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(54) Title: ANTI-EGFR ANTIBODY DRUG CONJUGATES

(57) Abstract: The invention relates to anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugates (ADCs) which inhibit Bcl-xL, including compositions and methods of using said ADCs.

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ANTI-EGFR ANTIBODY DRUG CONJUGATES

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/347,333, filed on June 8, 2016, the entire contents of which are expressly incorporated herein by reference.

SEQUENCE LISTING

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 June 2, 2017, is named 117813-12420_SL.txt and is 142,535 bytes in size.

BACKGROUND OF THE INVENTION

The human epidermal growth factor receptor (also known as HER-1 or Erb-B1, and referred to herein as "EGFR") is a 170 kDa transmembrane receptor encoded by the c-erbB protooncogene, and exhibits intrinsic tyrosine kinase activity (Modjtahedi et al., Br. J. Cancer 73:228-235 (1996); Herbst and Shin, Cancer 94:1593-1611 (2002)). SwissProt database entry P00533 provides the sequence of human EGFR. EGFR regulates numerous cellular processes via tyrosine-kinase mediated signal transduction pathways, including, but not limited to, activation of signal transduction pathways that control cell proliferation, differentiation, cell survival, apoptosis, angiogenesis, mitogenesis, and metastasis (Atalay et al., Ann. Oncology 14:1346-1363 (2003); Tsao and Herbst, Signal 4:4-9 (2003); Herbst and Shin, Cancer 94:1593-1611 (2002); Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)).

Known ligands of EGFR include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding by EGFR triggers receptor homo- and/or heterodimerization and autophosphorylation of key cytoplasmic residues. The phosphorylated EGFR recruits adapter proteins like GRB2 which in turn activate complex downstream signaling cascades, including at least the following major downstream signaling cascades: the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC, and STATs modules. This autophosphorylation also elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to cell proliferation. Ligand binding by EGFR may also activate the NF-kappa-B signaling cascade. Ligand binding also directly phosphorylates other proteins like RGS16, activating its GTPase

activity and potentially coupling the EGF receptor signaling to G protein-coupled receptor signaling. Ligand binding also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin.

Overexpression of EGFR has been reported in numerous human malignant conditions, including cancers of the bladder, brain, head and neck, pancreas, lung, breast, ovary, colon, prostate, and kidney. (Atalay et al., Ann. Oncology 14:1346-1363 (2003); Herbst and Shin, Cancer 94:1593-1611 (2002); and Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)). In many of these conditions, the overexpression of EGFR correlates or is associated with poor prognosis of the patients. (Herbst and Shin, Cancer 94:1593-1611 (2002); and Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)). EGFR is also expressed in the cells of normal tissues, particularly the epithelial tissues of the skin, liver, and gastrointestinal tract, although at generally lower levels than in malignant cells (Herbst and Shin, Cancer 94:1593-1611 (2002)).

A significant proportion of tumors containing amplifications of the EGFR gene also co-express a truncated version of the receptor (Wikstrand et al. (1998) J. Neurovirol. 4, 148-158) known as de2-7 EGFR, ΔEGFR, EGFRvIII, or Δ2-7 (terms used interchangeably herein) (Olapade-Olaopa et al. (2000) Br. J. Cancer. 82, 186-94). The rearrangement seen in the de2-7 EGFR results in an in-frame mature mRNA lacking 801 nucleotides spanning exons 2-7 (Wong et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 2965-9; Yamazaki et al. (1990) Jpn. J. Cancer Res. 81, 773-9; Yamazaki et al. (1988) Mol. Cell. Biol. 8, 1816-20; and Sugawa et al. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8602-6). The corresponding EGFR protein has a 267 amino acid deletion comprising residues 6-273 of the extracellular domain and a novel glycine residue at the fusion junction (Sugawa et al., 1990). This deletion, together with the insertion of a glycine residue, produces a unique junctional peptide at the deletion interface (Sugawa et al., 1990).

EGFRvIII has been reported in a number of tumor types including glioma, breast, lung, ovarian and prostate (Wikstrand et al. (1997) Cancer Res. 57, 4130-40; Olapade-Olaopa et al. (2000) Br. J. Cancer. 82, 186-94; Wikstrand, et al. (1995) Cancer Res. 55, 3140-8; Garcia de Palazzo et al. (1993) Cancer Res. 53, 3217-20). While this truncated receptor does not bind ligand, it possesses low constitutive activity and imparts a significant growth advantage to glioma cells grown as tumor xenografts in nude mice (Nishikawa et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7727-31) and is able to transform NIH3T3 cells (Batra et al. (1995) Cell Growth Differ. 6, 1251-9) and MCF-7 cells. The cellular mechanisms utilized by the de2-7 EGFR in glioma cells are not fully defined but are reported to include a decrease in apoptosis (Nagane et al. (1996) Cancer Res. 56, 5079-86) and a small enhancement of proliferation (Nagane et al., 1996). As expression of this truncated receptor is restricted to tumor cells it represents a highly specific target for antibody therapy.

Antibody drug conjugates (ADC) represent a new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen. Given the role of EGFR in cancer, there remains a need in the art for anti-EGFR ADCs that can be used for treatment of cancer.

SUMMARY OF THE INVENTION

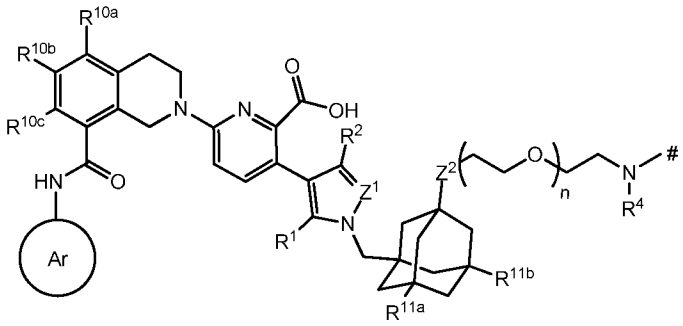
It has been discovered that small molecule inhibitors of Bcl-xL are efficacious when administered in the form of antibody drug conjugates (ADCs) that bind to antigens expressed on the surface of cells, *e.g.* cells that express EGFR, where inhibition of Bcl-xL and consequent induction of apoptosis would be beneficial. This discovery provides the ability to target Bcl-xL inhibitory therapies to specific cells and/or tissues that express EGFR, such that the Bcl-xL inhibitor is delivered internally to a transformed cancer cell expressing EGFR. One advantage of the invention is the potential for lowering serum levels necessary to achieve desired therapeutic benefit and/or avoiding and/or ameliorating potential side effects associated with systemic administration of the small molecule Bcl-xL inhibitors *per se*.

ADCs may increase the therapeutic efficacy of antibodies in treating disease, *e.g.*, cancer, due to the ability of the ADC to selectively deliver one or more drug moiety(s) to target tissues, such as a tumor-associated antigen, *e.g.*, EGFR expressing tumors. Thus, in certain embodiments, the invention provides anti-EGFR ADCs for therapeutic use, *e.g.*, treatment of cancer.

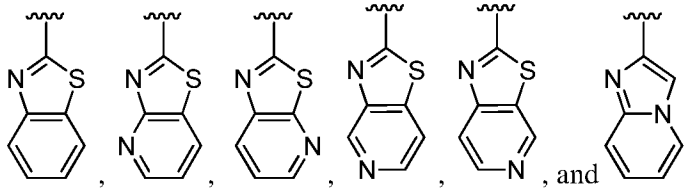
In one aspect, the invention features an anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising an anti-hEGFR antibody, *i.e.*, an antibody that specifically binds to human EGFR, linked to one or more Bcl-xL inhibitor(s).

In another aspect, the invention features an anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

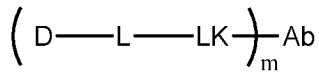
represents a point of attachment to a linker;

wherein the anti-hEGFR antibody has the following characteristics:

binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) or competes with a second anti-hEGFR antibody for binding to epidermal growth factor receptor variant III (EGFRvIII) (SEQ ID NO: 33) in a competitive binding assay, wherein the second anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5; and

binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K_d) of about 1 x 10^{-6} M or less, as determined by surface plasmon resonance.

In one embodiment, the ADC is a compound according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

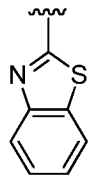
L is the linker;

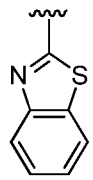
Ab is the anti-hEGFR antibody;

LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and

m is an integer ranging from 1 to 20.

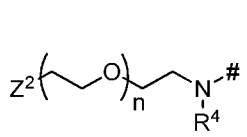
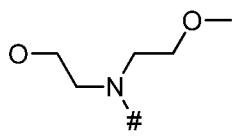
In another embodiment, the Ar is unsubstituted.

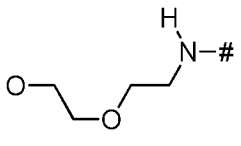
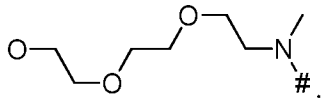
In a further embodiment, Ar is  .

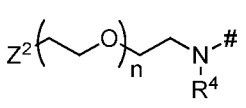
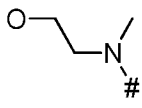
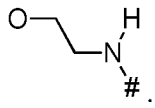
In one embodiment, R^{10a}, R^{10b}, and R^{10c} are each hydrogen. In another embodiment, one of R^{10a}, R^{10b} and R^{10c} is halo and the others are hydrogen. In another embodiment, Z^1 is N. In another embodiment, R^1 is methyl or chloro. In another embodiment, R^2 is hydrogen or methyl. In another embodiment, R^2 is hydrogen. In another embodiment, R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with -OCH_3. In another embodiment, Z^1 is N; R^1 is methyl; R^2 is hydrogen; R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with -OCH_3; one of R^{10a}, R^{10b} and R^{10c} is hydrogen or halo, and the others are hydrogen; R^{11a} and R^{11b} are each methyl, and Ar is  .

In another embodiment, Z^2 is CH_2 or O.

In another embodiment, n is 0, 1 or 2.

In another embodiment, the group  is  ,

 , or  .

In another embodiment, the group  is  or  .

In another embodiment, Z^2 is oxygen, R^4 is hydrogen or C_{1-4} alkanyl optionally substituted with OCH_3, and n is 0, 1 or 2.

In one embodiment, the Bcl-xL inhibitor is selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

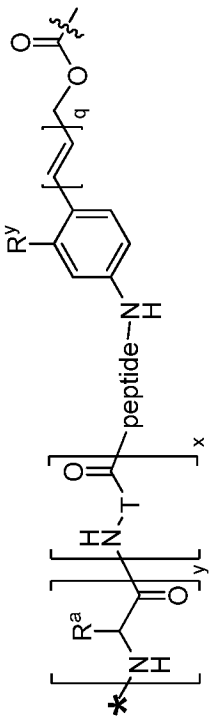
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

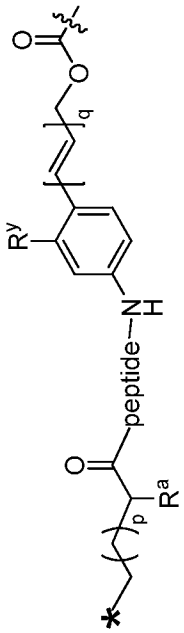
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

In one embodiment of any one of the aspects and embodiments herein, the linker is cleavable by a lysosomal enzyme. In a further embodiment, the lysosomal enzyme is Cathepsin B.

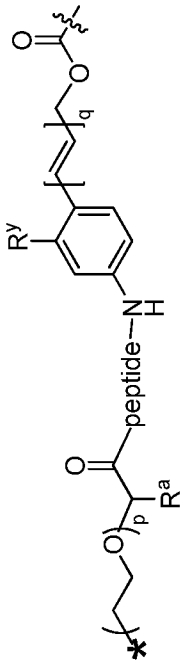
In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (IVa), (IVb), (IVc), or (IVd):



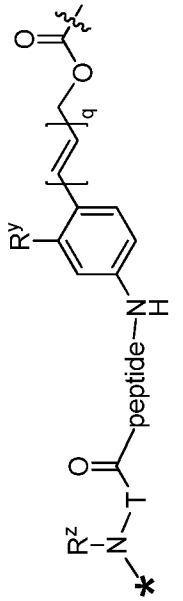
(IVa)



(IVb)



(IVc)



(IVd)

wherein:

peptide represents a peptide (illustrated N→C, wherein peptide includes the amino and carboxy "termini") cleavable by a lysosomal enzyme;

T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof;

R^a is selected from hydrogen, C_{1-6} alkyl, SO_3H and CH_2SO_3H;

R^y is hydrogen or C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^1 or C_{1-4} alkyl-(N)-[(C_{1-4} alkylene)-G^1]$_2$;

R^z is C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^2;

G^1 is SO_3H, CO_2H, PEG 4-32, or sugar moiety;

G^2 is SO_3H, CO_2H, or PEG 4-32 moiety;

r is 0 or 1;

s is 0 or 1;

p is an integer ranging from 0 to 5;

q is 0 or 1;

x is 0 or 1;

y is 0 or 1;

 represents the point of attachment of the linker to the Bcl-xL inhibitor; and

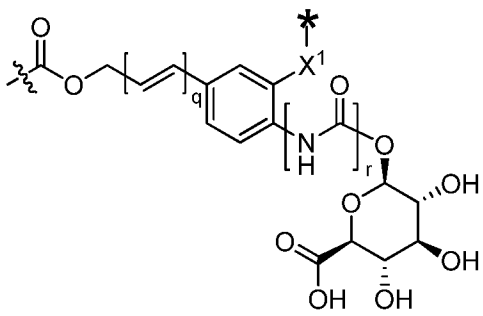
* represents the point of attachment to the remainder of the linker.

In a further embodiment, the peptide is selected from the group consisting of 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.

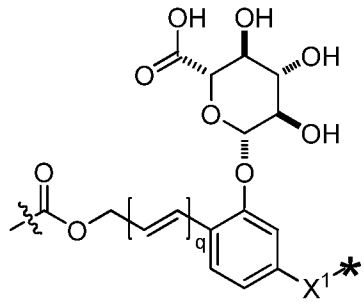
In one embodiment, the lysosomal enzyme is β-glucuronidase or β-galactosidase.

