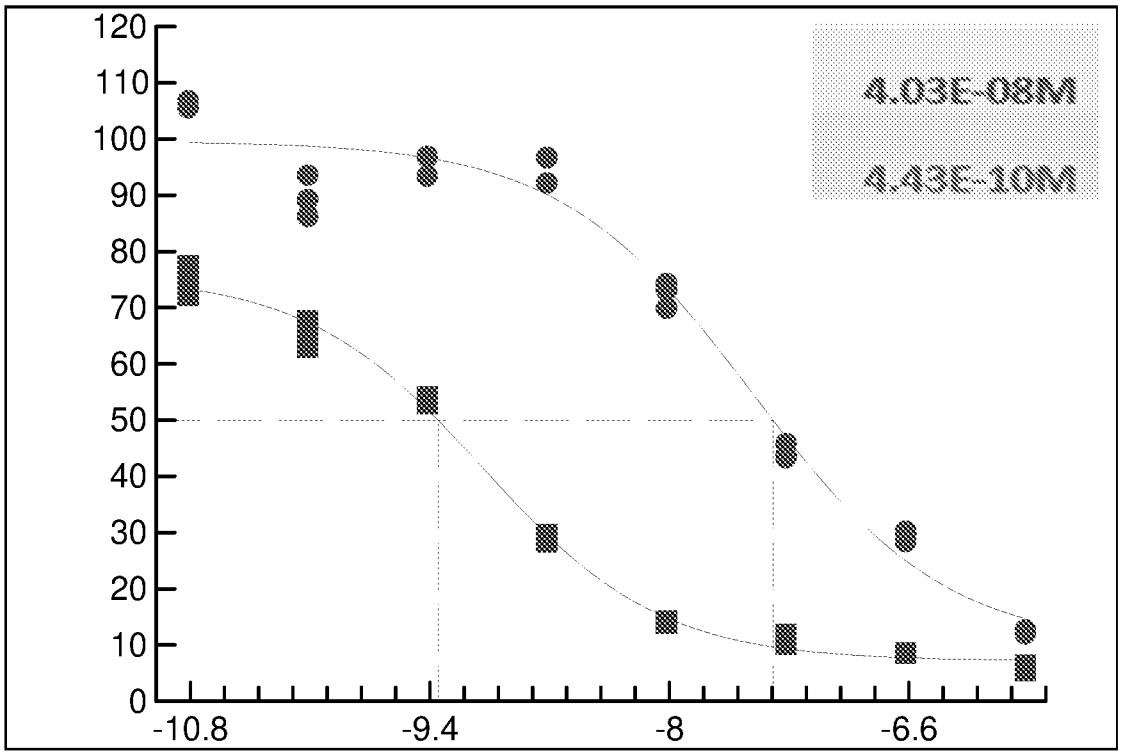
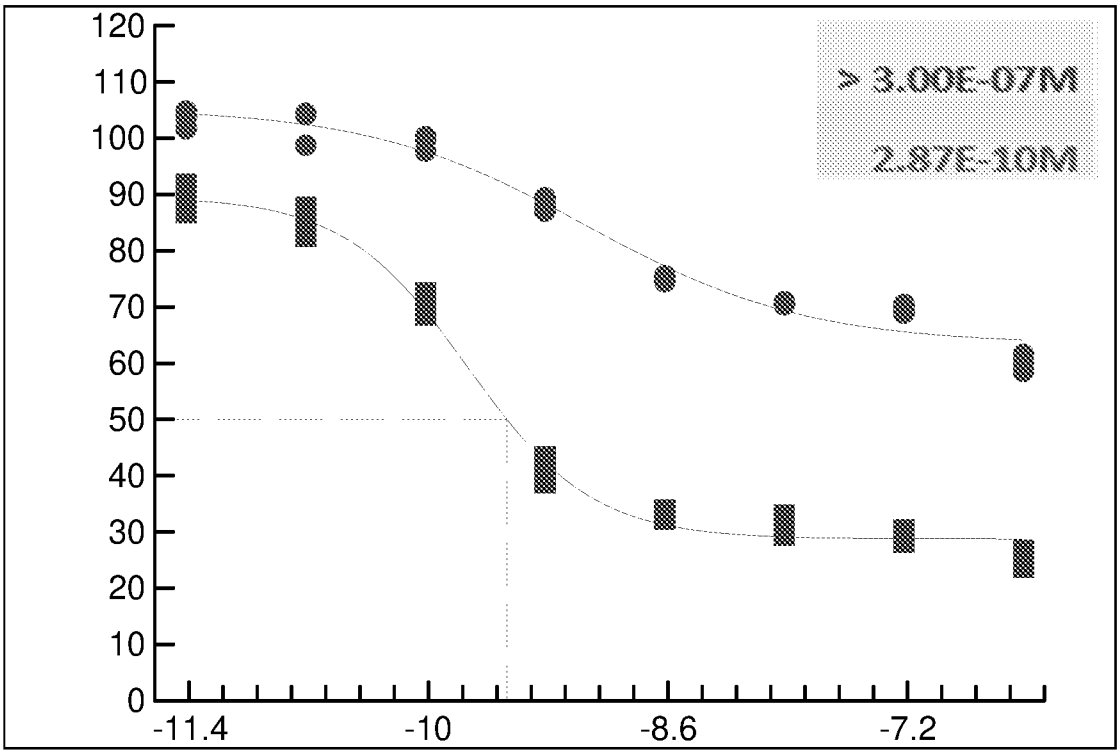


FIG. 6

Ab T – L23C-P7



naked anti-HER2 mAb

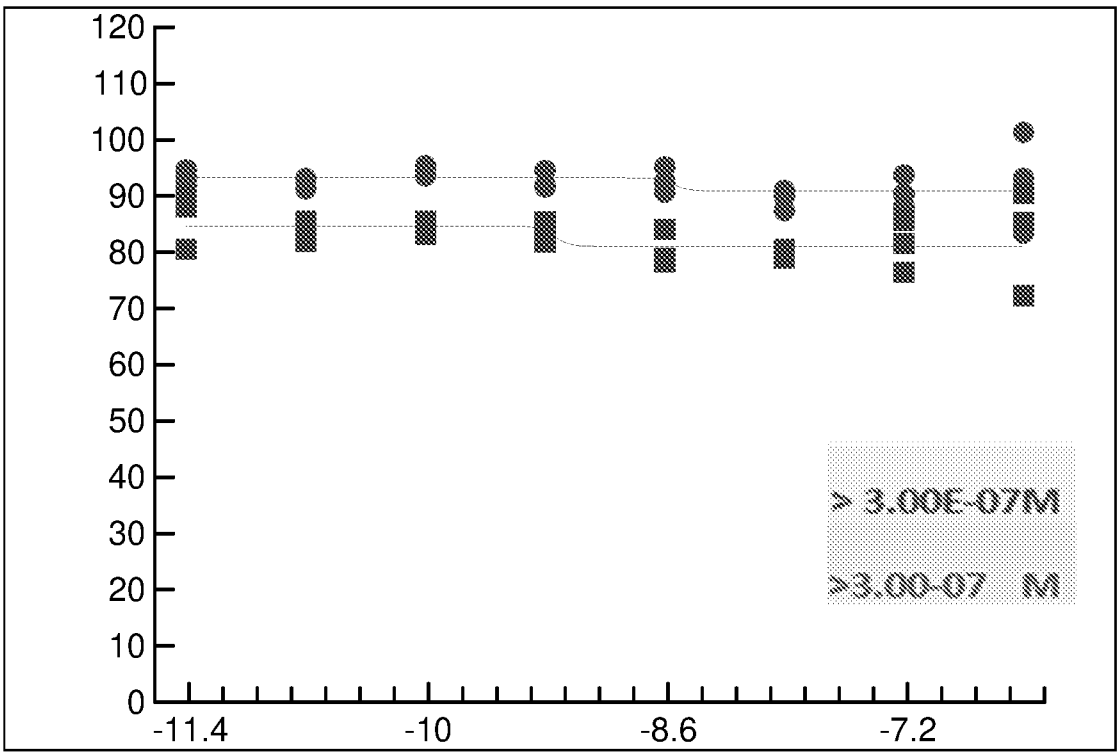
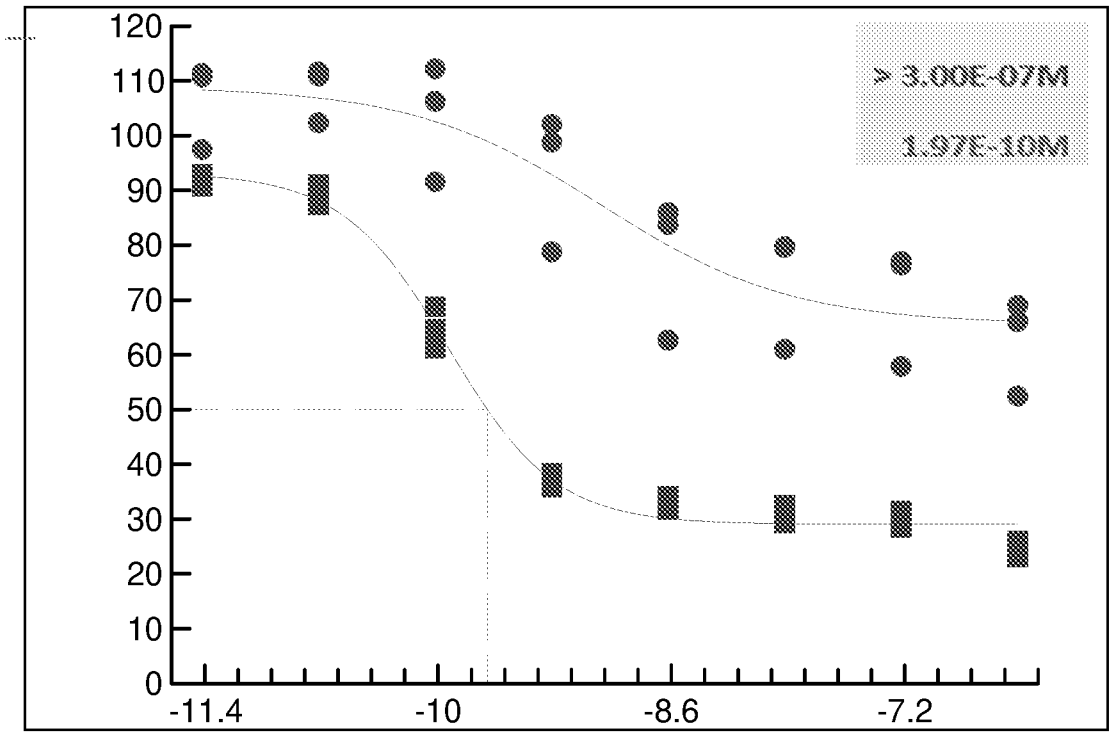


FIG. 6

Ab T – L110C-P7



Legend:

ADC or Payload

ADC or Payload + 10nM of Paclitaxel

FIG. 6

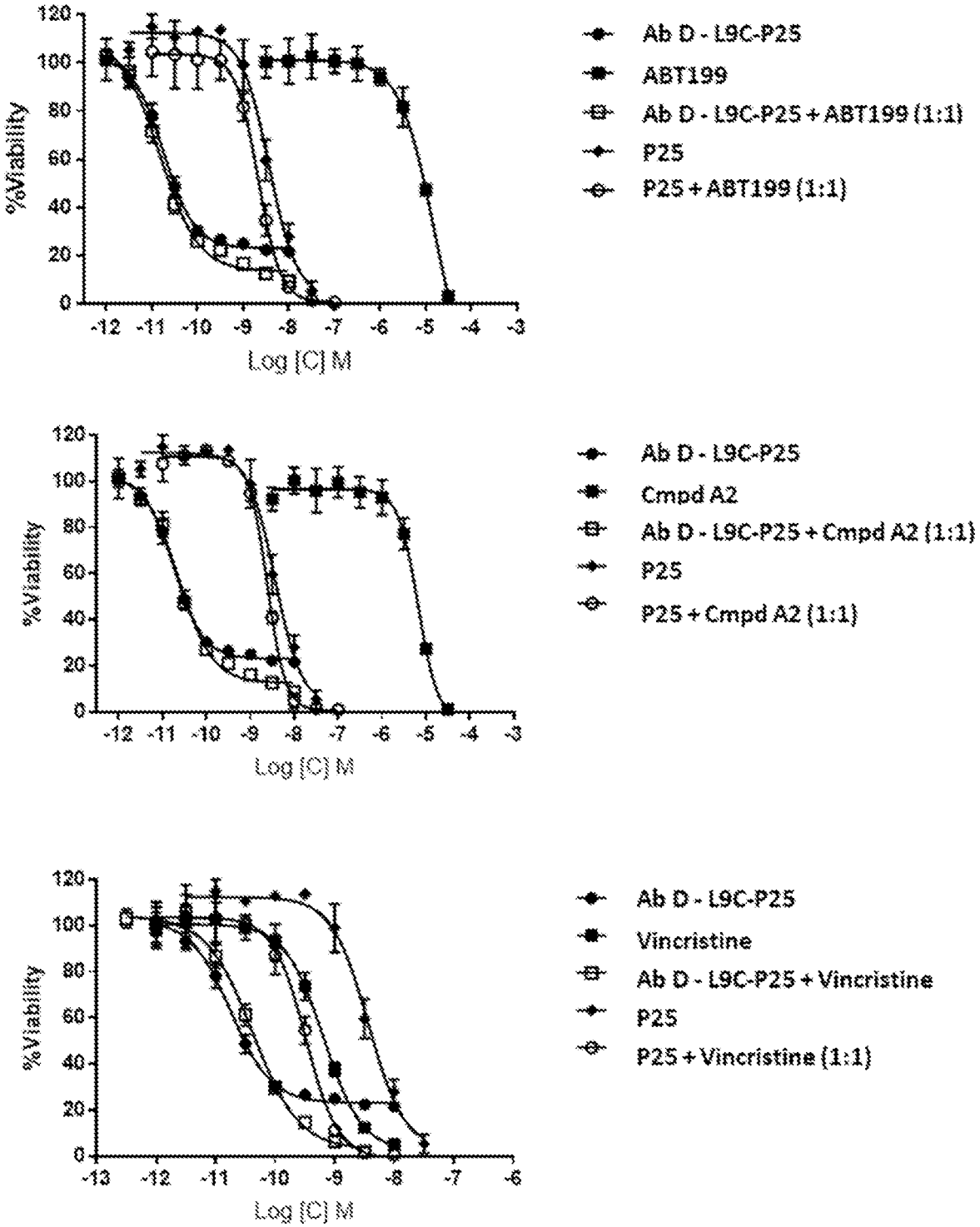

%Viability
Log [C] M
Ab D - L9C-P25
ABT199
Ab D - L9C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
%Viability
Log [C] M
Ab D - L9C-P25
Cmpd A2
Ab D - L9C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)
%Viability
Log [C] M
Ab D - L9C-P25
Vincristine
Ab D - L9C-P25 + Vincristine
P25
P25 + Vincristine (1:1)

FIG. 7A

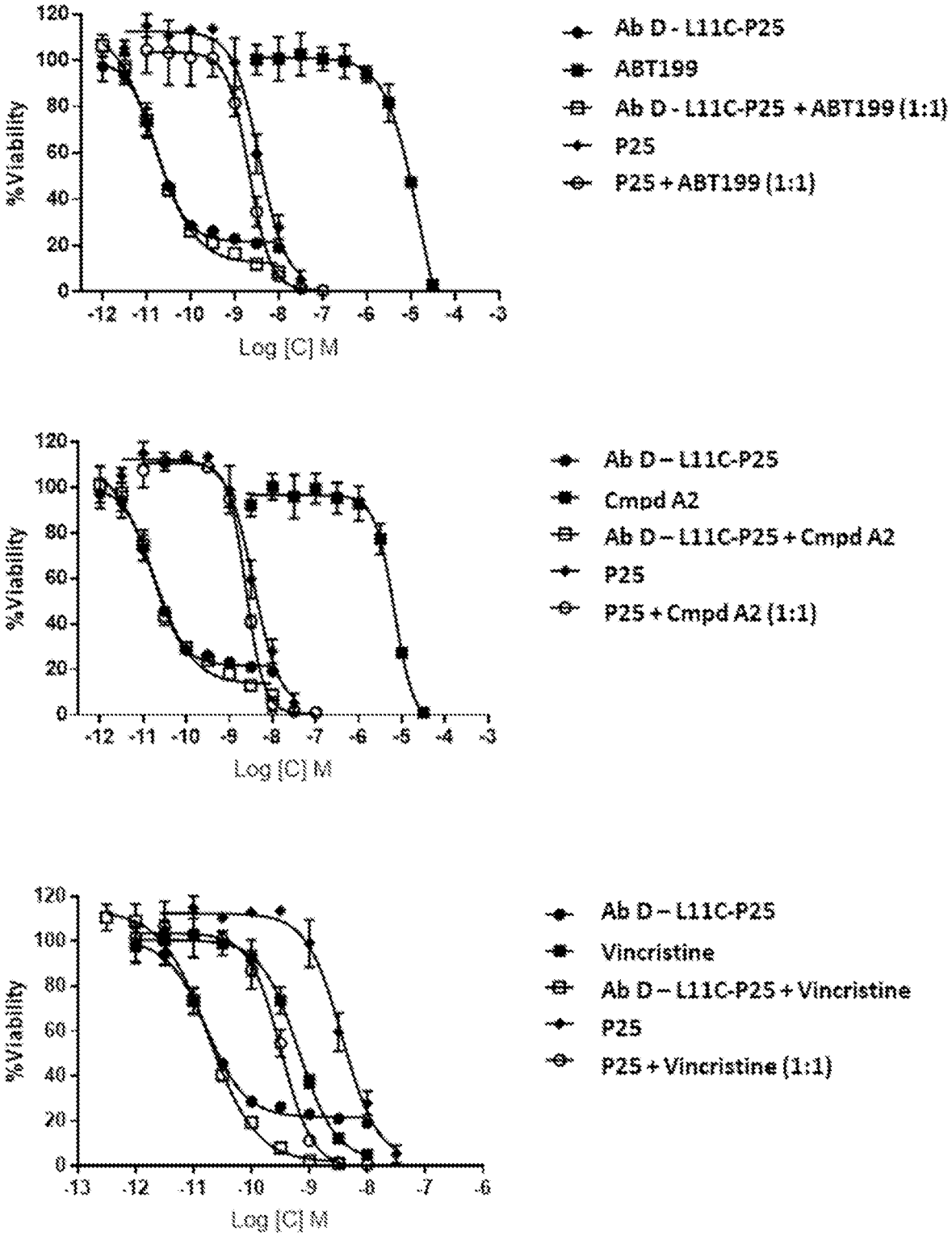

%Viability
Log [C] M
Ab D - L11C-P25
ABT199
Ab D - L11C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
%Viability
Log [C] M
Ab D – L11C-P25
Cmpd A2
Ab D – L11C-P25 + Cmpd A2
P25
P25 + Cmpd A2 (1:1)
%Viability
Log [C] M
Ab D – L11C-P25
Vincristine
Ab D – L11C-P25 + Vincristine
P25
P25 + Vincristine (1:1)

FIG. 7 A

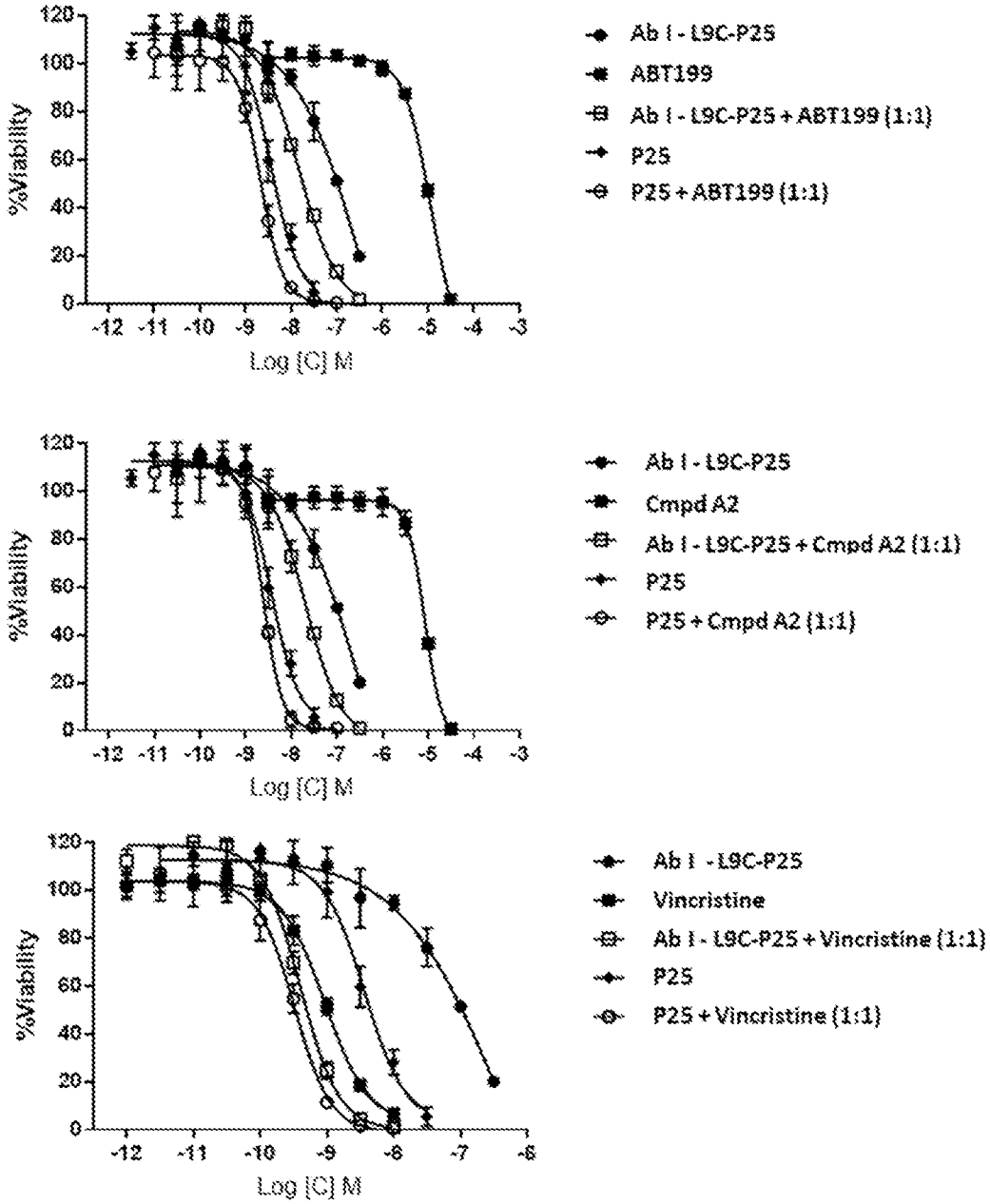

%Viability
Log [C] M
Ab I - L9C-P25
ABT199
Ab I - L9C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
Ab I - L9C-P25
Cmpd A2
Ab I - L9C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)
Ab I - L9C-P25
Vincristine
Ab I - L9C-P25 + Vincristine (1:1)
P25
P25 + Vincristine (1:1)