In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

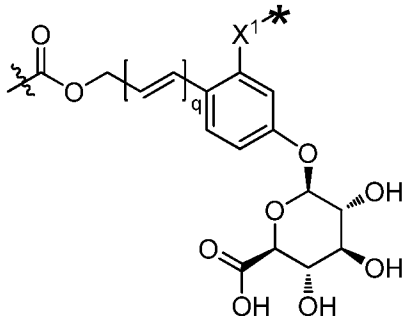
(Va)



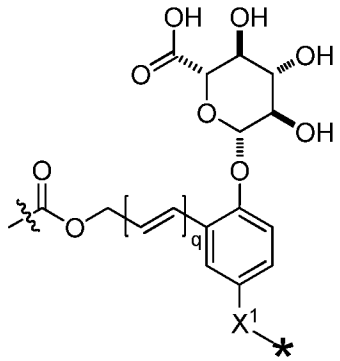
(Vb)



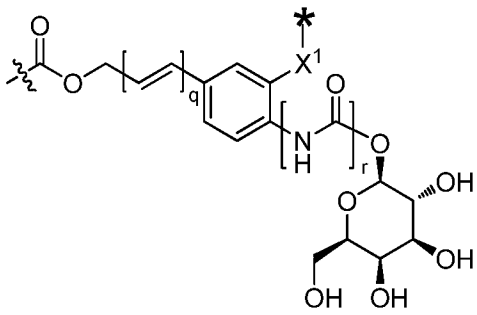
(Vc)



(Vd)



(Ve)



wherein:

q is 0 or 1;

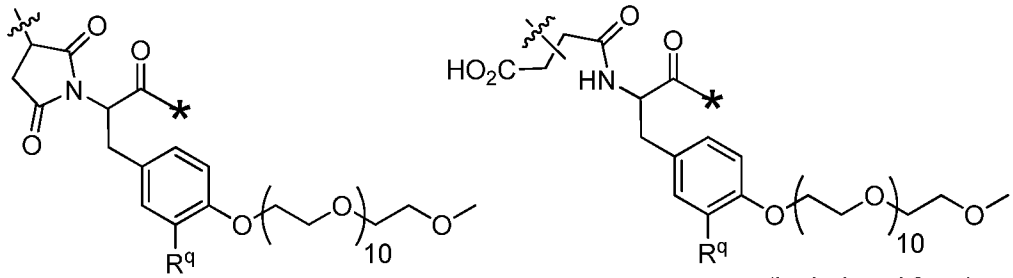
r is 0 or 1;

X^1 is CH_2, O or NH;

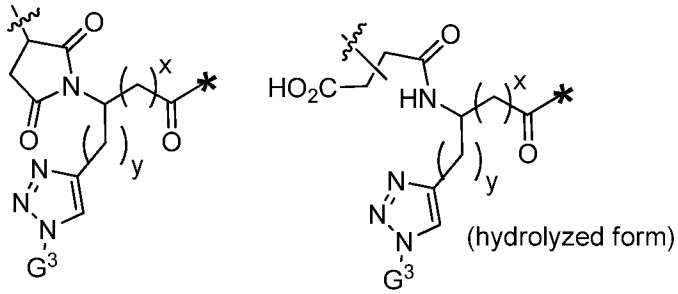
~ represents the point of attachment of the linker to the drug; and

* represents the point of attachment to the remainder of the linker.

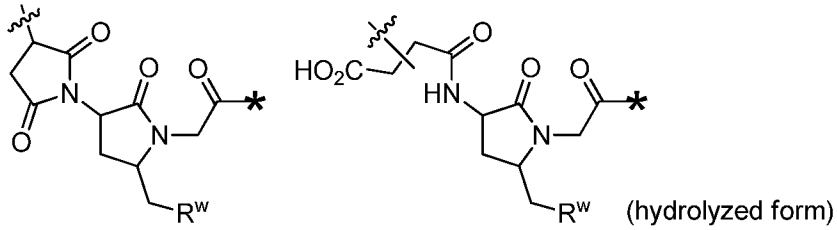
In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (VIIIa), (VIIIb), or (VIIIc):



(VIIIa)



(VIIIb)



(VIIIc)

or a hydrolyzed derivative thereof, wherein:

R^q is H or $-O-(CH_2CH_2O)_{11}-CH_3$;

x is 0 or 1;

y is 0 or 1;

G^3 is $-CH_2CH_2CH_2SO_3H$ or $-CH_2CH_2O-(CH_2CH_2O)_{11}-CH_3$;

R^w is $-O-CH_2CH_2SO_3H$ or $-NH(CO)-CH_2CH_2O-(CH_2CH_2O)_{12}-CH_3$;

* represents the point of attachment to the remainder of the linker; and

~ represents the point of attachment of the linker to the antibody.

In one embodiment of any one of the aspects and embodiments herein, the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

In one embodiment of any one of the aspects and embodiments herein, m is 2, 3 or 4.

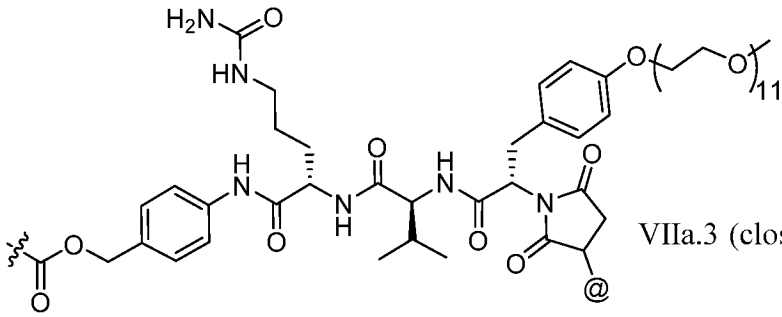
In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from IVa or IVb.

In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-V1c.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 in the closed or open form.

In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

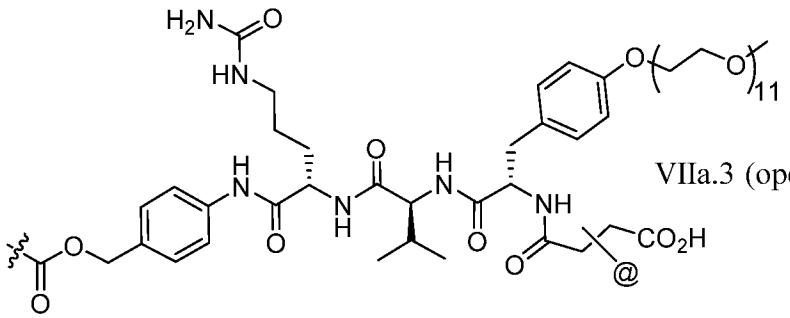
In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVc.5, IVc.6, IVd.4, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of VIIa.3, IVc.6, VIIc.1, and VIIc.5, wherein  is the attachment point to drug D and @ is the attachment point to the LK, wherein when the linker is in the open form as shown below, @ can be either at the α-position or β-position of the carboxylic acid next to it:



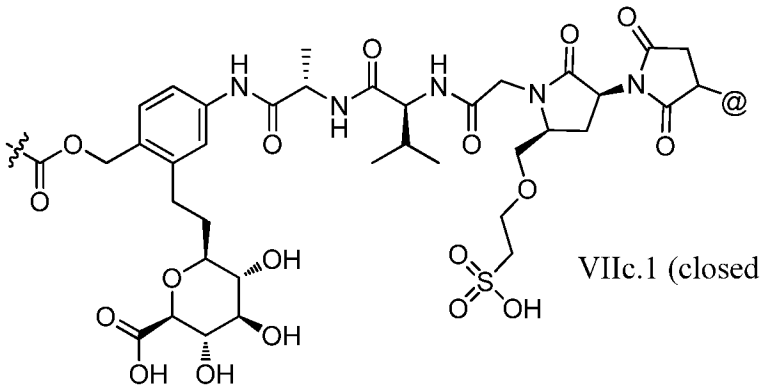
VIIa.3 (closed form)

,



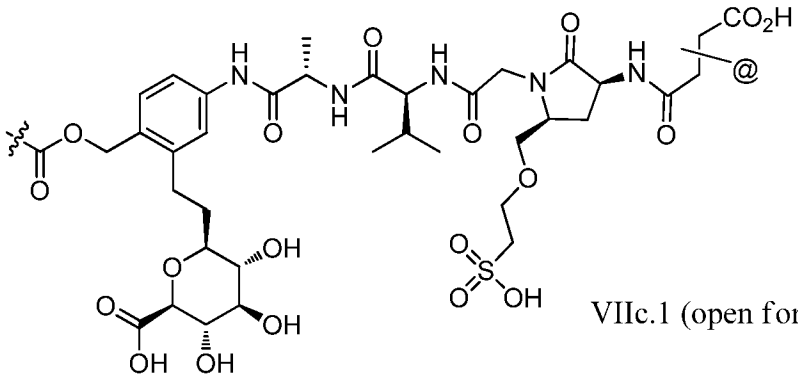
VIIa.3 (open form)

,



VIIc.1 (closed form)

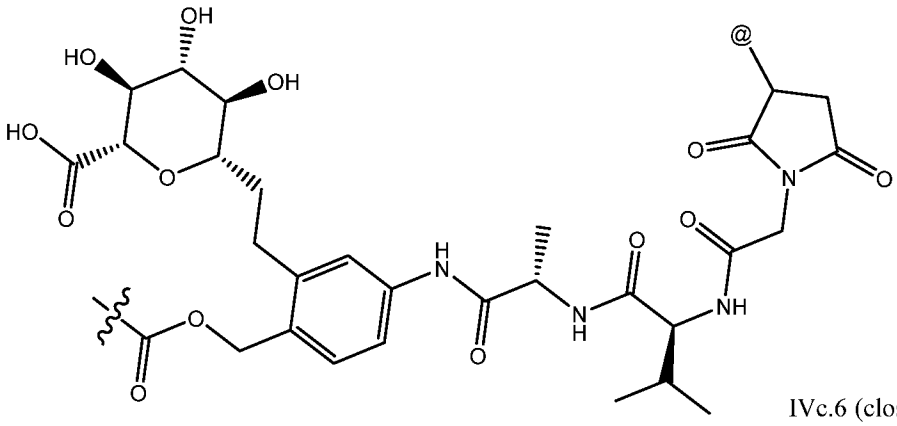
,



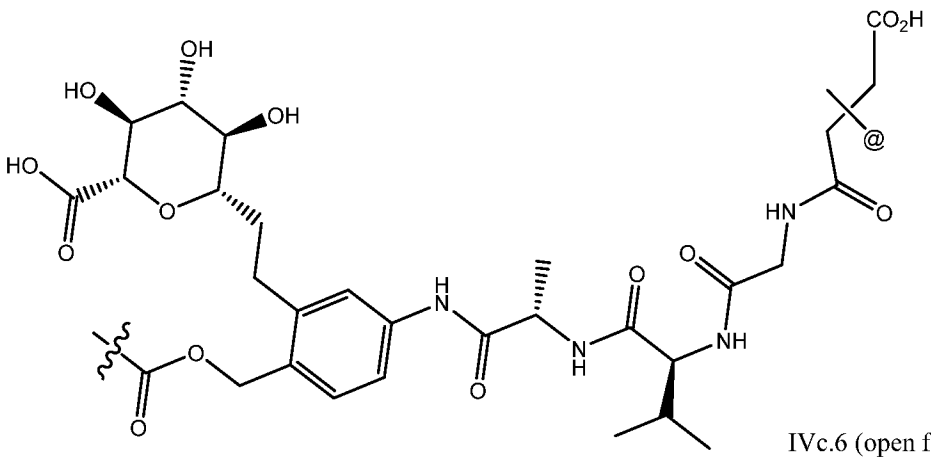
VIIc.1 (open form)

,

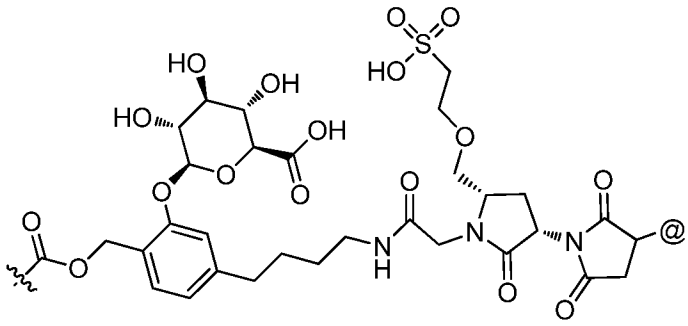
,



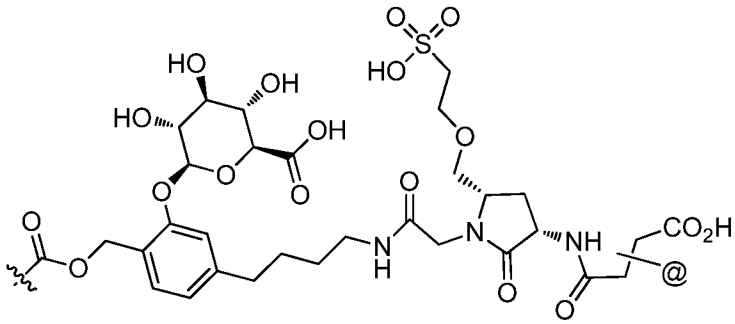
IVc.6 (closed form),



IVc.6 (open form),



VIIc.5 (closed form), and



VIIc.5 (open form)..

In one embodiment of any one of the aspects and embodiments herein, LK is a linkage formed with an amino group on the anti-hEGFR antibody Ab. In a further embodiment, LK is an amide or a thiourea.

In one embodiment of any one of the aspects and embodiments herein, LK is a linkage formed with a sulfhydryl group on the anti-hEGFR antibody Ab. In a further embodiment, LK is a thioether.

In one embodiment of any one of the aspects and embodiments herein, LK is selected from the group consisting of amide, thiourea and thioether; and m is an integer ranging from 1 to 8.

In one embodiment, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-V1c.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 wherein each linker has reacted with the anti-hEGFR antibody, Ab, forming a covalent attachment;

LK is thioether; and

m is an integer ranging from 1 to 8.

In one embodiment, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; and

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

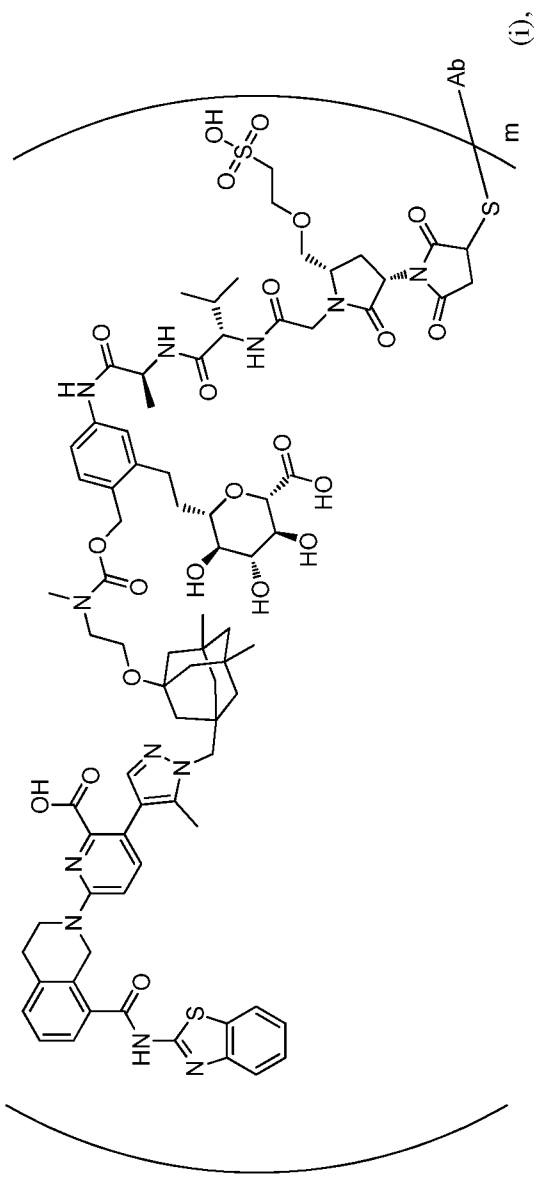
L is selected from the group consisting of linkers Vc.5, IVc.6, IVd.4, VIIa.1, VIIc.1, VIIc.3, VIIc.4, and VIIc.5 in either closed or open form;

LK is thioether; and

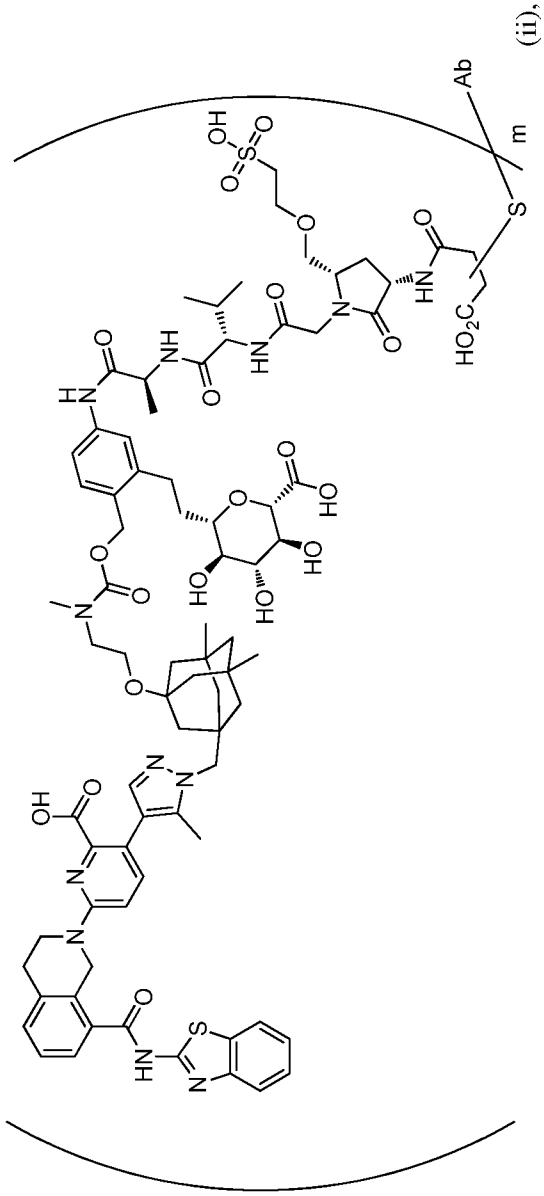
m is an integer ranging from 2 to 4.

In one embodiment, the ADC is selected from the group consisting of AbA-WD, AbA-LB, AbA-VD, AbB-WD, AbB-LB, AbB-VD, AbG-WD, AbG-LB, AbG-VD, AbK-WD, AbK-LB, and AbK-VD, wherein WD, LB, and VD are synthons disclosed in Table 5, and wherein the synthons are either in open or closed form.

In one embodiment, the ADC is selected from the group consisting of formulas i-vi:

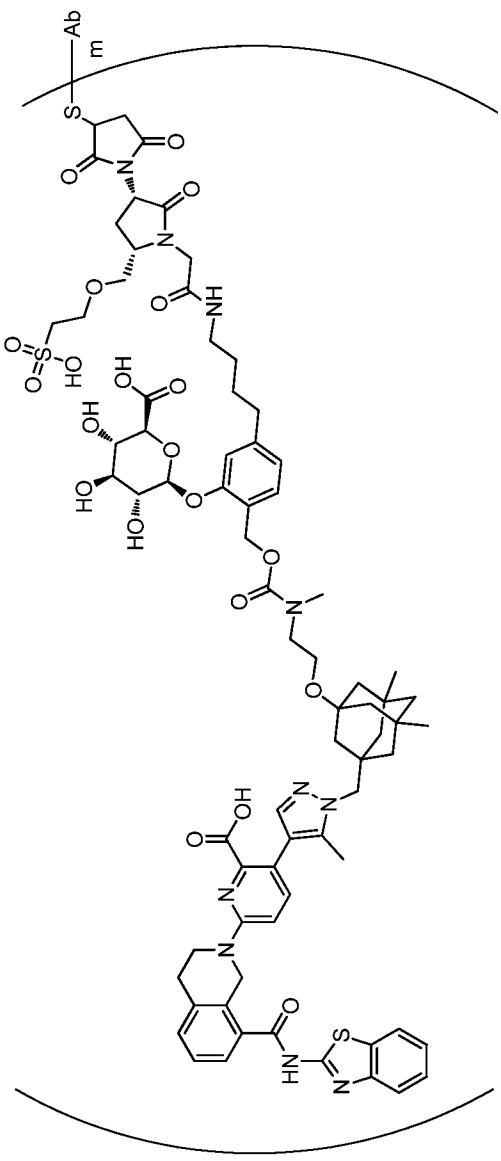


(i),

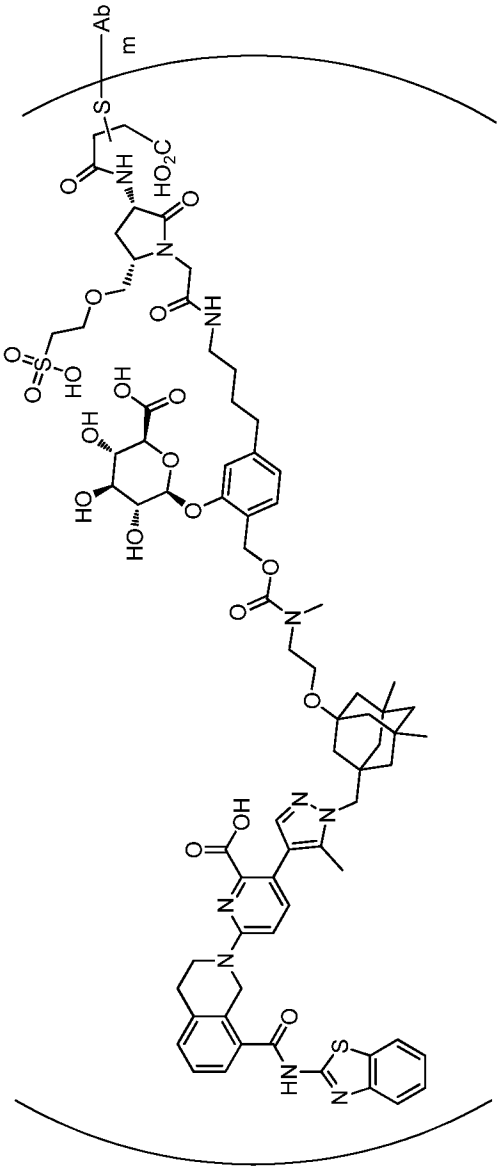


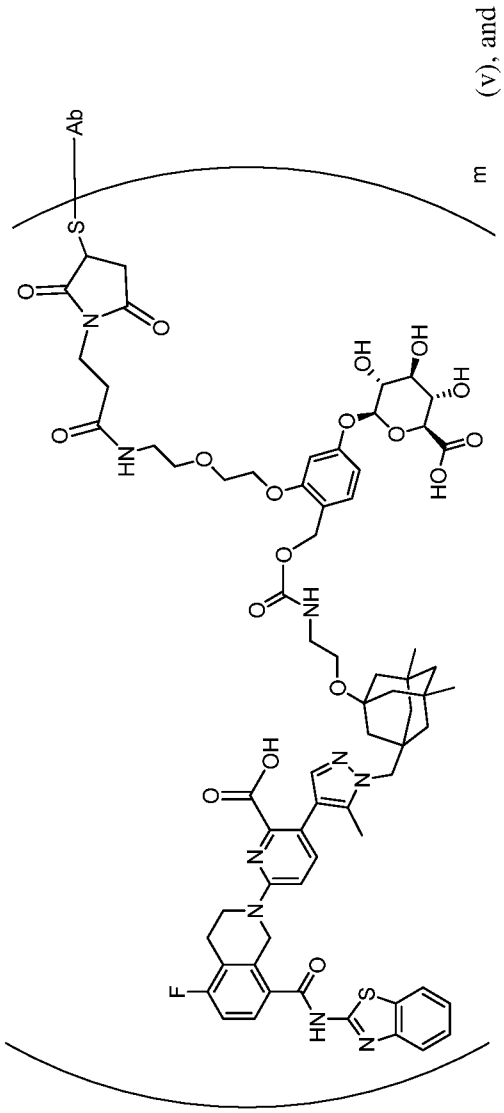
(ii),

(iii),

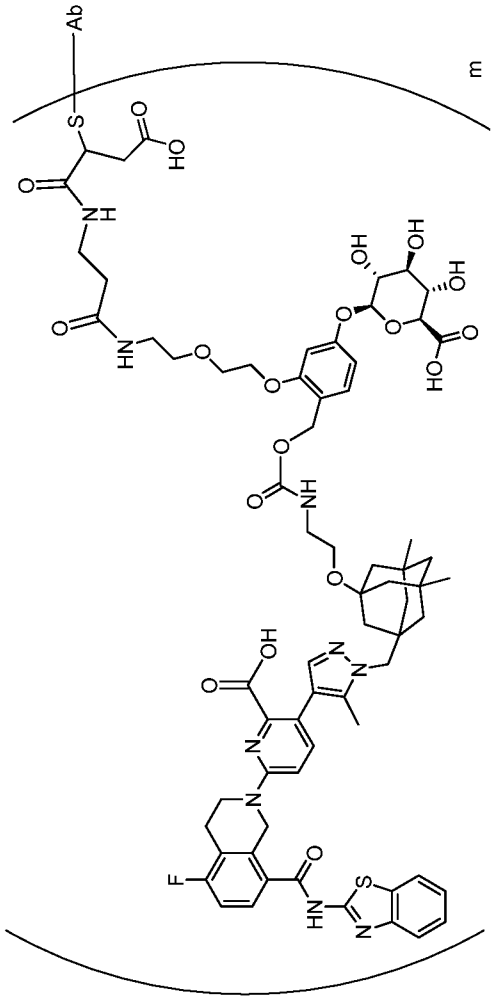


(iv),





m (v), and



m (vi),

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG.

In a further embodiment, m is an integer from 2 to 6.

In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-10} M, as determined by surface plasmon resonance.

In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-7} M, as determined by surface plasmon resonance.

In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of about 8.2 x 10^{-9} M or less, as determined by surface plasmon resonance.

In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of between about 8.2 x 10^{-9} M and about 6.3 x 10^{-10} M, as determined by surface plasmon resonance.

In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of between about 8.2 x 10^{-9} M and about 2.0 x 10^{-9} M, as determined by surface plasmon resonance.

In one embodiment of any one of the aspects and embodiments herein, the anti-hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85, wherein the antibody does not comprise both the heavy chain CDR set of SEQ ID NOs: 2, 3, and 4, and the light chain CDR set of SEQ ID NOs: 6, 7, and 8.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO:

23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 64, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65.

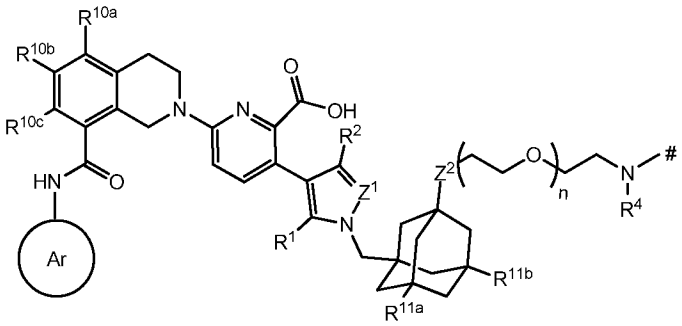
In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

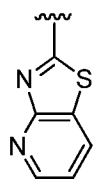
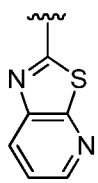
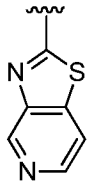
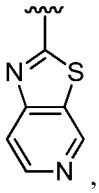
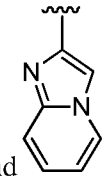
In one embodiment, the antibody is an IgG1 antibody having four polypeptide chains, two heavy chains and two light chains.