FIG. 7B

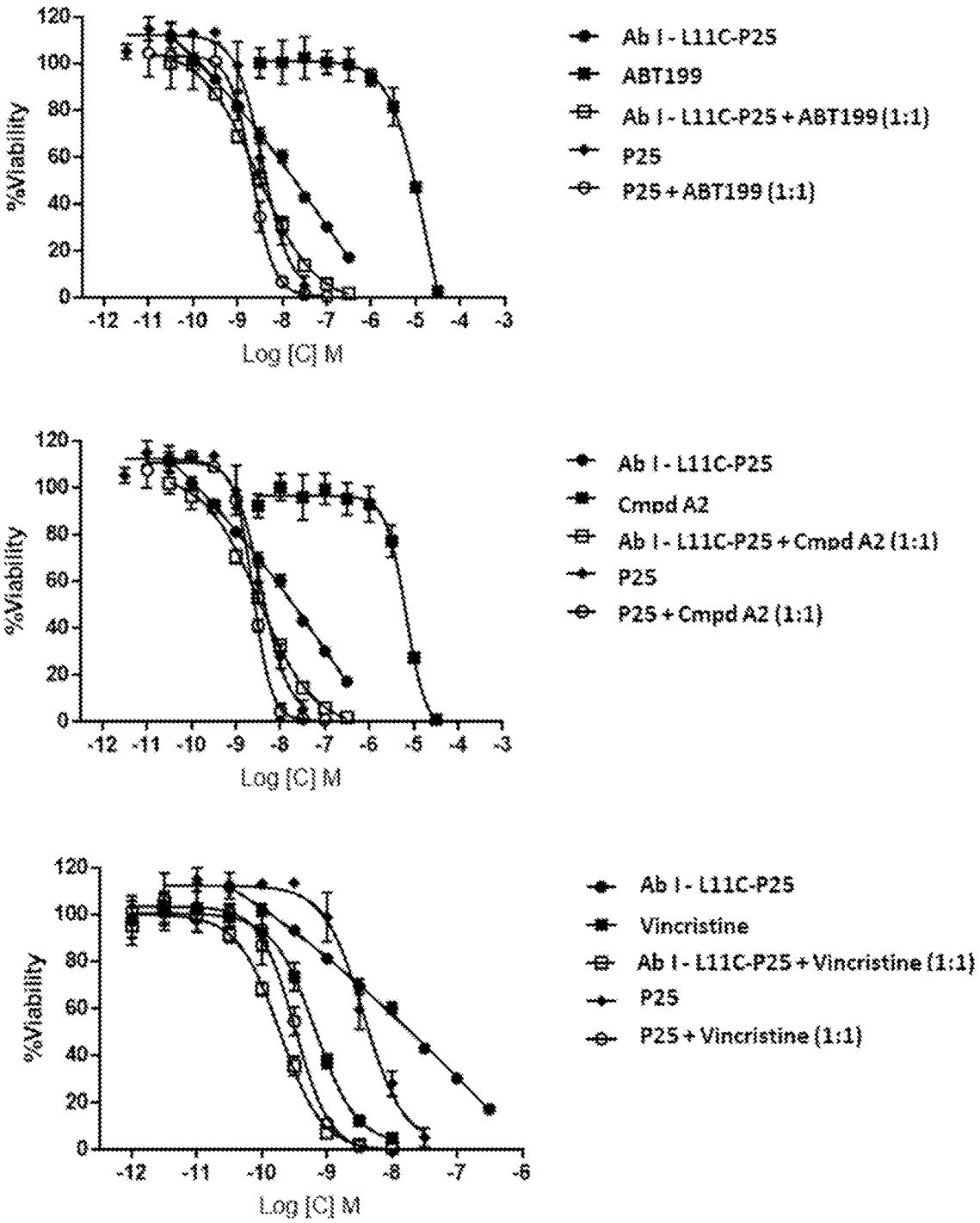

%Viability
Log [C] M
Ab I - L11C-P25
ABT199
Ab I - L11C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
Ab I - L11C-P25
Cmpd A2
Ab I - L11C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)
Ab I - L11C-P25
Vincristine
Ab I - L11C-P25 + Vincristine (1:1)
P25
P25 + Vincristine (1:1)

FIG. 7B

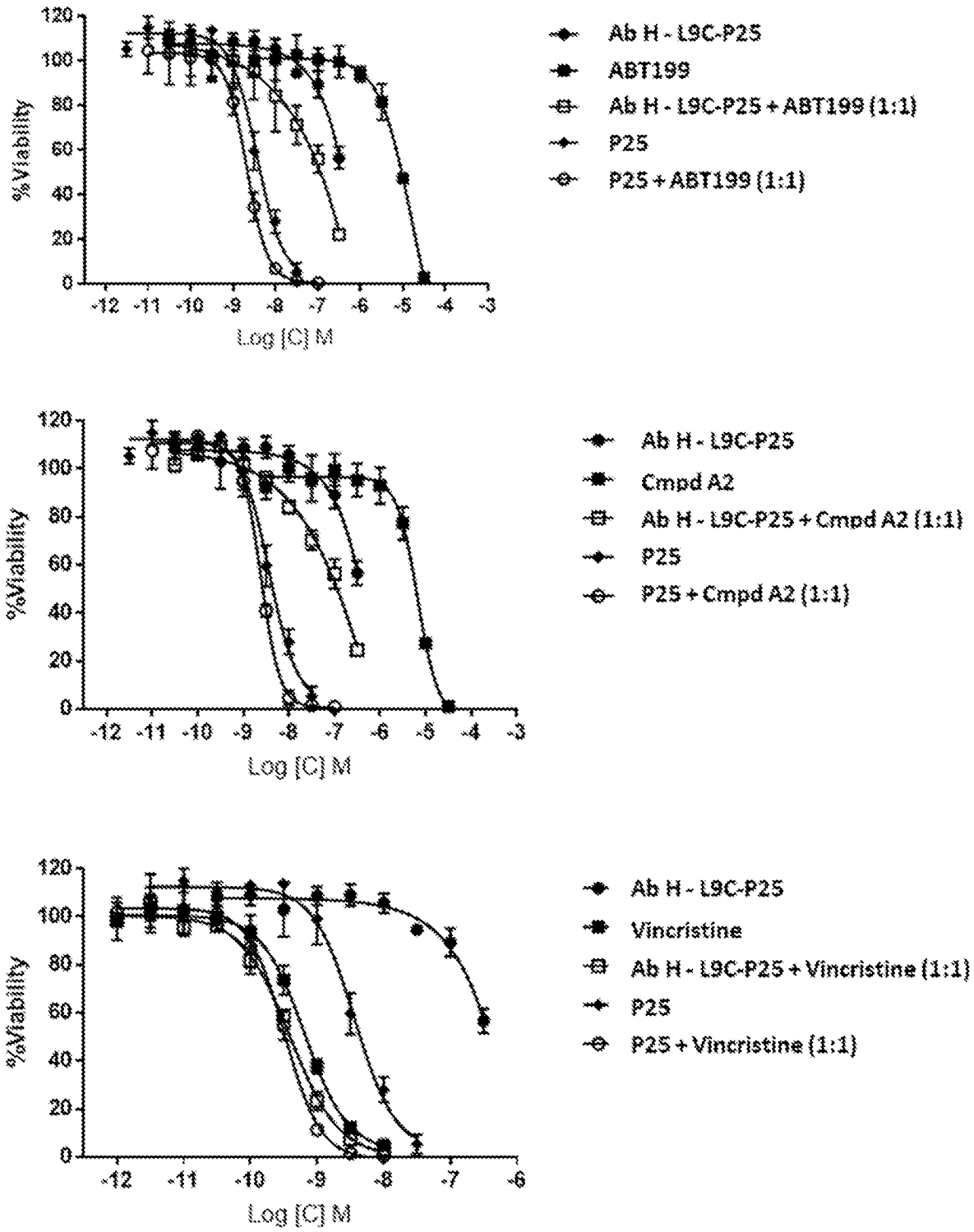

%Viability
Log [C] M
Ab H - L9C-P25
ABT199
Ab H - L9C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
%Viability
Log [C] M
Ab H - L9C-P25
Cmpd A2
Ab H - L9C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)
%Viability
Log [C] M
Ab H - L9C-P25
Vincristine
Ab H - L9C-P25 + Vincristine (1:1)
P25
P25 + Vincristine (1:1)