In one embodiment, the antibody comprises a kappa light chain. In one embodiment, the antibody comprises a lambda light chain.

In one aspect, the invention features an anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa) 

wherein:

Ar is selected from  ,  ,  ,  , and  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

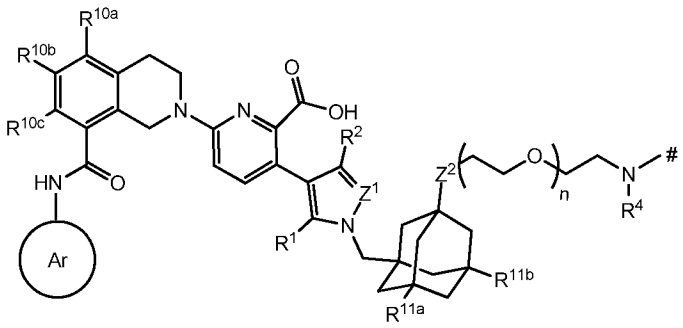
represents a point of attachment to a linker; and

wherein the anti-hEGFR antibody is a monoclonal IgG antibody and comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6.

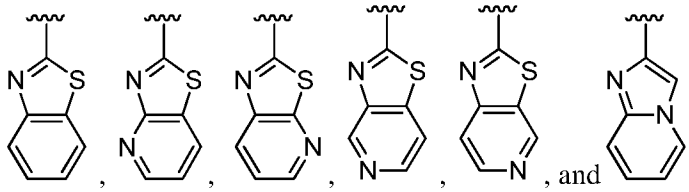
In one embodiment, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

In another aspect, the invention features an anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

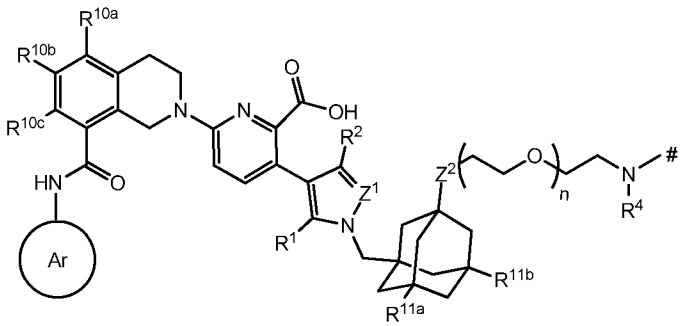
represents a point of attachment to a linker; and

wherein the antibody is a monoclonal IgG antibody and comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

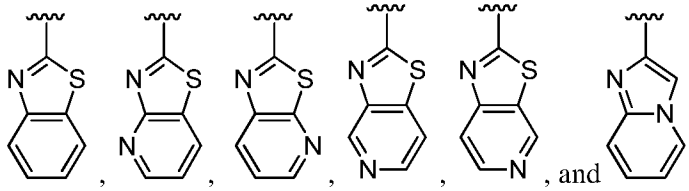
In one embodiment, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

In another aspect, the invention features an anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

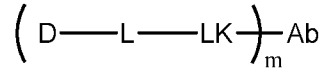
n is 0, 1, 2 or 3; and

represents a point of attachment to a linker; and

wherein the antibody is a monoclonal IgG antibody and comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

In one embodiment, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

In one embodiment of any one of the aspects and embodiments herein, the ADC is a compound according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

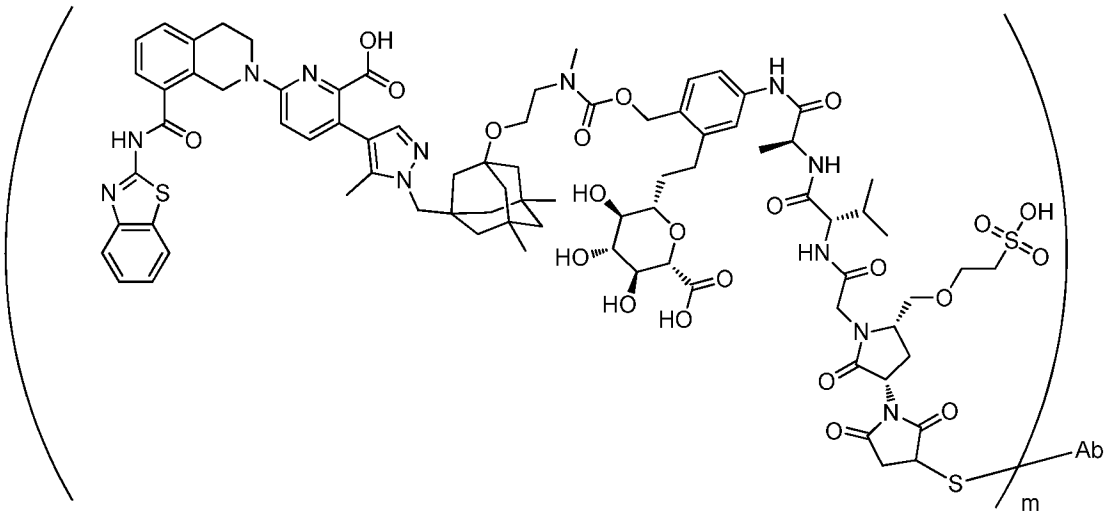
L is the linker;

Ab is the anti-hEGFR antibody;

LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and

m is an integer ranging from 1 to 20.

In one embodiment, the ADC is a compound according to structural formula (i)

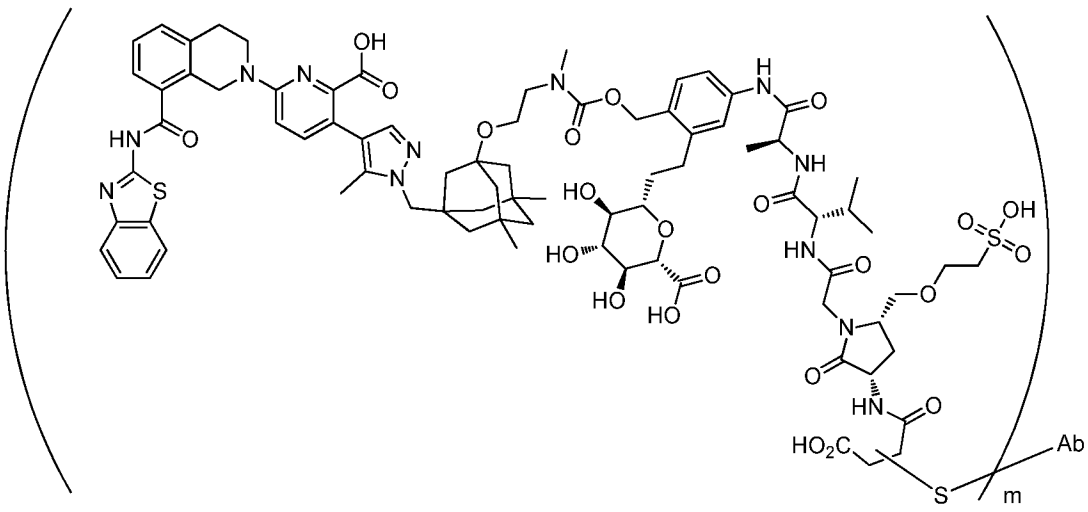


(i),

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light

chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

In one embodiment, the ADC is a compound according to structural formula (ii)

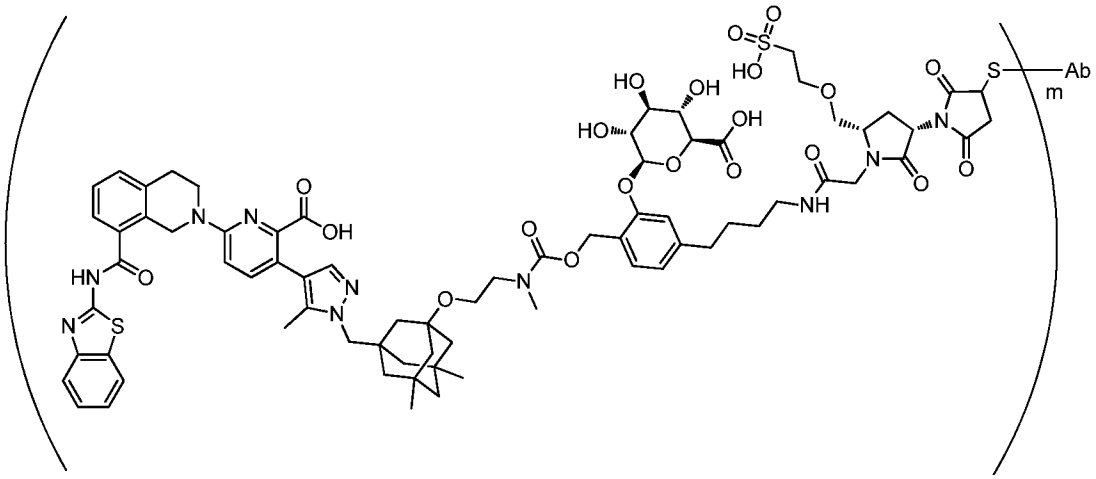


(ii),

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable

region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

In one embodiment, the ADC is a compound according to structural formula (iii)

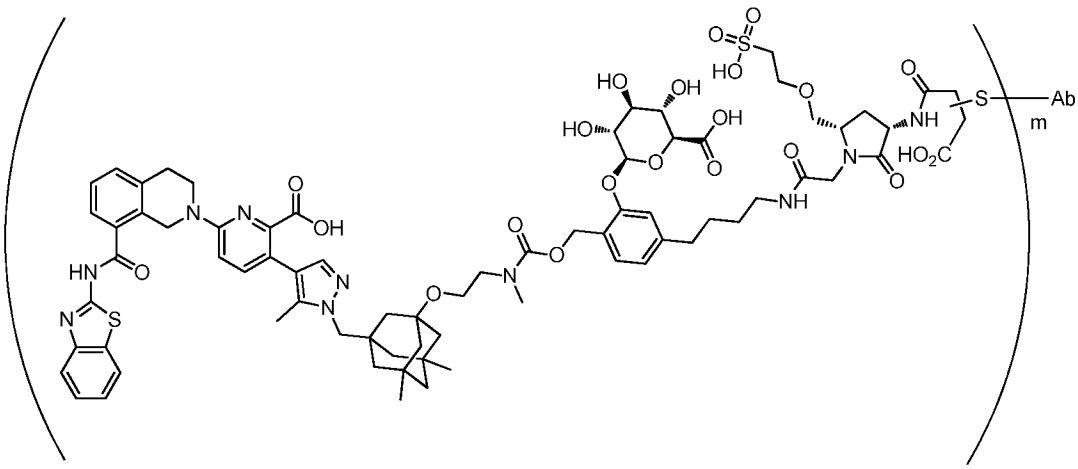


(iii)

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light

chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

In one embodiment, the ADC is a compound according to structural formula (iv)



(iv),

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light

chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

In another aspect, the present invention features a pharmaceutical composition comprising an effective amount of an ADC according to any one of the aspects and embodiments herein, and a pharmaceutically acceptable carrier.

In another aspect, the invention features a pharmaceutical composition comprising an ADC mixture comprising a plurality of the ADC of any one of the aspects or embodiments herein, and a pharmaceutically acceptable carrier.

In one embodiment, the ADC mixture has an average drug to antibody ratio (DAR) of 2 to 4.

In one embodiment, the ADC mixture comprises ADCs each having a DAR of 2 to 8.

In one embodiment, the ADC mixture comprises ADCs each having a DAR of 1.5-4.

In one embodiment, the ADC mixture comprises ADCs each having a DAR of 1.5-8.

In another aspect, the invention features a method for treating cancer, comprising administering a therapeutically effective amount of an ADC of any one of the embodiments or aspects herein to a subject in need thereof.

In one embodiment, the cancer is selected from the group consisting of breast cancer, lung cancer, a glioblastoma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, and kidney cancer.

In another embodiment, the cancer is a squamous cell carcinoma. In a further embodiment, the squamous cell carcinoma is squamous lung cancer or squamous head and neck cancer.

In one embodiment, the cancer is triple negative breast cancer.

In one embodiment, the cancer is non-small cell lung cancer.

In one embodiment of any one of the aspects and embodiments herein, the cancer is characterized as having EGFR overexpression.

In one embodiment of any one of the aspects and embodiments herein, the cancer is characterized as having an activating EGFR mutation. In a further embodiment, the activating EGFR mutation is selected from the group consisting of an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

In another aspect, the invention features a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering the ADC of any one of the embodiments or aspects herein to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased.

In one embodiment, the solid tumor is a non-small cell lung carcinoma or a glioblastoma.

In one embodiment, the solid tumor is a squamous cell carcinoma.

In one embodiment of any one of the aspects and embodiments herein, the solid tumor is an EGFRvIII positive solid tumor or is an EGFR-expressing solid tumor.

In one embodiment of any one of the aspects and embodiments herein, the solid tumor overexpresses EGFR.

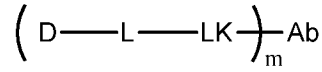
In one embodiment of any one of the aspects and embodiments herein, the ADC is administered in combination with an additional agent or an additional therapy.

In another embodiment, the additional agent is selected from the group consisting of an anti-PD1 antibody (e.g. pembrolizumab), an anti-PD-L1 antibody (e.g. atezolizumab), an anti-CTLA-4 antibody (e.g. ipilimumab), a MEK inhibitor (e.g. trametinib), an ERK inhibitor, a BRAF inhibitor (e.g. dabrafenib), osimertinib, erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g. dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-xL inhibitor, a Bcl-2 inhibitor (e.g. venetoclax), ibrutinib, a mTOR inhibitor (e.g. everolimus), a PI3K inhibitor (e.g. buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g. lapatinib), a taxane (e.g. docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g. rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g. TDM1), a TRAIL agonist, a proteasome inhibitor (e.g. bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor.