FIG. 7C

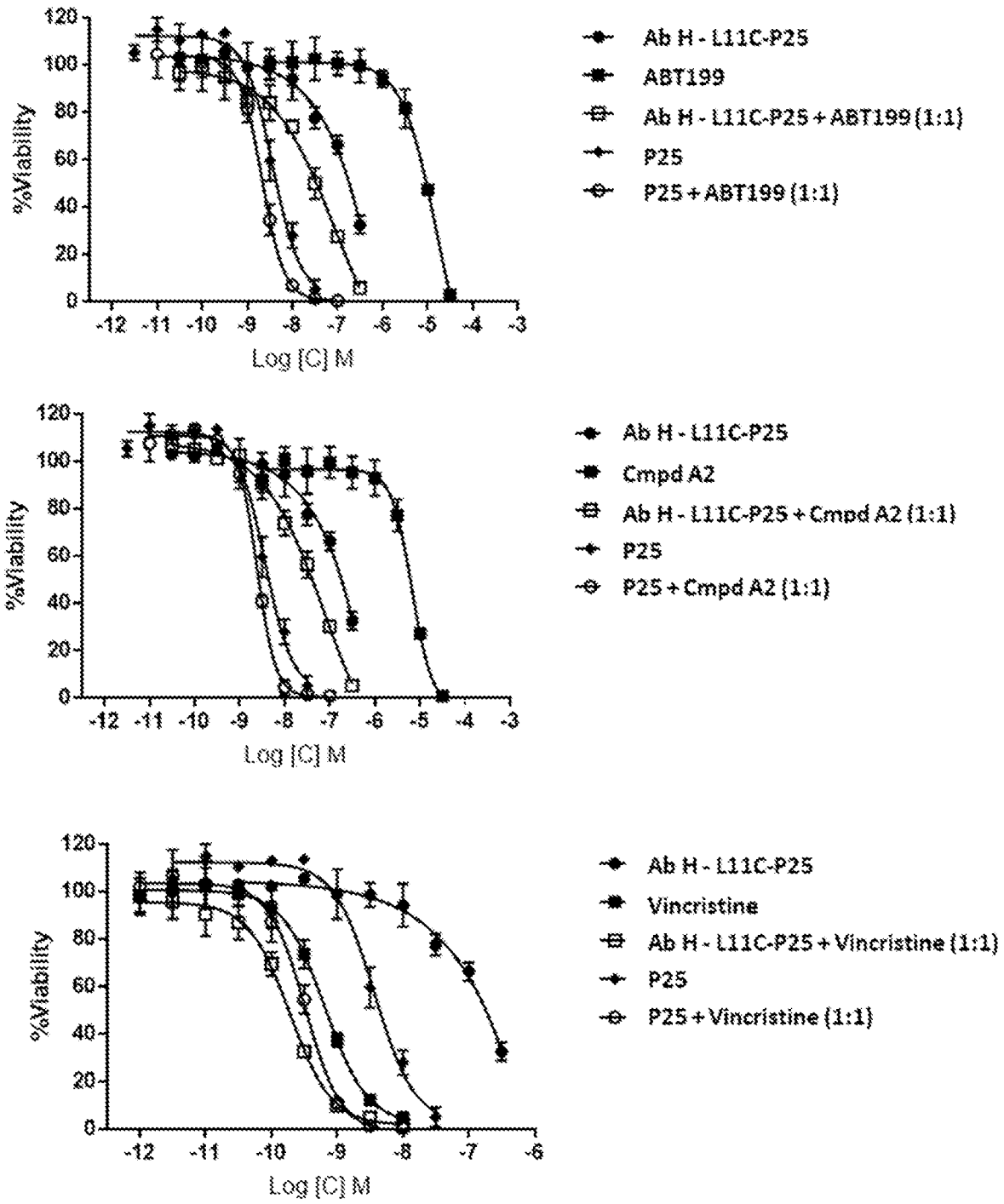

%Viability
Log [C] M
Ab H - L11C-P25
ABT199
Ab H - L11C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
Ab H - L11C-P25
Cmpd A2
Ab H - L11C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)
Ab H - L11C-P25
Vincristine
Ab H - L11C-P25 + Vincristine (1:1)
P25
P25 + Vincristine (1:1)

FIG. 7C

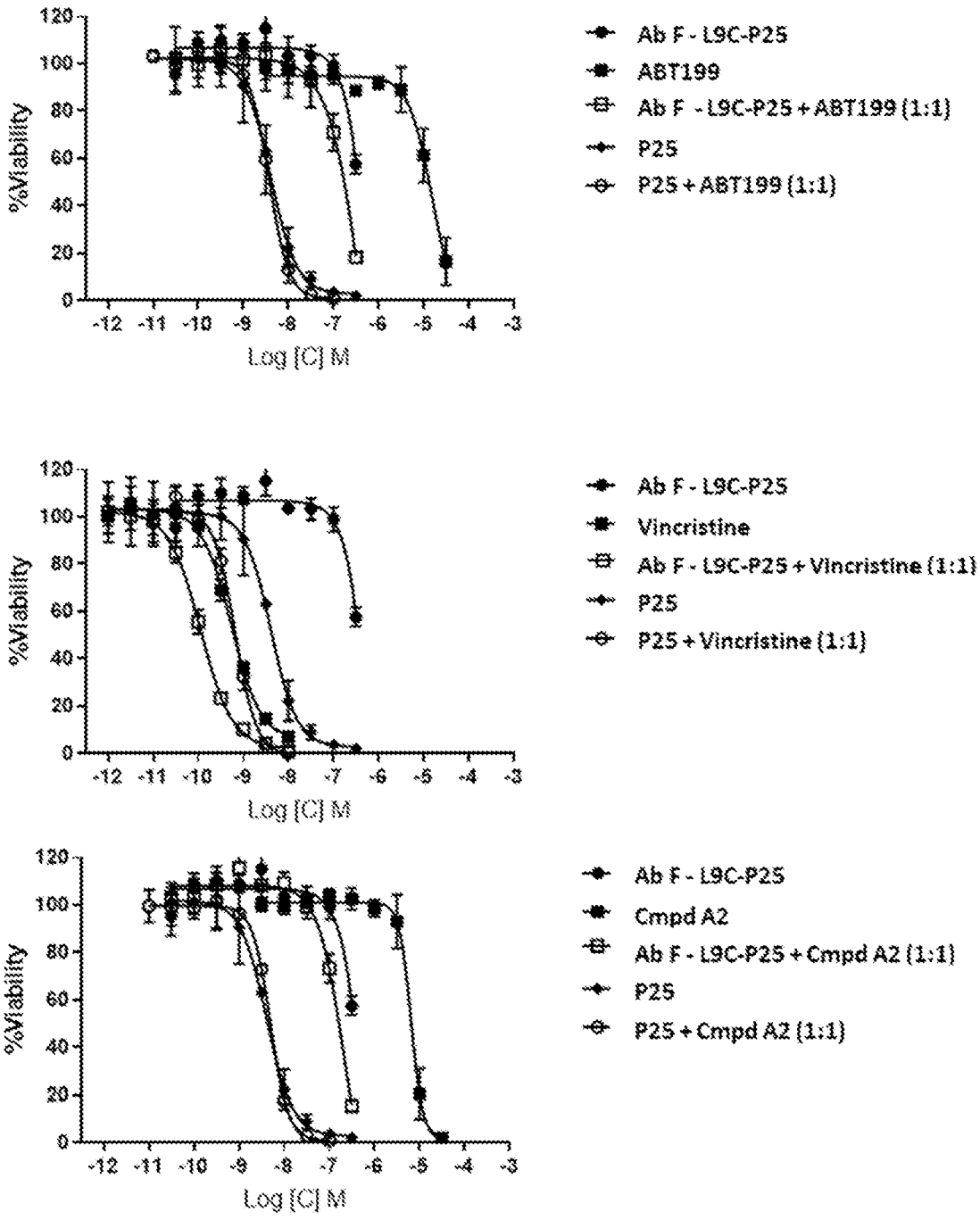

%Viability
Log [C] M
Ab F - L9C-P25
ABT199
Ab F - L9C-P25 + ABT199 (1:1)
P25
P25 + ABT199 (1:1)
%Viability
Log [C] M
Ab F - L9C-P25
Vincristine
Ab F - L9C-P25 + Vincristine (1:1)
P25
P25 + Vincristine (1:1)
%Viability
Log [C] M
Ab F - L9C-P25
Cmpd A2
Ab F - L9C-P25 + Cmpd A2 (1:1)
P25
P25 + Cmpd A2 (1:1)