In one embodiment, the additional therapy is radiation.

In one embodiment, the additional agent is a chemotherapeutic agent.

In another aspect, the present invention features a process for the preparation of an ADC according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

L is the linker;

Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA, AbB, AbG, and AbK;

LK represents a covalent linkage linking linker L to antibody Ab; and

m is an integer ranging from 1 to 20.

the process comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.31 and 2.34 to 2.63;

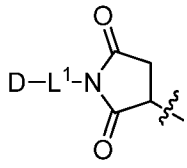
adjusting the pH of the solution to a pH of 7.5 to 8.5; and

allowing the reaction to run for 48 to 80 hours..

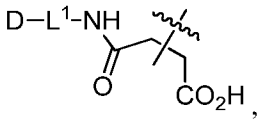
wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry

In another embodiment, the invention features an ADC prepared by the process described in the aspects and embodiments herein.

In one embodiment of any one of the aspects and embodiments herein, the ADC is formed by contacting an antibody that binds a hEGFR cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the synthon covalently links to the antibody through a maleimide moiety as shown in formulae (IId) and (IIe),



(IId)



(IIe),

wherein D is the Bcl-xL inhibitor drug of formula (IIa); and L^1 is the portion of the linker not formed from the maleimide upon attachment of the synthon to the antibody; and wherein the drug-linker synthon is selected from the list below:

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl) carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy) methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({(1s,3s)-3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[(2S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-

(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-

1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid;

1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-

yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,20-dioxo-7,10,13,16-tetraoxa-3,19-diazadocos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-methyl-10,26-dioxo-3,6,13,16,19,22-hexaoxa-9,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl](methyl)amino}ethoxy)ethoxy]ethoxy}-

5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetratriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,26-dioxo-7,10,13,16,19,22-hexaoxa-3,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-

yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide;

(6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid;

(6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{4-[({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)amino]butyl}phenyl beta-D-glucopyranosiduronic acid;

3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-

dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium; and

(6S)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{[N-({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid.

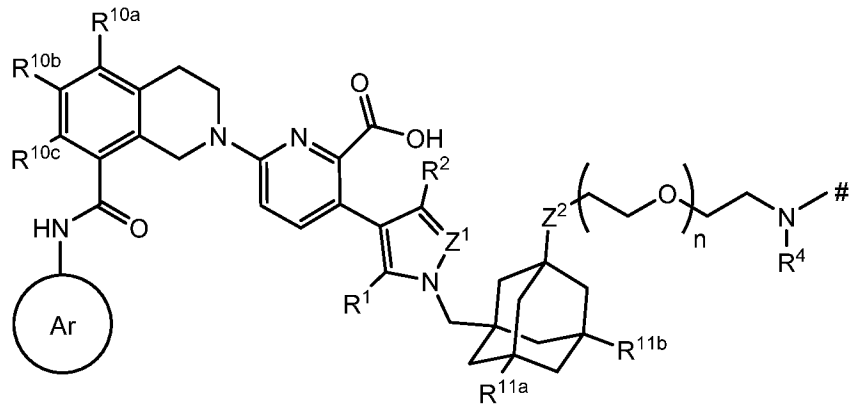
In one embodiment, the contacting step is carried out under conditions such that the ADC has a DAR of 2, 3 or 4.

In another aspect, the present invention features a synthon according to structural formula D-L^2-R^x, wherein:

D is the Bcl-xL inhibitor drug according to structural formula (IIa);

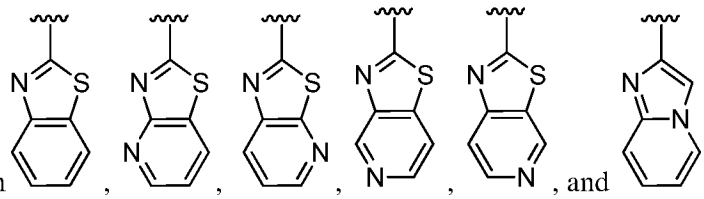
L^2 is the linker selected from the group consisting of IVa.8, IVb.16-IVb.19, IVc.3-IVc.6, IVd.1-IVd.4, Vb.5-Vb.10, Vc.11, Vd.3-Vd.6, VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8 and VIIc.1-VIIc.6; and

R^x is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,



(IIa)

wherein:

Ar is selected from  , and , which is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents the point of attachment to linker L^2.

In one embodiment, R^x comprises a maleimide, an acetyl halide, or a vinyl sulfone.

In one embodiment, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

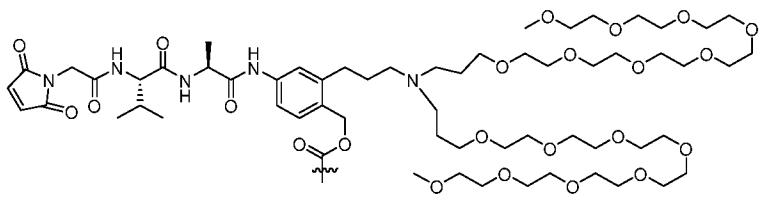
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

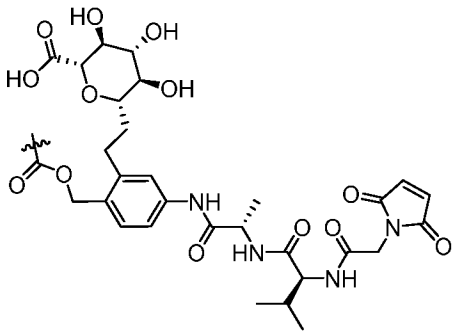
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

In one embodiment, linker L^2 is selected from the group consisting of:

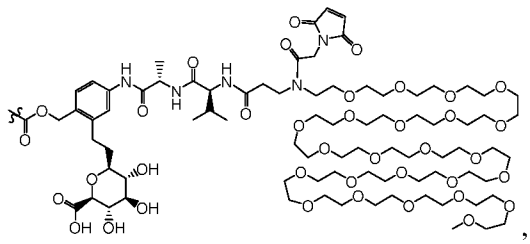
(IVc.5)



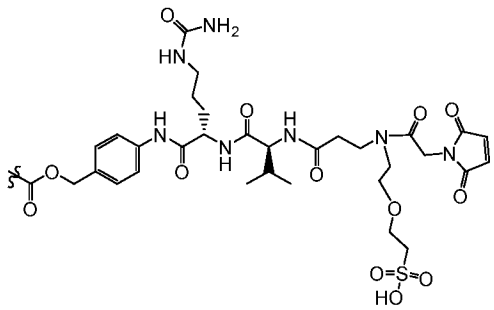
(IVc.6)



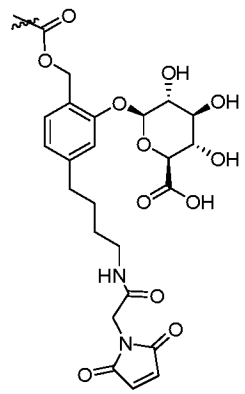
(IVd.3)



(IVd.4)



(Vb.9)



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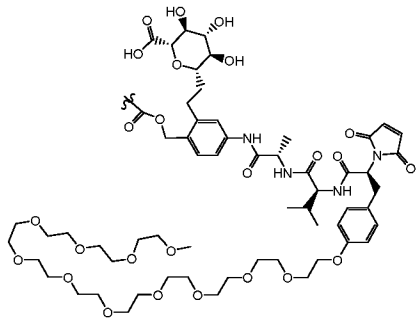
(VIIa.1)



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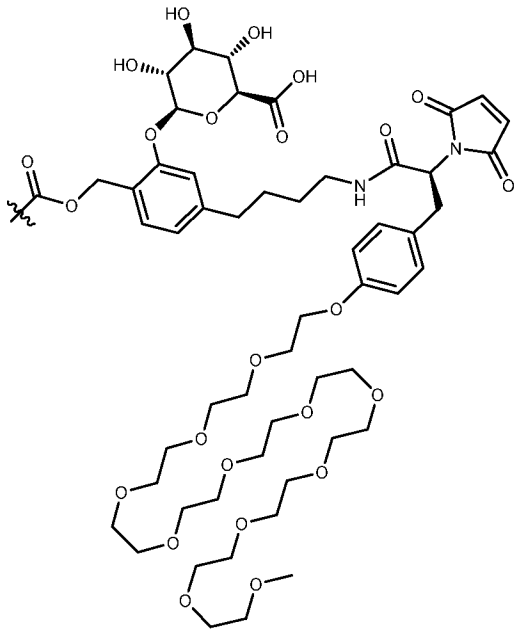
(VIIa.2)



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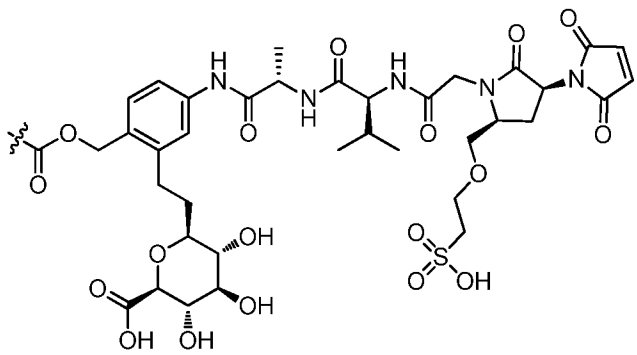
(VIIc.1)



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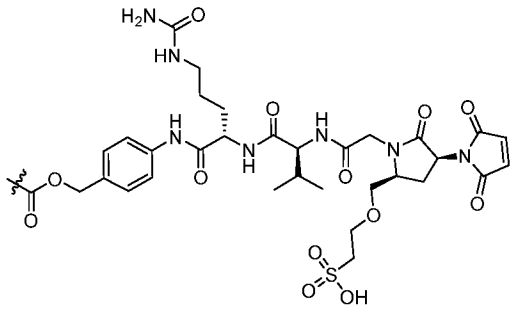
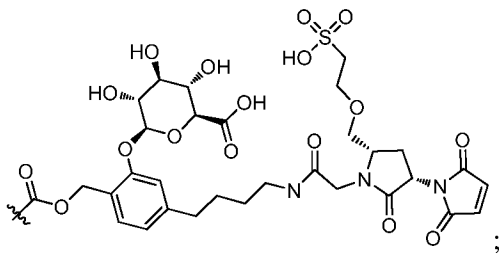
(VIIc.4)



, and

(VIIc.5)

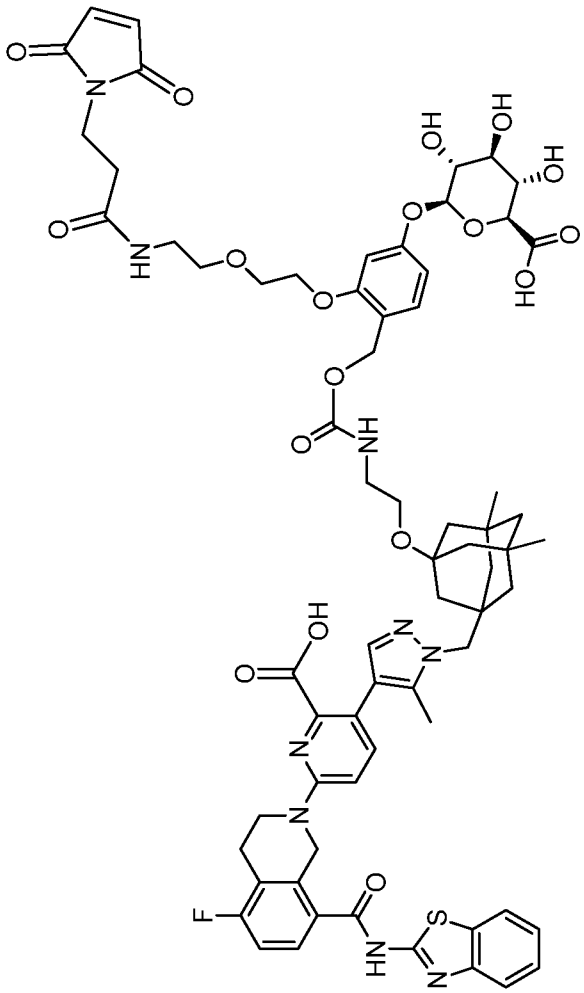


;

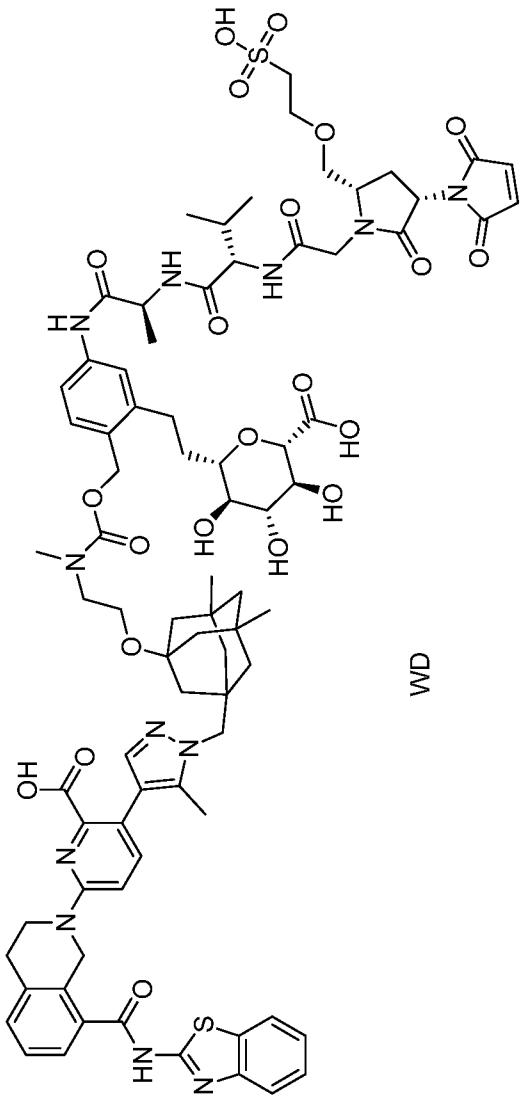
wherein, ~ represents the point of attachment of the linker L^2 to the Bcl-xL inhibitor.

In one embodiment, the synthon is selected from the group consisting of synthon examples 2.42 (LB), 2.54 (LX), 2.55 (MJ), 2.56 (NH), 2.57 (OV), 2.58 (QS), 2.59 (SG), 2.60 (UF), 2.61 (VD), 2.62 (VX), 2.63 (WD).

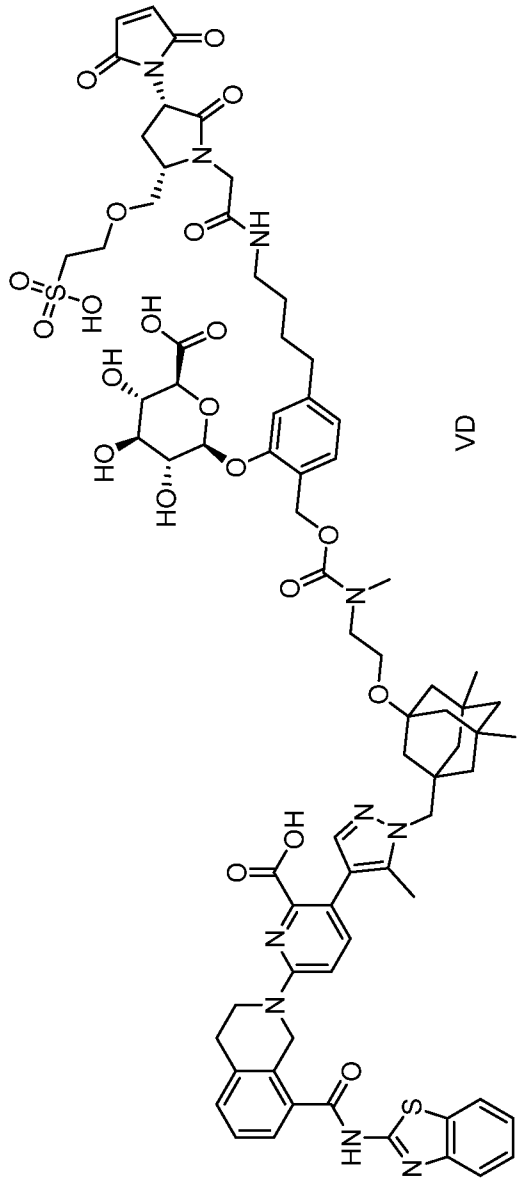
In one embodiment, the synthon is selected from the group consisting of synthon examples 2.41 (LB), 2.61 (VD) and 2.63 (WD).



LB

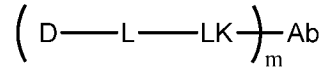


WD



VD

In another aspect, the invention features a process for the preparation of an ADC according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa) as disclosed herein;

L is the linker as disclosed herein;

Ab is an hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA; AbB; AbG; or AbK;

LK represents a covalent linkage linking linker L to antibody Ab; and

m is an integer ranging from 1 to 20;

the process comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to2.63 (Table 5);

adjusting the pH of the solution to a pH of 7.5 to 8.5;

allowing the reaction to run for 48 to 80 hours to form the ADC;

wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

In one embodiment, m is 2.

In another aspect, the invention features an ADC prepared by the process as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic of EGFR and the regions bound by Ab1 and Ab2.

Figure 2 provides the variable heavy (VH) and variable light (VL) chain region amino acid sequences of Ab1 (SEQ ID NOs: 1 and 5) and AbA (SEQ ID NOs: 9 and 5). CDR sequences within the VH and VL regions are boxed, and differences between the Ab1 VH sequence and the AbA VH sequence are shaded.

Figure 3 describes the full length light and heavy chains for Ab1 (SEQ ID NOs: 13 and 14) and AbA (SEQ ID NOs: 13 and 15). Differences between the Ab1 sequence and the AbA sequence in the heavy chain are highlighted.

Figure 4 shows a representation of antibody reduction, modification with a maleimide derivative to give a thiosuccinimide intermediate, and subsequent hydrolysis of thiosuccinimide moiety.

Figure 5 shows mass spectrometry (MS) characterization of light chain and heavy chain of an exemplary antibody 1) prior to conjugation, 2) after conjugation to a maleimide derivative to give a thiosuccinimide intermediate and 3) post pH8-mediated hydrolysis of the thiosuccinimide ring.

DETAILED DESCRIPTION OF THE INVENTION

Numerous Bcl-xL inhibitors have been developed for treatment of diseases (*e.g.*, cancer) that involve dysregulated apoptotic pathways. However, Bcl-xL inhibitors can act on cells other than the target cells (*e.g.*, cancer cells). For instance, pre-clinical studies have shown that pharmacological inactivation of Bcl-xL reduces platelet half-life and causes thrombocytopenia (see Mason *et al.*, 2007, Cell 128:1173-1186).

Given the importance of Bcl-xL in regulating apoptosis, there remains a need in the art for agents that inhibit Bcl-xL activity, either selectively or non-selectively, as an approach towards the treatment of diseases in which apoptosis is dysregulated via expression or over-expression of anti-apoptotic Bcl-2 family proteins, such as Bcl-xL. Accordingly, new Bcl-xL inhibitors with reduced dose-limiting toxicity are needed.

One potential means of delivering a drug to a cell which has not been explored for Bcl-xL inhibitors is delivery through the use of antibody drug conjugates (ADCs). Antibody drug conjugates (ADC) represent a new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen.

Accordingly, the development of new ADCs that can selectively deliver Bcl-xL to target cancer cells, *e.g.*, EGFRvIII expressing cells, would be a significant discovery.

Various aspects of the invention relate to new anti-EGFR antibody drug conjugates (ADCs; also called immunoconjugates), and pharmaceutical compositions thereof. In particular, the present disclosure concerns new anti-EGFR ADCs comprising Bcl-xL inhibitors, synthons useful for synthesizing the ADCs, compositions comprising the ADCs, methods of making the ADCs, and various methods of using the ADCs.

As will be appreciated by skilled artisans, the ADCs disclosed herein are "modular" in nature. Throughout the instant disclosure, various specific embodiments of the various "modules" comprising the ADCs, as well as the synthons useful for synthesizing the ADCs, are described. As specific non-limiting examples, specific embodiments of antibodies, linkers, and

Bcl-xL inhibitors that may comprise the ADCs and synthons are described. It is intended that all of the specific embodiments described may be combined with each other as though each specific combination were explicitly described individually.

It will also be appreciated by skilled artisans that the various Bcl-xL inhibitors, ADCs and/or ADC synthons described herein may be in the form of salts, and in certain embodiments, particularly pharmaceutically acceptable salts. The compounds of the present disclosure that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion, e.g., a halide such as a bromide, chloride, or fluoride.

Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, etc. Base addition salts include those derived from inorganic bases, such as ammonium and alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like.

In the disclosure below, if both structural diagrams and nomenclature are included and if the nomenclature conflicts with the structural diagram, the structural diagram controls.

I. Definitions

In order that the invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention. Further, unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art.

Various chemical substituents are defined below. In some instances, the number of carbon atoms in a substituent (*e.g.*, alkyl, alkanyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl) is indicated by the prefix "C_x-C_y" or "C_{x}-$_y$," wherein x is the minimum and y is the maximum number of carbon atoms. Thus, for example, "C_1-C_6 alkyl" refers to an alkyl containing from 1 to 6 carbon atoms. Illustrating further, "C_3-C_8 cycloalkyl" means a saturated hydrocarbon ring containing from 3 to 8 carbon ring atoms. If a substituent is described as being "substituted," a hydrogen atom on a carbon or nitrogen is replaced with a non-hydrogen group. For example, a substituted alkyl substituent is an alkyl substituent in which at least one hydrogen atom on the alkyl is replaced with a non-hydrogen group. To illustrate, monofluoroalkyl is alkyl substituted with a fluoro radical, and difluoroalkyl is alkyl substituted with two fluoro radicals. It

should be recognized that if there is more than one substitution on a substituent, each substitution may be identical or different (unless otherwise stated). If a substituent is described as being "optionally substituted", the substituent may be either (1) not substituted or (2) substituted. Possible substituents include, but are not limited to, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, cycloalkyl, heterocyclyl, heteroaryl, halogen, C_1-C_6 haloalkyl, oxo, -CN, NO_2, $-OR^{xa}$, $-OC(O)R^z$, $-OC(O)N(R^{xa})_2$, $-SR^{xa}$, $-S(O)_2R^{xa}$, $-S(O)_2N(R^{xa})_2$, $-C(O)R^{xa}$, $-C(O)OR^{xa}$, $-C(O)N(R^{xa})_2$, $-C(O)N(R^{xa})S(O)_2R^z$, $-N(R^{xa})_2$, $-N(R^{xa})C(O)R^z$, $-N(R^{xa})S(O)_2R^z$, $-N(R^{xa})C(O)O(R^z)$, $-N(R^{xa})C(O)N(R^{xa})_2$, $-N(R^{xa})S(O)_2N(R^{xa})_2$, -($C_1$-$C_6$ alkylenyl)-CN, -(C_1-C_6 alkylenyl)-OR^{xa}, -(C_1-C_6 alkylenyl)-$OC(O)R^z$, -(C_1-C_6 alkylenyl)-$OC(O)N(R^{xa})_2$, -(C_1-C_6 alkylenyl)-SR^{xa}, -(C_1-C_6 alkylenyl)-$S(O)_2R^{xa}$, -(C_1-C_6 alkylenyl)-$S(O)_2N(R^{xa})_2$, -(C_1-C_6 alkylenyl)-$C(O)R^{xa}$, -(C_1-C_6 alkylenyl)-$C(O)OR^{xa}$, -(C_1-C_6 alkylenyl)-$C(O)N(R^{xa})_2$, -(C_1-C_6 alkylenyl)-$C(O)N(R^{xa})S(O)_2R^z$, -(C_1-C_6 alkylenyl)-$N(R^{xa})_2$, -(C_1-C_6 alkylenyl)-$N(R^{xa})C(O)R^z$, -(C_1-C_6 alkylenyl)-$N(R^{xa})S(O)_2R^z$, -(C_1-C_6 alkylenyl)-$N(R^{xa})C(O)O(R^z)$, -(C_1-C_6 alkylenyl)-$N(R^{xa})C(O)N(R^{xa})_2$, or -(C_1-C_6 alkylenyl)-$N(R^{xa})S(O)_2N(R^{xa})_2$; wherein R^{xa}, at each occurrence, is independently hydrogen, aryl, cycloalkyl, heterocyclyl, heteroaryl, C_1-C_6 alkyl, or C_1-C_6 haloalkyl; and R^z, at each occurrence, is independently aryl, cycloalkyl, heterocyclyl, heteroaryl, C_1-C_6 alkyl or C_1-C_6 haloalkyl.

Various ADCs, synthons and Bcl-xL inhibitors comprising the ADCs and/or synthons are described in some embodiments herein by reference to structural formulae including substituents, for example substituents Ar, Z^1, Z^2, R^1, R^2, R^4, R^{10a}, R^{10b}, R^{10c}, R^{11a}, R^{11b}, L, R^x, F^x, LK, Ab, *n*, and/or *m*. It is to be understood that the various groups comprising substituents may be combined as valence and stability permit. Combinations of substituents and variables envisioned by this disclosure are only those that result in the formation of stable compounds. As used herein, the term "stable" refers to compounds that possess stability sufficient to allow manufacture and that maintain the integrity of the compound for a sufficient period of time to be useful for the purpose detailed herein.

As used herein, the following terms are intended to have the following meanings:

The term "alkoxy" refers to a group of the formula $-OR^{xa}$, where R^{xa} is an alkyl group. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula $-R^bOR^{xa}$ where R^b is an alkylene group and R^{xa} is an alkyl group.

The term "alkyl" by itself or as part of another substituent refers to a saturated or unsaturated branched, straight-chain or cyclic monovalent hydrocarbon radical that is derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene or alkyne. Typical alkyl groups include, but are not limited to, methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl,

prop-1-en-2-yl, prop-2-en-1-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl, prop-1-yn-1-yl , prop-2-yn-1-yl, *etc.*; butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl , but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, *etc.*; and the like. Where specific levels of saturation are intended, the nomenclature "alkanyl," "alkenyl" and/or "alkynyl" are used, as defined below. The term "lower alkyl" refers to alkyl groups with 1 to 6 carbons.

The term "alkanyl" by itself or as part of another substituent refers to a saturated branched, straight-chain or cyclic alkyl derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane. Typical alkanyl groups include, but are not limited to, methyl; ethanyl; propanyls such as propan-1-yl, propan-2-yl (isopropyl), cyclopropan-1-yl, *etc.*; butanyls such as butan-1-yl, butan-2-yl (*sec*-butyl), 2-methyl-propan-1-yl (isobutyl), 2-methyl-propan-2-yl (*t*-butyl), cyclobutan-1-yl, *etc.*; and the like.

The term "alkenyl" by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl having at least one carbon-carbon double bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkene. Typical alkenyl groups include, but are not limited to, ethenyl; propenyls such as prop-1-en-1-yl , prop-1-en-2-yl, prop-2-en-1-yl, prop-2-en-2-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl ; butenyls such as but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, *etc.*; and the like.

The term "alkynyl" by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl having at least one carbon-carbon triple bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkyne. Typical alkynyl groups include, but are not limited to, ethynyl; propynyls such as prop-1-yn-1-yl , prop-2-yn-1-yl, *etc.*; butynyls such as but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl , *etc.*; and the like.

The term "alkylamine" refers to a group of the formula -NHR^{xa} and "dialkylamine" refers to a group of the formula –$NR^{xa}R^{xa}$, where each R^{xa} is, independently of the others, an alkyl group.