FIG. 7D

HPAF-II

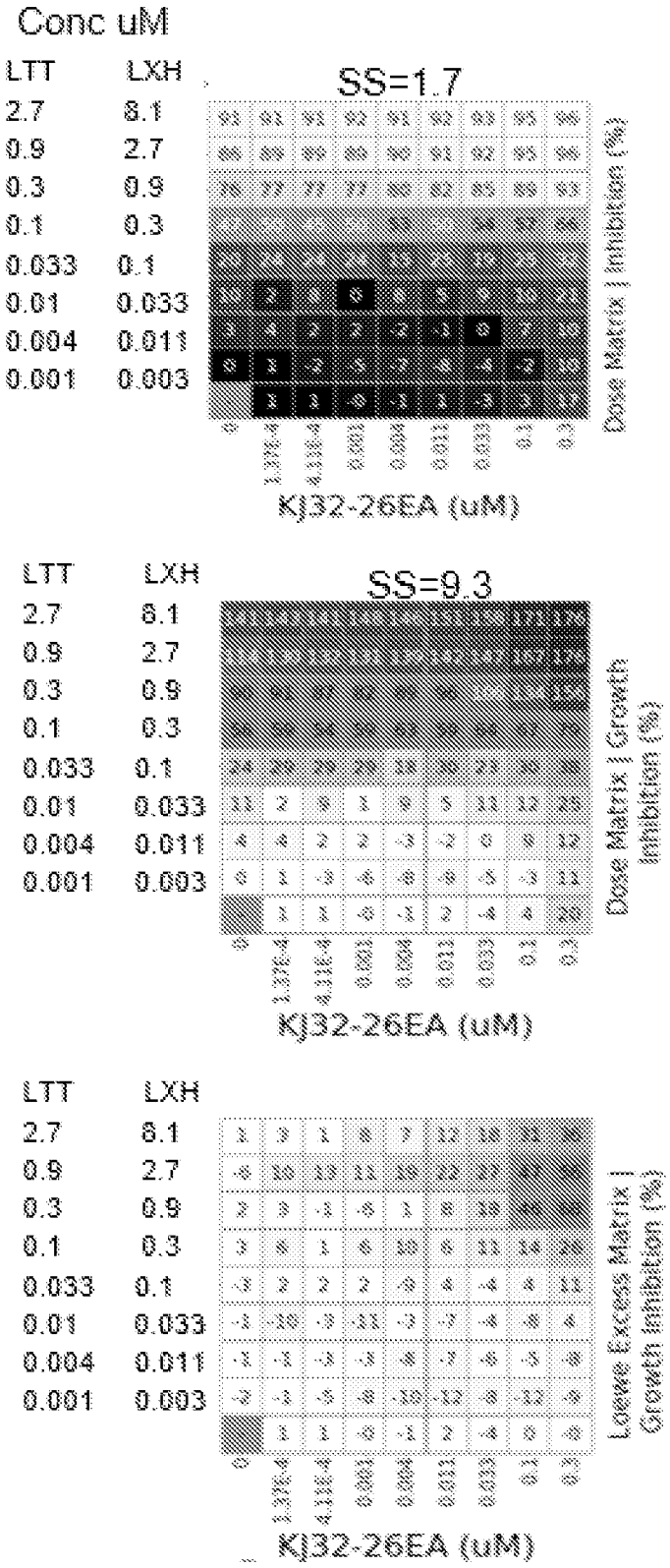


FIG. 8A

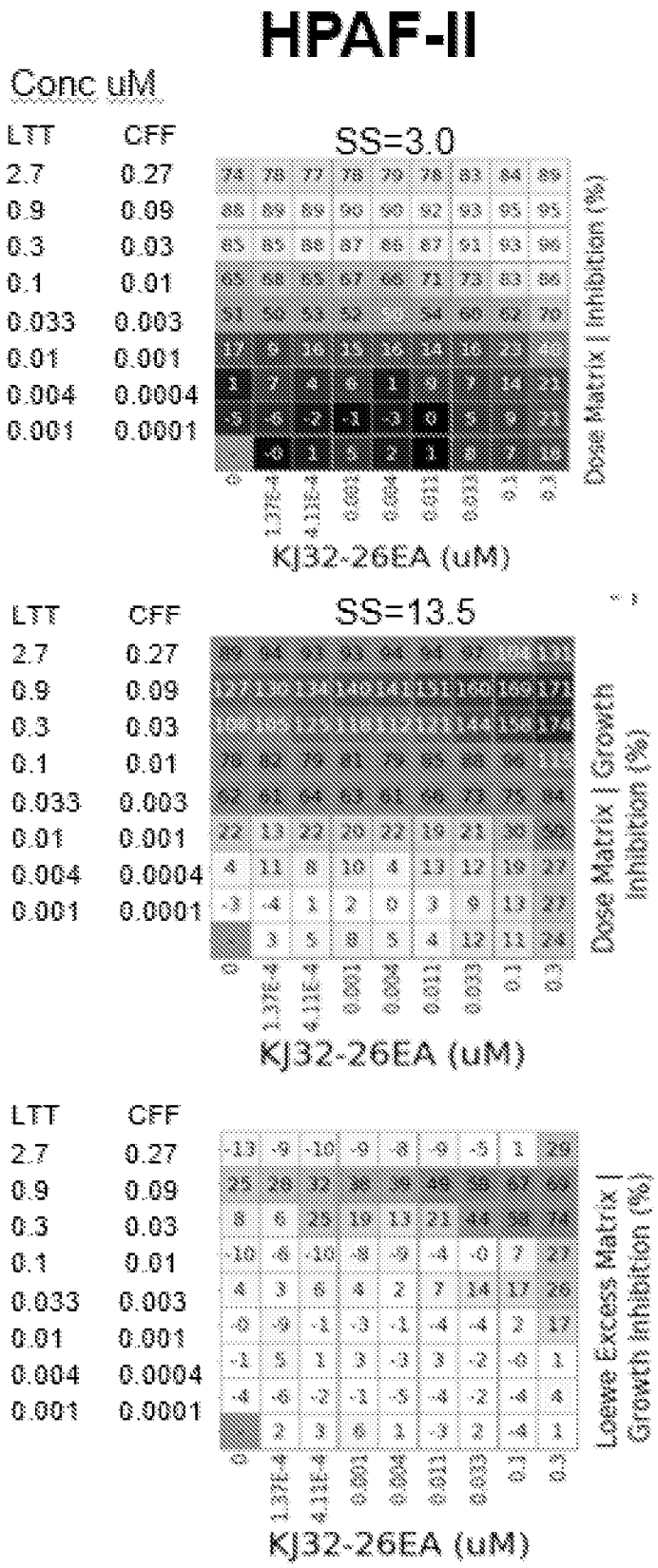

HPAF-II
Conc uM
LTT
CFF
2.7
0.27
0.9
0.09
0.3
0.03
0.1
0.01
0.033
0.003
0.01
0.001
0.004
0.0004
0.001
0.0001
SS=3.0
Dose Matrix | Inhibition (%)
KJ32-26EA (uM)
SS=13.5
Dose Matrix | Growth Inhibition (%)
KJ32-26EA (uM)
Loewe Excess Matrix | Growth Inhibition (%)
KJ32-26EA (uM)

FIG. 8A

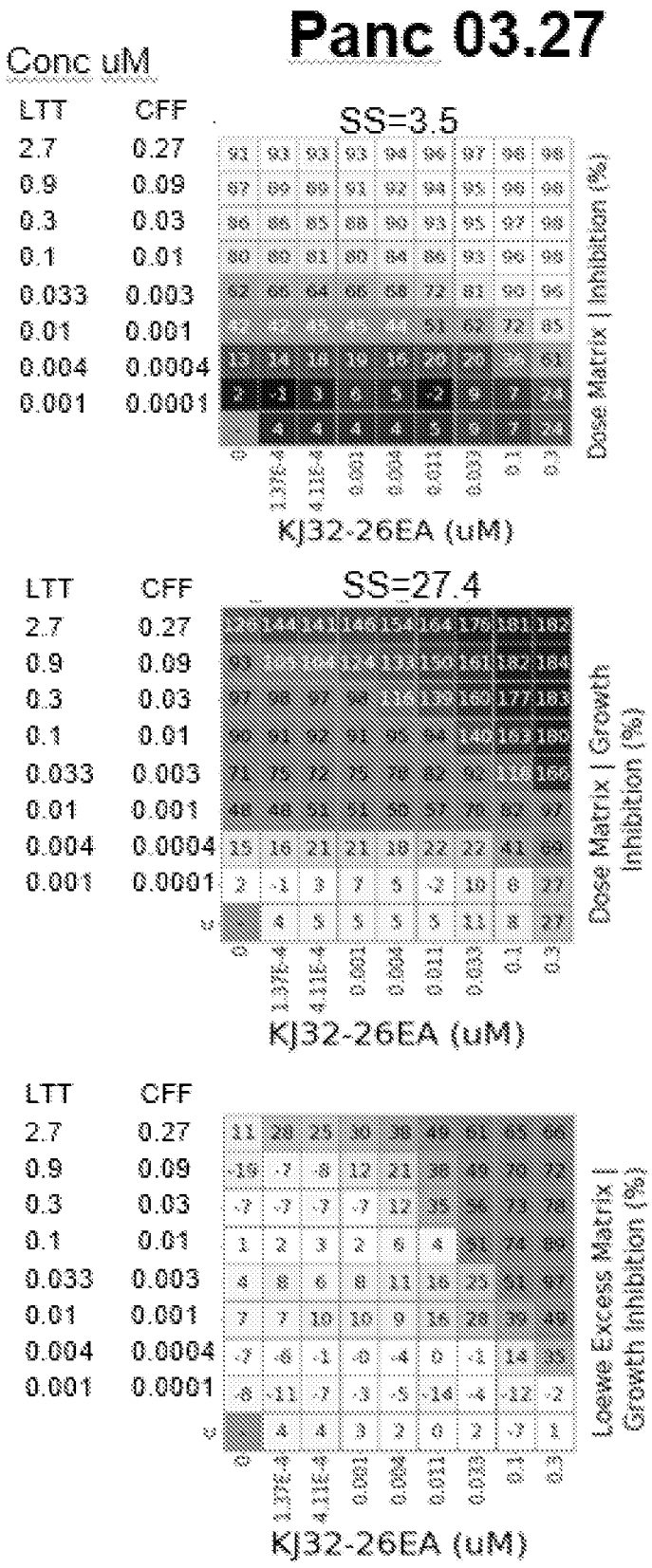

Panc 03.27
Conc uM
LTT
CFF
2.7 0.27
0.9 0.09
0.3 0.03
0.1 0.01
0.033 0.003
0.01 0.001
0.004 0.0004
0.001 0.0001
SS=3.5
Dose Matrix | Inhibition (%)
KJ32-26EA (uM)
SS=27.4
Dose Matrix | Growth Inhibition (%)
KJ32-26EA (uM)
Loewe Excess Matrix | Growth Inhibition (%)
KJ32-26EA (uM)

FIG. 8B

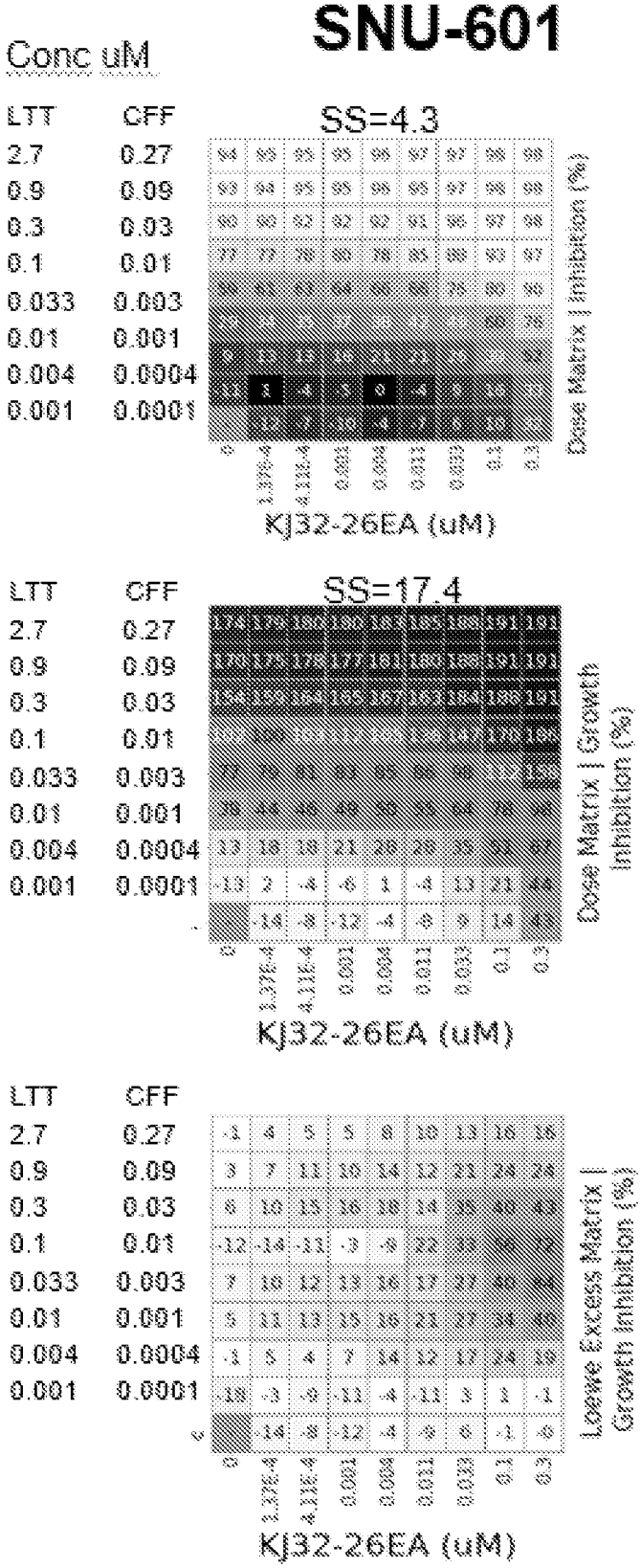

SNU-601
Conc uM
LTT
CFF
2.7
0.27
0.9
0.09
0.3
0.03
0.1
0.01
0.033
0.003
0.01
0.001
0.004
0.0004
0.001
0.0001
SS=4.3
Dose Matrix | Inhibition (%)
KJ32-26EA (uM)
SS=17.4
Dose Matrix | Growth Inhibition (%)
KJ32-26EA (uM)
Loewe Excess Matrix | Growth Inhibition (%)
KJ32-26EA (uM)

FIG. 8 B

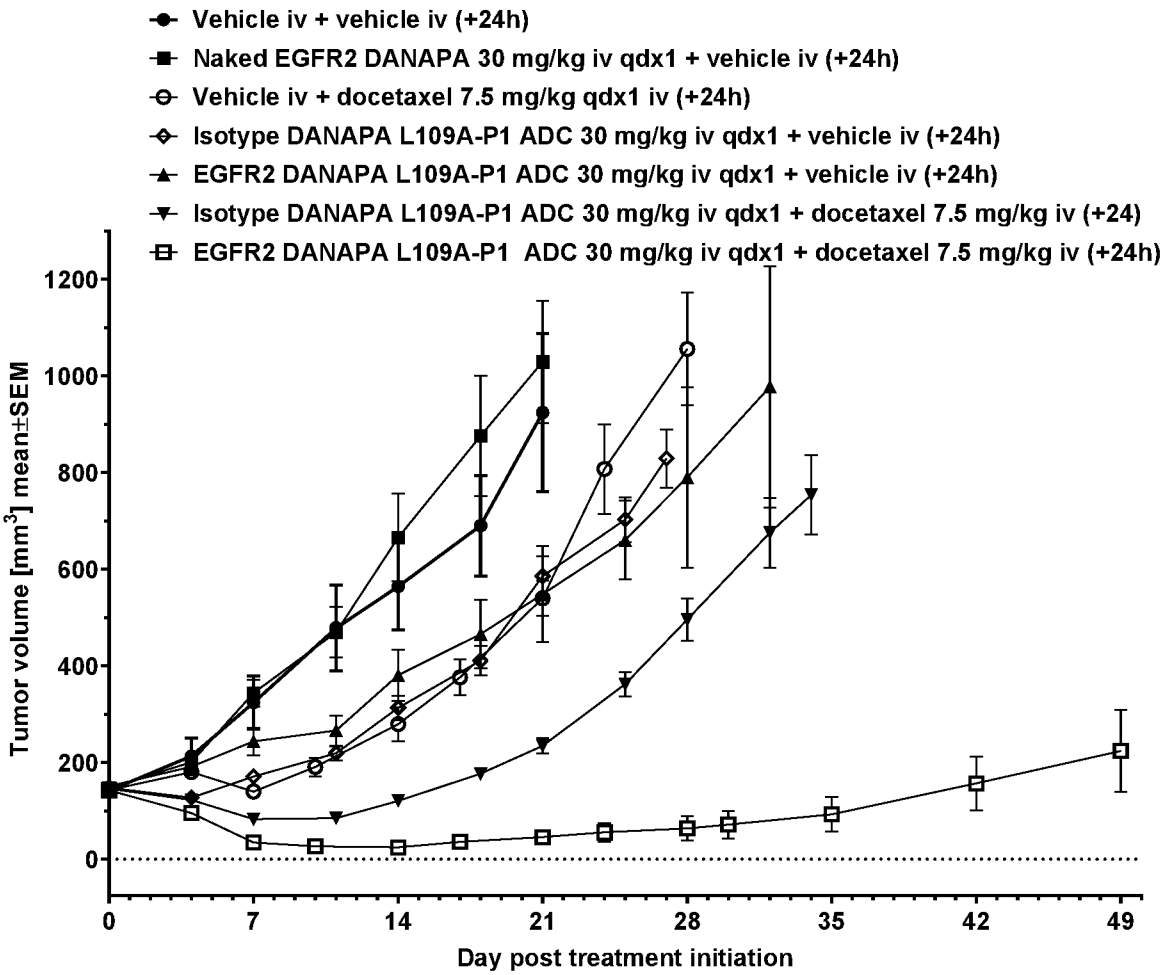

Vehicle iv + vehicle iv (+24h)
Naked EGFR2 DANAPA 30 mg/kg iv qdx1 + vehicle iv (+24h)
Vehicle iv + docetaxel 7.5 mg/kg qdx1 iv (+24h)
Isotype DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv (+24h)
EGFR2 DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv (+24h)
Isotype DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv (+24)
EGFR2 DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv (+24h)
Tumor volume [mm3] mean±SEM
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
49
Day post treatment initiation

FIG. 9

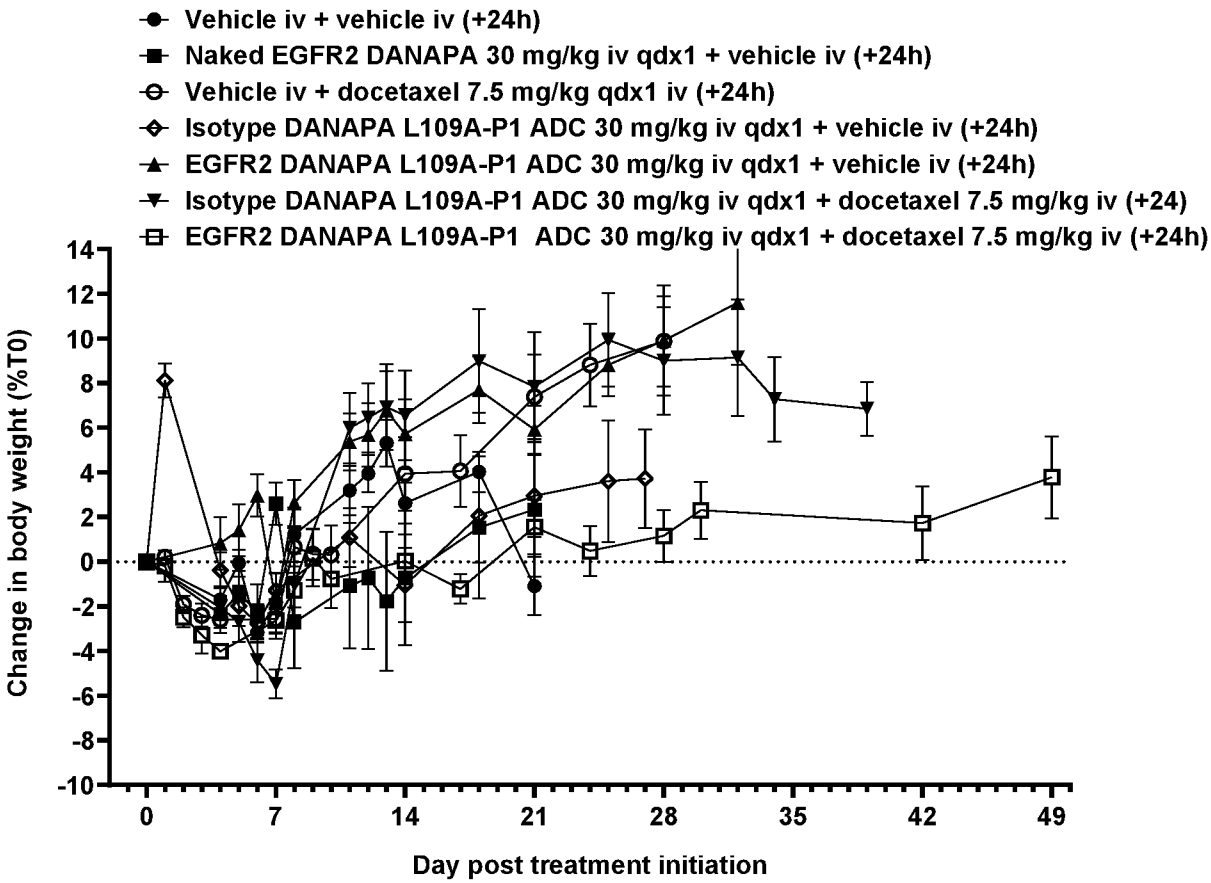

Vehicle iv + vehicle iv (+24h)
Naked EGFR2 DANAPA 30 mg/kg iv qdx1 + vehicle iv (+24h)
Vehicle iv + docetaxel 7.5 mg/kg qdx1 iv (+24h)
Isotype DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv (+24h)
EGFR2 DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + vehicle iv (+24h)
Isotype DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv (+24)
EGFR2 DANAPA L109A-P1 ADC 30 mg/kg iv qdx1 + docetaxel 7.5 mg/kg iv (+24h)
Change in body weight (%T0)
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
0
7
14
21
28
35
42
49
Day post treatment initiation

FIG. 10

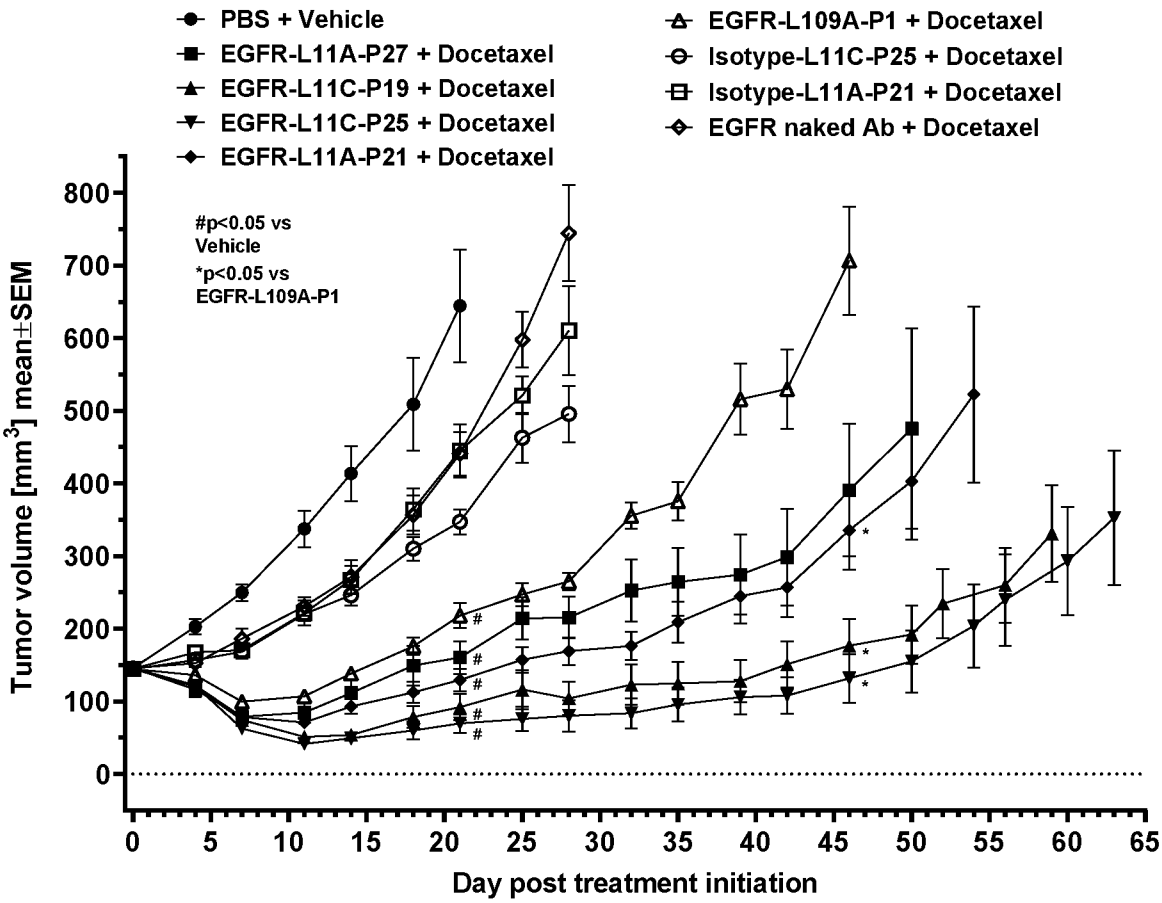

PBS + Vehicle
EGFR-L11A-P27 + Docetaxel
EGFR-L11C-P19 + Docetaxel
EGFR-L11C-P25 + Docetaxel
EGFR-L11A-P21 + Docetaxel
EGFR-L109A-P1 + Docetaxel
Isotype-L11C-P25 + Docetaxel
Isotype-L11A-P21 + Docetaxel
EGFR naked Ab + Docetaxel
#p<0.05 vs Vehicle
*p<0.05 vs EGFR-L109A-P1
Tumor volume [mm3] mean±SEM
Day post treatment initiation
0
100
200
300
400
500
600
700
800
0
5
10
15
20
25
30
35
40
45
50
55
60
65