The term "alkylene" refers to an alkane, alkene or alkyne group having two terminal monovalent radical centers derived by the removal of one hydrogen atom from each of the two terminal carbon atoms. Typical alkylene groups include, but are not limited to, methylene; and saturated or unsaturated ethylene; propylene; butylene; and the like. The term "lower alkylene" refers to alkylene groups with 1 to 6 carbons.

The term "aryl" means an aromatic carbocyclyl containing from 6 to 14 carbon ring atoms. An aryl may be monocyclic or polycyclic (*i.e.*, may contain more than one ring). In the case of polycyclic aromatic rings, only one ring in the polycyclic system is required to be aromatic while the remaining ring(s) may be saturated, partially saturated or unsaturated. Examples of aryls include phenyl, naphthalenyl, indenyl, indanyl, and tetrahydronaphthyl.

The prefix "halo" indicates that the substituent which includes the prefix is substituted with one or more independently selected halogen radicals. For example, haloalkyl means an alkyl substituent in which at least one hydrogen radical is replaced with a halogen radical. Typical halogen radicals include chloro, fluoro, bromo and iodo. Examples of haloalkyls include chloromethyl, 1-bromoethyl, fluoromethyl, difluoromethyl, trifluoromethyl, and 1,1,1-trifluoroethyl. It should be recognized that if a substituent is substituted by more than one halogen radical, those halogen radicals may be identical or different (unless otherwise stated).

The term "haloalkoxy" refers to a group of the formula $-OR^c$, where R^c is a haloalkyl.

The terms "heteroalkyl," "heteroalkanyl," "heteroalkenyl," "heteroalkynyl," and "heteroalkylene" refer to alkyl, alkanyl, alkenyl, alkynyl, and alkylene groups, respectively, in which one or more of the carbon atoms, *e.g.*, 1, 2 or 3 carbon atoms, are each independently replaced with the same or different heteroatoms or heteroatomic groups. Typical heteroatoms and/or heteroatomic groups which can replace the carbon atoms include, but are not limited to, O, S, SO, NR^c, PH, S(O), $S(O)_2$, $S(O)NR^c$, $S(O)_2NR^c$, and the like, including combinations thereof, where each R^c is independently hydrogen or C_1-C_6 alkyl.

The terms "cycloalkyl" and "heterocyclyl" refer to cyclic versions of "alkyl" and "heteroalkyl" groups, respectively. For heterocyclyl groups, a heteroatom can occupy the position that is attached to the remainder of the molecule. A cycloalkyl or heterocyclyl ring may be a single-ring (monocyclic) or have two or more rings (bicyclic or polycyclic).

Monocyclic cycloalkyl and heterocyclyl groups will typically contain from 3 to 7 ring atoms, more typically from 3 to 6 ring atoms, and even more typically 5 to 6 ring atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl; cyclobutyls such as cyclobutanyl and cyclobutenyl; cyclopentyls such as cyclopentanyl and cyclopentenyl; cyclohexyls such as cyclohexanyl and cyclohexenyl; and the like. Examples of monocyclic heterocyclyls include, but are not limited to, oxetane, furanyl, dihydrofuranyl, tetrahydrofuranyl, tetrahydropyranyl, thiophenyl (thiofuranyl), dihydrothiophenyl, tetrahydrothiophenyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, triazolyl, tetrazolyl, oxazolyl, oxazolidinyl, isoxazolidinyl, isoxazolyl, thiazolyl, isothiazolyl, thiazolinyl, isothiazolinyl, thiazolidinyl, isothiazolidinyl, thiodiazolyl, oxadiazolyl (including 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl (furazanyl), or 1,3,4-oxadiazolyl), oxatriazolyl (including 1,2,3,4-oxatriazolyl or 1,2,3,5-oxatriazolyl), dioxazolyl

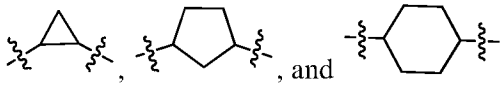
(including 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, or 1,3,4-dioxazolyl), 1,4-dioxanyl, dioxothiomorpholinyl, oxathiazolyl, oxathiolyl, oxathiolanyl, pyranyl, dihydropyranyl, thiopyranyl, tetrahydrothiopyranyl, pyridinyl (azinyl), piperidinyl, diazinyl (including pyridazinyl (1,2-diazinyl), pyrimidinyl (1,3-diazinyl), or pyrazinyl (1,4-diazinyl)), piperazinyl, triazinyl (including 1,3,5-triazinyl, 1,2,4-triazinyl, and 1,2,3-triazinyl)), oxazinyl (including 1,2-oxazinyl, 1,3-oxazinyl, or 1,4-oxazinyl)), oxathiazinyl (including 1,2,3-oxathiazinyl, 1,2,4-oxathiazinyl, 1,2,5-oxathiazinyl, or 1,2,6-oxathiazinyl)), oxadiazinyl (including 1,2,3-oxadiazinyl, 1,2,4-oxadiazinyl, 1,4,2-oxadiazinyl, or 1,3,5-oxadiazinyl)), morpholinyl, azepinyl, oxepinyl, thiepinyl, diazepinyl, pyridonyl (including pyrid-2(1H)-onyl and pyrid-4(1H)-onyl), furan-2(5H)-onyl, pyrimidonyl (including pyramid-2(1H)-onyl and pyramid-4(3H)-onyl), oxazol-2(3H)-onyl, 1H-imidazol-2(3H)-onyl, pyridazin-3(2H)-onyl, and pyrazin-2(1H)-onyl.

Polycyclic cycloalkyl and heterocyclyl groups contain more than one ring, and bicyclic cycloalkyl and heterocyclyl groups contain two rings. The rings may be in a bridged, fused or spiro orientation. Polycyclic cycloalkyl and heterocyclyl groups may include combinations of bridged, fused and/or spiro rings. In a spirocyclic cycloalkyl or heterocyclyl, one atom is common to two different rings. An example of a spirocycloalkyl is spiro[4.5]decane and an example of a spiroheterocyclyls is a spiropyrazoline.

In a bridged cycloalkyl or heterocyclyl, the rings share at least two common non-adjacent atoms. Examples of bridged cycloalkyls include, but are not limited to, adamantyl and norbornanyl rings. Examples of bridged heterocyclyls include, but are not limited to, 2-oxatricyclo[3.3.1.$1^{3,7}$]decanyl.

In a fused-ring cycloalkyl or heterocyclyl, two or more rings are fused together, such that two rings share one common bond. Examples of fused-ring cycloalkyls include decalin, naphthylene, tetralin, and anthracene. Examples of fused-ring heterocyclyls containing two or three rings include imidazopyrazinyl (including imidazo[1,2-a]pyrazinyl), imidazopyridinyl (including imidazo[1,2-a]pyridinyl), imidazopyridazinyl (including imidazo[1,2-b]pyridazinyl), thiazolopyridinyl (including thiazolo[5,4-c]pyridinyl, thiazolo[5,4-b]pyridinyl, thiazolo[4,5-b]pyridinyl, and thiazolo[4,5-c]pyridinyl), indolizinyl, pyranopyrrolyl, 4H-quinolizinyl, purinyl, naphthyridinyl, pyridopyridinyl (including pyrido[3,4-b]-pyridinyl, pyrido[3,2-b]-pyridinyl, or pyrido[4,3-b]-pyridinyl), and pteridinyl. Other examples of fused-ring heterocyclyls include benzo-fused heterocyclyls, such as dihydrochromenyl, tetrahydroisoquinolinyl, indolyl, isoindolyl (isobenzazolyl, pseudoisoindolyl), indoleninyl (pseudoindolyl), isoindazolyl (benzpyrazolyl), benzazinyl (including quinolinyl (1-benzazinyl) or isoquinolinyl (2-benzazinyl)), phthalazinyl, quinoxalinyl, quinazolinyl, benzodiazinyl (including cinnolinyl (1,2-benzodiazinyl) or quinazolinyl (1,3-benzodiazinyl)), benzopyranyl (including chromanyl or isochromanyl), benzoxazinyl (including 1,3,2-benzoxazinyl, 1,4,2-benzoxazinyl, 2,3,1-benzoxazinyl, or 3,1,4-

benzoxazinyl), benzo[d]thiazolyl, and benzisoxazinyl (including 1,2-benzisoxazinyl or 1,4-benzisoxazinyl).

The term "cycloalkylene" refers to a cycloalkyl group having two monovalent radical centers derived by the removal of one hydrogen atom from each of two ring carbons. Exemplary cycloalkylene groups include:  .

The term "heteroaryl" refers to an aromatic heterocyclyl containing from 5 to 14 ring atoms. A heteroaryl may be a single ring or 2 or 3 fused rings. Examples of heteroaryls include 6-membered rings such as pyridyl, pyrazyl, pyrimidinyl, pyridazinyl, and 1,3,5-, 1,2,4- or 1,2,3-triazinyl; 5-membered ring substituents such as triazolyl, pyrrolyl, imidazyl, furanyl, thiophenyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, 1,2,3-, 1,2,4-, 1,2,5-, or 1,3,4-oxadiazolyl and isothiazolyl; 6/5-membered fused ring substituents such as imidazopyrazinyl (including imidazo[1,2-a]pyrazinyl)imidazopyridinyl (including imidazo[1,2-a]pyridinyl), imidazopyridazinyl (including imidazo[1,2-b]pyridazinyl), thiazolopyridinyl (including thiazolo[5,4-c]pyridinyl, thiazolo[5,4-b]pyridinyl, thiazolo[4,5-b]pyridinyl, and thiazolo[4,5-c]pyridinyl), benzo[d]thiazolyl, benzothiofuranyl, benzisoxazolyl, benzoxazolyl, purinyl, and anthranilyl; and 6/6-membered fused rings such as benzopyranyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, and benzoxazinyl. Heteroaryls may also be heterocycles having aromatic (4N+2 pi electron) resonance contributors such as pyridonyl (including pyrid-2(1H)-onyl and pyrid-4(1H)-onyl), pyrimidonyl (including pyramid-2(1H)-onyl and pyramid-4(3H)-onyl), pyridazin-3(2H)-onyl and pyrazin-2(1H)-onyl.

The term "sulfonate" as used herein means a salt or ester of a sulfonic acid.

The term "methyl sulfonate" as used herein means a methyl ester of a sulfonic acid group.

The term "carboxylate" as used herein means a salt or ester of a carboxylic acid.

The term "sugar" as used herein in the context of linkers means an O-glycoside or *N*-glycoside carbohydrate derivatives of the monosaccharide class and may originate from naturally-occurring sources or may be synthetic in origin. For example "sugar" includes derivatives such as but not limited to those derived from beta-glucuronic acid and beta-galactose. Suitable sugar substitutions include but are not limited to hydroxyl, amine, carboxylic acid, esters, and ethers.

The term "NHS ester" means the N-hydroxysuccinimide ester derivative of a carboxylic acid.

The term salt when used in context of "or salt thereof" includes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention.

Where a salt is intended to be administered to a patient (as opposed to, for example, being in use in an *in vitro* context), the salt preferably is pharmaceutically acceptable and/or physiologically compatible. The term "pharmaceutically acceptable" is used adjectivally in this patent application to mean that the modified noun is appropriate for use as a pharmaceutical product or as a part of a pharmaceutical product. The term "pharmaceutically acceptable salt" includes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention.

The term "anti-Epidermal Growth Factor Receptor (EGFR) antibody" as used herein, refers to an antibody that specifically binds to EGFR. An antibody "which binds" an antigen of interest, *i.e.,* EGFR, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. In a preferred embodiment, the antibody specifically binds to human EGFR (hEGFR). Examples of anti-EGFR antibodies are disclosed below. Unless otherwise indicated, the term "anti-EGFR antibody" is meant to refer to an antibody which binds to wild type EGFR or any variant of EGFR, such as EGFRvIII.

The amino acid sequence of wild type human EGFR is provided below as SEQ ID NO: 32, where the signal peptide (amino acid residues 1-24) is underlined, and the amino acid residues of the extracellular domain (ECD, amino acid residues 25-645) are highlighted in bold. A truncated wild type ECD of the EGFR (also referred to herein as EGFR(1-525)) corresponds to SEQ ID NO: 47 and is equivalent to amino acids 1-525 of SEQ ID NO: 32. The mature form of wild type EGFR corresponds to the protein without the signal peptide, *i.e.,* amino acid residues 25 to 1210 of SEQ ID NO: 32.

1 mrpsgtagaa llallaalcp asra**leekkv cqgtsnkltq lgtfedhfls lqrmfnncev**
61 **vlgnleityv qrnydlsflk tiqevagyvl ialntverip lenlqiirgn myyensyala**
121 **vlsnydankt glkelpmrnl qeilhgavrf snnpalcnve siqwrdivss dflsnmsmdf**
181 **qnhlgscqkc dpscpngscw gageencqkl tkiicaqqcs grcrgkspsd cchnqcaagc**
241 **tgpresdclv crkfrdeatc kdtcpplmly npttyqmdvn pegkysfgat cvkkcprnyv**
301 **vtdhgscvra cgadsyemee dgvrkckkce gpcrkvcngi gigefkdsls inatnikhfk**
361 **nctsisgdlh ilpvafrgds fthtppldpq eldilktvke itgflliqaw penrtdlhaf**
421 **enleiirgrt kqhgqfslav vslnitslgl rslkeisdgd viisgnknlc yantinwkkl**
481 **fgtsgqktki isnrgensck atgqvchalc spegcwgpep rdcvscrnvs rgrecvdkcn**
541 **llegeprefv enseciqchp eclpqamnit ctgrgpdnci qcahyidgph cvktcpagvm**

601 **genntlvwky adaghvchlc hpnctygctg pglegcptng pkips**iatgm vgallllvv
661 algiglfmrr rhivrkrtlr rllqerelve pltpsgeapn qallrilket efkkikvlgs
721 gafgtvykgl wipegekvki pvaikelrea tspkankeil deayvmasvd nphvcrllgi
781 cltstvqlit qlmpfgclld yvrehkdnig sqyllnwcvq iakgmnyled rrlvhrdlaa
841 rnvlvktpqh vkitdfglak llgaeekeyh aeggkvpikw malesilhri ythqsdvwsy
901 gvtvwelmtf gskpydgipa seissilekg erlpqppict idvymimvkc wmidadsrpk
961 freliiefsk mardpqrylv iqgdermhlp sptdsnfyra lmdeedmddv vdadeylipq
1021 qgffsspsts rtpllsslsa tsnnstvaci drnglqscpi kedsflqrys sdptgalted
1081 siddtflpvp eyinqsvpkr pagsvqnpvy hnqplnpaps rdphyqdphs tavgnpeyln
1141 tvqptcvnst fdspahwaqk gshqisldnp dyqqdffpke akpngifkgs taenaeylrv
1201 apqssefiga (SEQ ID NO: 32)

The amino acid sequence of the ECD of human EGFR is provided below as SEQ ID NO: 34, and includes the signal sequence (underlined).