FIG. 11

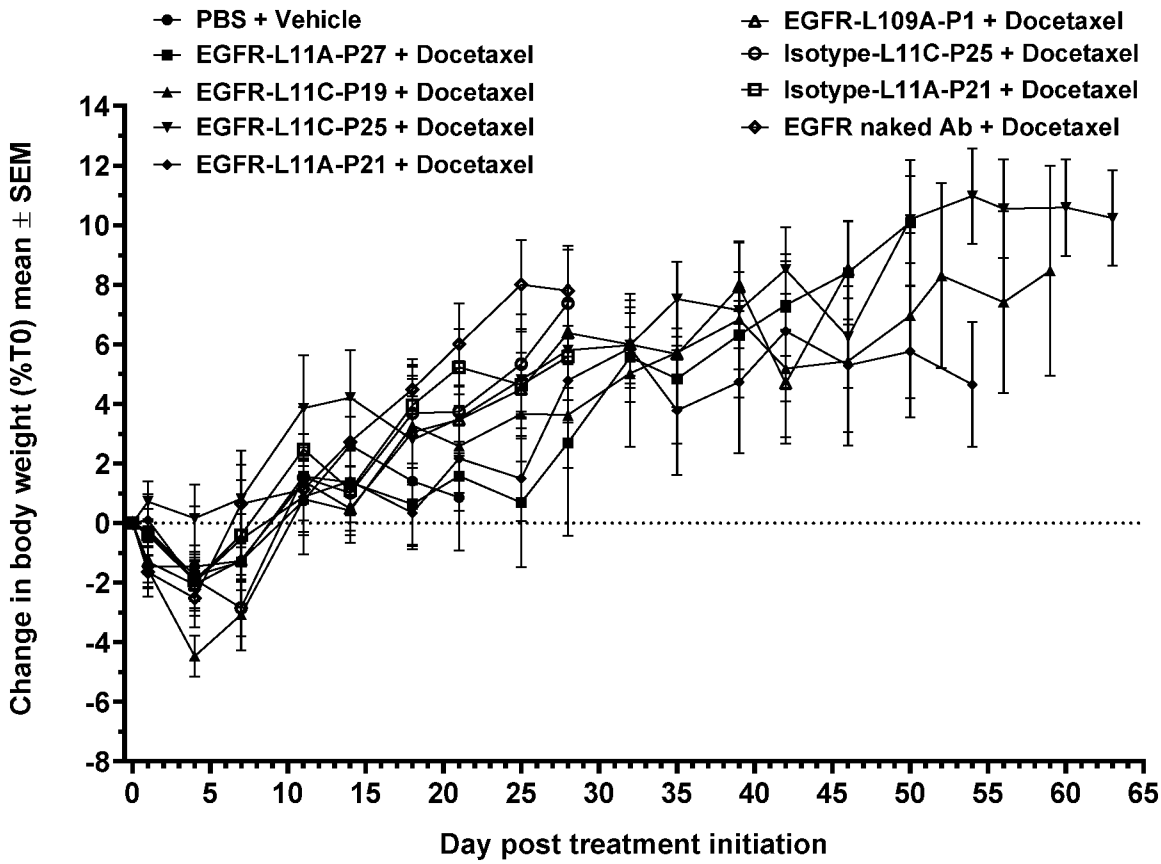

PBS + Vehicle
EGFR-L11A-P27 + Docetaxel
EGFR-L11C-P19 + Docetaxel
EGFR-L11C-P25 + Docetaxel
EGFR-L11A-P21 + Docetaxel
EGFR-L109A-P1 + Docetaxel
Isotype-L11C-P25 + Docetaxel
Isotype-L11A-P21 + Docetaxel
EGFR naked Ab + Docetaxel
Change in body weight (%T0) mean ± SEM
14
12
10
8
6
4
2
0
-2
-4
-6
-8
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Day post treatment initiation

FIG. 12

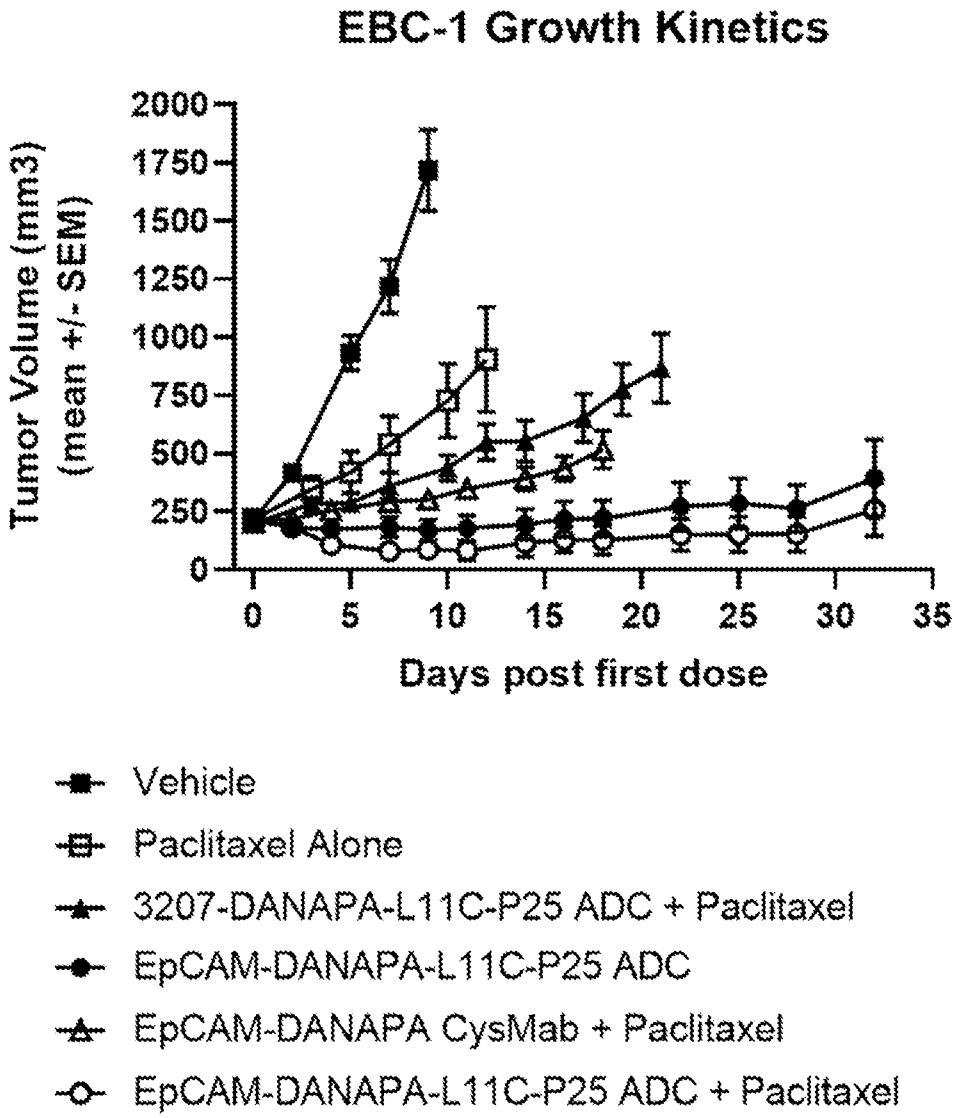

EBC-1 Growth Kinetics
Tumor Volume (mm3)
(mean +/- SEM)
2000
1750
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
Days post first dose
Vehicle
Paclitaxel Alone
3207-DANAPA-L11C-P25 ADC + Paclitaxel
EpCAM-DANAPA-L11C-P25 ADC
EpCAM-DANAPA CysMab + Paclitaxel
EpCAM-DANAPA-L11C-P25 ADC + Paclitaxel

FIG. 13

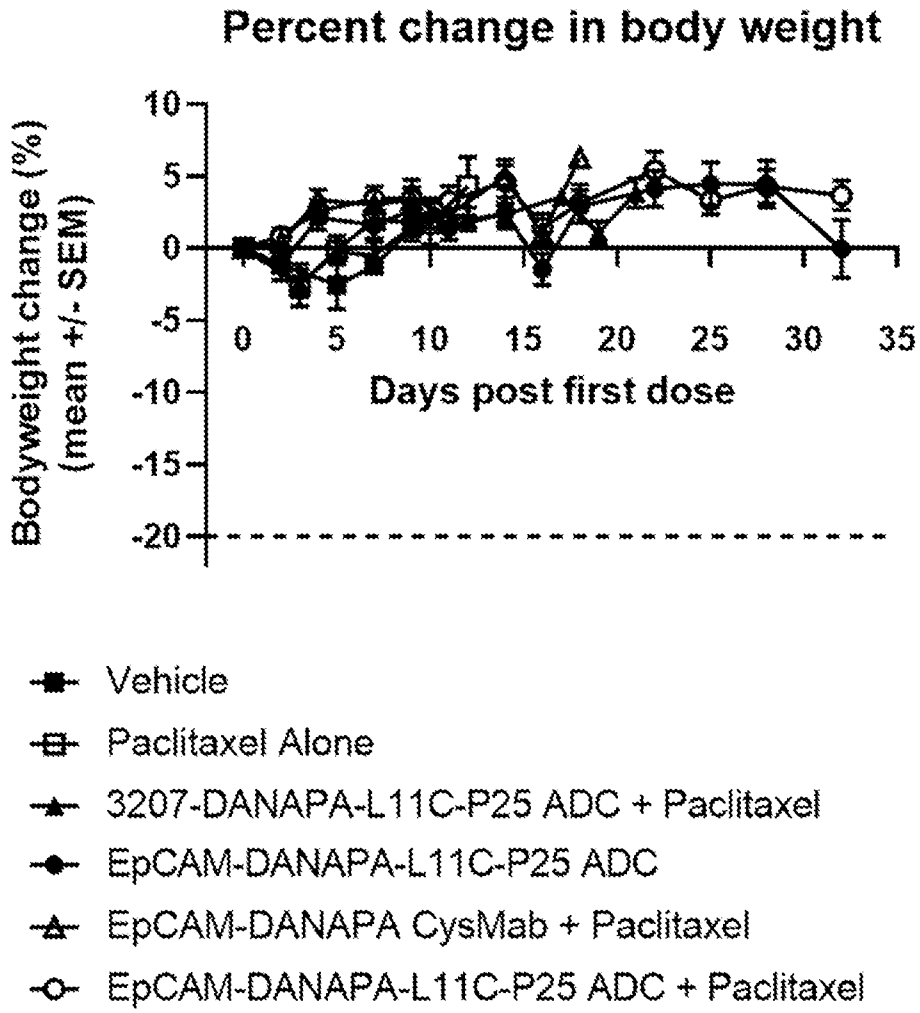

Percent change in body weight
Bodyweight change (%)
(mean +/- SEM)
10
5
0
-5
-10
-15
-20
0
5
10
15
20
25
30
35
Days post first dose
Vehicle
Paclitaxel Alone
3207-DANAPA-L11C-P25 ADC + Paclitaxel
EpCAM-DANAPA-L11C-P25 ADC
EpCAM-DANAPA CysMab + Paclitaxel
EpCAM-DANAPA-L11C-P25 ADC + Paclitaxel

FIG. 14

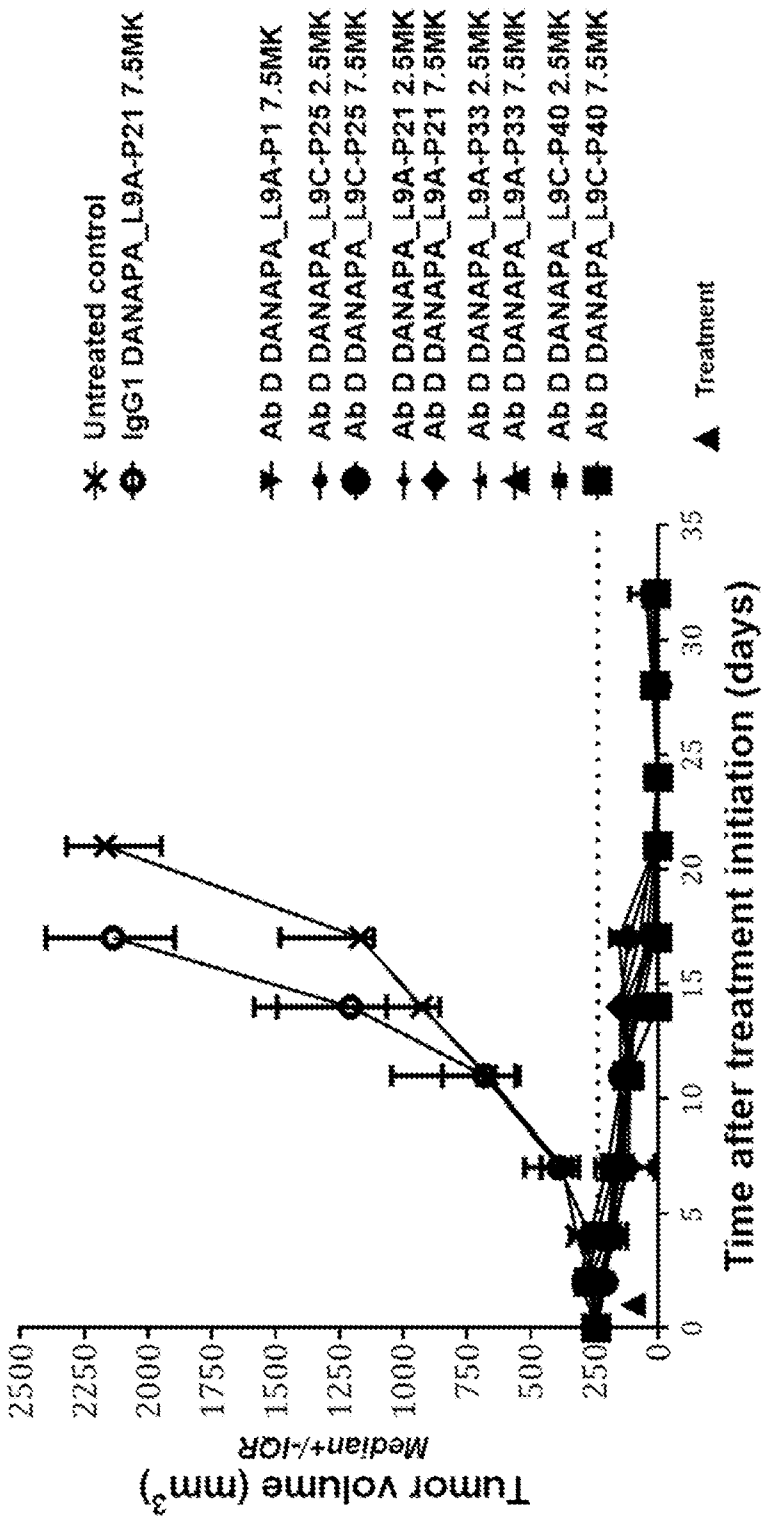

Untreated control
IgG1 DANAPA_L9A-P21 7.5MK
Ab D DANAPA_L9A-P1 7.5MK
Ab D DANAPA_L9C-P25 2.5MK
Ab D DANAPA_L9C-P25 7.5MK
Ab D DANAPA_L9A-P21 2.5MK
Ab D DANAPA_L9A-P21 7.5MK
Ab D DANAPA_L9A-P33 2.5MK
Ab D DANAPA_L9A-P33 7.5MK
Ab D DANAPA_L9C-P40 2.5MK
Ab D DANAPA_L9C-P40 7.5MK
Treatment
Tumor volume (mm³)
Median+/-IQR
Time after treatment initiation (days)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35

FIG. 15

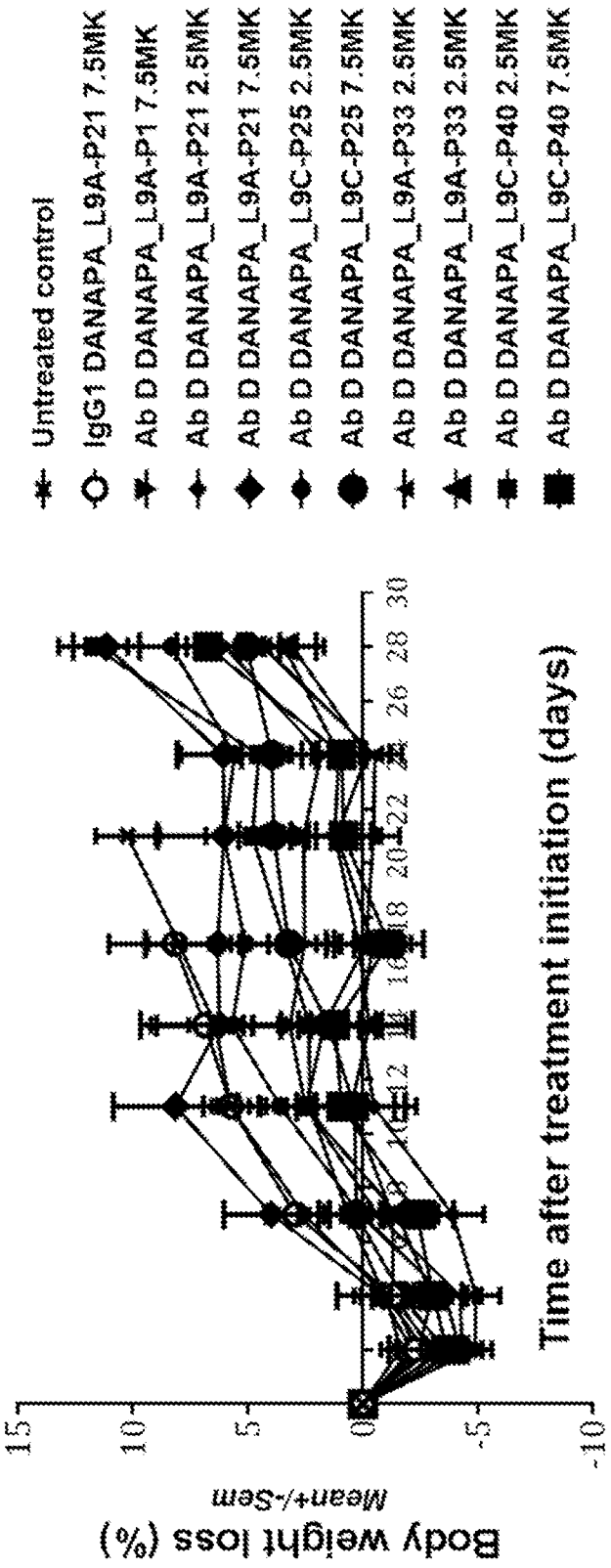

Untreated control
IgG1 DANAPA_L9A-P21 7.5MK
Ab D DANAPA_L9A-P1 7.5MK
Ab D DANAPA_L9A-P21 2.5MK
Ab D DANAPA_L9A-P21 7.5MK
Ab D DANAPA_L9C-P25 2.5MK
Ab D DANAPA_L9C-P25 7.5MK
Ab D DANAPA_L9A-P33 2.5MK
Ab D DANAPA_L9A-P33 2.5MK
Ab D DANAPA_L9C-P40 2.5MK
Ab D DANAPA_L9C-P40 7.5MK
Time after treatment initiation (days)
Body weight loss (%)
Mean+/-Sem

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/060620

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/68 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2017/214233 A1 (ABBVIE INC [US]) 14 December 2017 (2017-12-14) cited in the application claims and examples	1-96
A	WO 2016/094517 A1 (ABBVIE INC [US]) 16 June 2016 (2016-06-16) cited in the application claims and examples	1-96
A	WO 2016/094505 A1 (ABBVIE INC [US]) 16 June 2016 (2016-06-16) cited in the application claims and examples	1-96

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2022	01/03/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Burema, Shiri

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/060620

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/094509 A1 (ABBVIE INC [US]) 16 June 2016 (2016-06-16) cited in the application claims and examples	1-96
X,P	WO 2021/018858 A1 (SERVIER LAB [FR]; VERNALIS R&D LTD [GB]) 4 February 2021 (2021-02-04) the whole document	1-96
X,P	WO 2021/018857 A1 (SERVIER LAB [FR]; VERNALIS R&D LTD [GB]) 4 February 2021 (2021-02-04) the whole document	1-96

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/060620

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017214233 A1	14-12-2017	AU 2017277422 A1	03-01-2019
		BR 112018075639 A2	09-04-2019
		CA 3027173 A1	14-12-2017
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		EP 3468615 A1	17-04-2019
		EP 3888689 A1	06-10-2021
		JP 2019521114 A	25-07-2019
		US 2019153107 A1	23-05-2019
		WO 2017214233 A1	14-12-2017
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		AU 2020210220 A1	20-08-2020
		BR 112017012342 A2	27-02-2018
		CA 2970161 A1	16-06-2016
		CN 107207553 A	26-09-2017
		CN 111620862 A	04-09-2020
		EP 3230283 A1	18-10-2017
		JP 2018502839 A	01-02-2018
		JP 2020128378 A	27-08-2020
		US 2016158377 A1	09-06-2016
		US 2020239553 A1	30-07-2020
		WO 2016094517 A1	16-06-2016
WO 2016094505 A1	16-06-2016	AU 2015360609 A1	29-06-2017
		AU 2021205091 A1	12-08-2021
		BR 112017012377 A2	24-04-2018
		CA 2969908 A1	16-06-2016
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