1 mrpsgtagaa llallaalcp asraleekkv cqgtsnkltq lgtfedhfls lqrmfnncev
61 vlgnleityv qrnydlsflk tiqevagyvl ialntverip lenlqiirgn myyensyala
121 vlsnydankt glkelpmrnl qeilhgavrf snnpalcnve siqwrdivss dflsnmsmdf
181 qnhlgscqkc dpscpngscw gageencqkl tkiicaqqcs grcrgkspsd cchnqcaagc
241 tgpresdclv crkfrdeatc kdtcpplmly npttyqmdvn pegkysfgat cvkkcprnyv
301 vtdhgscvra cgadsyemee dgvrkckkce gpcrkvcngi gigefkdsls inatnikhfk
361 nctsisgdlh ilpvafrgds fthtppldpq eldilktvke itgflliqaw penrtdlhaf
421 enleiirgrt kqhgqfslav vslnitslgl rslkeisdgd viisgnknlc yantinwkkl
481 fgtsgqktki isnrgensck atgqvchalc spegcwgpep rdcvscrnvs rgrecvdkcn
541 llegeprefv enseciqchp eclpqamnit ctgrgpdnci qcahyidgph cvktcpagvm
601 genntlvwky adaghvchlc hpnctygctg pglegcptng pkips (SEQ ID NO: 34)

The overall structure of EGFR is described in Figure 1. The ECD of EGFR has four domains (Cochran *et al.* (2004) *J. Immunol. Methods*, 287, 147-158). Domains I and III have been suggested to contribute to the formation of high affinity binding sites for ligands. Domains II and IV are cysteine rich, laminin-like regions that stabilize protein folding and contain a possible EGFR dimerization interface.

EGFR variants may result from gene rearrangement accompanied by EGFR gene amplification.

EGFRvIII is the most commonly occurring variant of the EGFR in human cancers (Kuan et al. Endocr Relat Cancer. 8(2):83-96 (2001)). During the process of gene amplification, a 267 amino acid deletion occurs in the extracellular domain of EGFR with a glycine residue inserted at the fusion junction. Thus, EGFRvIII lacks amino acids 6-273 of the extracellular domain of wild type EGFR and includes a glycine residue insertion at the junction. The EGFRvIII variant of EGFR contains a deletion of 267 amino acid residues in the extracellular domain where a glycine is inserted at the deletion junction. The EGFRvIII amino acid sequence is shown below as SEQ ID NO: 33 (the ECD is highlighted in bold and corresponds to SEQ ID NO: 46 the signal sequence is underlined).

mrpsgtagaallallaalcpasra**leekkgnyvvtdhgscvracgadsyemeedgvrkckkcegp crkvcngigigefkdslsinatnikhfknctsisgdlhilpvafrgdsfthtppldpqeldilkt vkeitgflliqawpenrtdlhafenleiirgrtkqhgqfslavvslnitslglrslkeisdgdvi isgnknlcyantinwkklfgtsgqktkiisnrgensckatgqvchalcspegcwgpeprdcvscr nvsrgrecvdkcnllegeprefvenseciqchpeclpqamnitctgrgpdnciqcahyidgphcv ktcpagvmgenntlvwkyadaghvchlchpnctygctgpglegcptngp**kipsiatgmvgallll lvvalgiglfmrrrhivrkrtlrrllqerelvepltpsgeapnqallrilketefkkikvlgsga fgtvykglwipegekvkipvaikelreatspkankeildeayvmasvdnphvcrllgicltstvq litqlmpfgclldyvrehkdnigsqyllnwcvqiakgmnyledrrlvhrdlaarnvlvktpqhvk itdfglakllgaeekeyhaeggkvpikwmalesilhriythqsdvwsygvtvwelmtfgskpydg ipaseissilekgerlpqppictidvymimvkcwmidadsrpkfreliiefskmardpqrylviq gdermhlpsptdsnfyralmdeedmddvvdadeylipqqgffsspstsrtpllsslsatsnnstv acidrnglqscpikedsflqryssdptgaltedsiddtflpvpeyinqsvpkrpagsvqnpvyhn qplnpapsrdphyqdphstavgnpeylntvqptcvnstfdspahwaqkgshqisldnpdyqqdff pkeakpngifkgstaenaeylrvapqssefiga (SEQ ID NO: 33)

EGFRvIII contributes to tumor progression through constitutive signaling in a ligand independent manner. EGFRvIII is not known to be expressed in normal tissues (Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Olapade-Olaopa et al. Br J Cancer. 82(1):186-94 (2000)), but shows significant expression in tumor cells, including breast cancers, gliomas, NSCL cancers, ovarian cancers, and prostate cancers (Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Ge et al. Int J Cancer. 98(3):357-61 (2002); Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Moscatello et al. Cancer Res. 55(23):5536-9 (1995); Garcia de Palazzo et al. Cancer Res. 53(14):3217-20 (1993); Moscatello et al. Cancer Res. 55(23):5536-9 (1995); and Olapade-Olaopa et al. 2(1):186-94 (2000)).

"Biological activity of EGFR " as used herein, refers to all inherent biological properties of the EGFR, including, but not limited to, binding to epidermal growth factor (EGF), binding to tumor growth factor α (TGFα), homodimerization, activation of JAK2 kinase activity, activation of MAPK kinase activity, and activation of transmembrane receptor protein tyrosine kinase activity.

The term "gene amplification", as used herein, refers to a cellular process characterized by the production of multiple copies of any particular piece of DNA. For example, a tumor cell may amplify, or copy, chromosomal segments as a result of cell signals and sometimes environmental events. The process of gene amplification leads to the production of additional copies of the gene. In one embodiment, the gene is EGFR, i.e., "EGFR amplification." In one embodiment, the compositions and methods disclosed herein are used to treat a subject having EGFR amplified cancer.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody or an ADC with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (*e.g.*, an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody or ADC is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody or ADC.

The phrase "specifically binds to hEGFR" or "specific binding to hEGFR", as used herein, refers to the ability of an anti-EGFR antibody or ADC to bind to hEGFR with an K_D of at least about $1x10^{-6}$ M, $1x10^{-7}$ M, $1x10^{-8}$ M, $1x10^{-9}$ M, $1x10^{-10}$ M, $1x10^{-11}$ M, $1x10^{-12}$ M, or more, and/or bind to an antigen with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen. It shall be understood, however, that the antibody or ADC may be capable of specifically binding to two or more antigens which are related in sequence. For example, in one

embodiment, an antibody can specifically bind to both human and a non-human (e.g., mouse or non-human primate) orthologs of EGFR. In one embodiment, the antigen is EGFR(1-525).

The term "antibody" refers to an immunoglobulin molecule that specifically binds to an antigen and comprises a heavy (H) chain(s) and a light (L chain(s). Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or 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 carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY) and class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass. While the term "antibody" is not intended to include antigen binding portions of an antibody (defined below), it is intended, in certain embodiments, to include an antibody having a small number of amino acid deletions from the carboxy end of the heavy chain(s). In one embodiment, an antibody comprises a heavy chain having 1-5 amino acid deletions the carboxy end of the heavy chain. In a one embodiment, an antibody is a monoclonal antibody which is an IgG, having four polypeptide chains, two heavy (H) chains, and two light (L chains) that can bind to hEGFR. In one embodiment, an antibody is a monoclonal IgG antibody comprising a lambda or a kappa light chain.

The term "antigen binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, hEGFR). It has been shown that the antigen binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they

can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen binding portion" of an antibody. In certain embodiments of the invention, scFv molecules may be incorporated into a fusion protein. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

An IgG is a class of antibody comprising two heavy chains and two light chains arranged in a Y-shape. Exemplary human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and represented below in Table 1.

Table 1: Sequence of human IgG heavy chain constant domain and light chain constant domain

Protein	Sequence Identifier	Sequence
Ig gamma-1 constant region	SEQ ID NO:41	ASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
Ig gamma-1 constant region mutant	SEQ ID NO:42	ASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK

Protein	Sequence Identifier	Sequence
Ig Kappa constant region	SEQ ID NO:43	RTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
Ig Lambda constant region	SEQ ID NO:44	QPKAAPSVTLFPPSSEELQANKATLVCLI SDFYPGAVTVAWKADSSPVKAGVETTTPS KQSNNKYAASSYLSLTPEQWKSHRSYSCQ VTHEGSTVEKTVAPTECS

Still further, an antibody or antigen binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds EGFR is substantially free of antibodies that specifically bind antigens other than EGFR). An isolated antibody that specifically binds EGFR may, however, have cross-reactivity to other antigens, such as EGFR molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a nonhuman species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. In particular, the term "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises

substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In other embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

The terms "Kabat numbering," "Kabat definitions," and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.,* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad, Sci.* 190:382-391 and, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain (HC) and the light chain (LC), which are designated CDR1, CDR2 and CDR3 (or specifically HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3), for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering

system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia &Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, CDR-L2, and CDR-L3 of light chain and CDR-H1, CDR-H2, and CDR-H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub- regions, and FRs represents two or more of the four sub- regions constituting a framework region.

The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the

framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See *e.g.*, Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

"Percent (%) amino acid sequence identity" with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In one embodiment, the invention includes an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to an amino acid sequence set forth in any one of SEQ ID NOs: 1 to 31, 35-40, or 50 to 85.

The term "multivalent antibody" is used herein to denote an antibody comprising two or more antigen binding sites. In certain embodiments, the multivalent antibody may be engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody.

The term "multispecific antibody" refers to an antibody capable of binding two or more unrelated antigens. In one embodiment, the multispecific antibody is a bispecific antibody that is capable of binding to two unrelated antigens, *e.g.,* a bispecific antibody, or antigen-binding portion thereof, that binds EGFR (*e.g.,* EGFRvIII) and CD3.

The term "activity" includes activities such as the binding specificity/affinity of an antibody or ADC for an antigen, for example, an anti-hEGFR antibody that binds to an hEGFR antigen and/or the neutralizing potency of an antibody, for example, an anti-hEGFR antibody whose binding to hEGFR inhibits the biological activity of hEGFR, *e.g.*, inhibition of phosphorylation of EGFR in an EGFR expressing cell line, *e.g.,* the human lung carcinoma cell line H292, or inhibition of proliferation of EGFR expressing cell lines, *e.g.,* human H292 lung carcinoma cells, human H1703 lung carcinoma cells, or human EBC1 lung carcinoma cells.

The term "non small-cell lung carcinoma (NSCLC) xenograft assay," as used herein, refers to an *in vivo* assay used to determine whether an anti-EGFR antibody or ADC, can inhibit tumor growth (*e.g.,* further growth) and/or decrease tumor growth resulting from the transplantation of NSCLC cells into an immunodeficient mouse. An NSCLC xenograft assay includes transplantation of NSCLC cells into an immunodeficient mouse such that a tumor grows to a desired size, *e.g.,* 200-250 mm^3, whereupon the antibody or ADC is administered to the mouse to determine whether the antibody or ADC can inhibit and/or decrease tumor growth. In certain embodiments, the activity of the antibody or ADC is determined according to the percent tumor growth inhibition (%TGI) relative to a control antibody, *e.g.,* a human IgG antibody (or collection thereof) which does not specifically bind tumor cells, *e.g.,* is directed to an antigen not associated with cancer or is obtained from a source which is noncancerous (*e.g.,* normal human serum). In such embodiments, the antibody (or ADC) and the control antibody are administered to the mouse at the same dose, with the same frequency, and via the same route. In one embodiment, the mouse used in the NSCLC xenograft assay is a severe combined immunodeficiency (SCID) mouse and/or an athymic CD-1 nude mouse. Examples of NSCLC cells that may be used in the NSCLC xenograft assay include, but are not limited to, H292 cells (*e.g.,* NCIH292 [H292] (ATCC CRL1848).

The term "epitope" refers to a region of an antigen that is bound by an antibody or ADC. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In one embodiment, the antibodies of the invention bind to an epitope defined by the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) (which corresponds to amino acid residues 287-302 of the mature form of hEGFR).

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277. In one embodiment, surface plasmon resonance is determined according to the methods described in Example 2

The term " k_{on}" or " k_a", as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex.

The term "k_{off}" or " k_d", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction (*e.g.,* AbA antibody and EGFR). K_D is calculated by k_a / k_d.

The term "competitive binding", as used herein, refers to a situation in which a first antibody competes with a second antibody, for a binding site on a third molecule, *e.g.,* an antigen. In one embodiment, competitive binding between two antibodies is determined using FACS analysis.

The term "competitive binding assay" is an assay used to determine whether two or more antibodies bind to the same epitope. In one embodiment, a competitive binding assay is a competition fluorescent activated cell sorting (FACS) assay which is used to determine whether two or more antibodies bind to the same epitope by determining whether the fluorescent signal of a labeled antibody is reduced due to the introduction of a non-labeled antibody, where competition for the same epitope will lower the level of fluorescence. An example of a competition binding FACS assay is provided in Example 3 where competition FACS assay is described using U87MG cells (which express EGFRvIII).

The term "antibody-drug-conjugate" or "ADC" refers to a binding protein, such as an antibody or antigen binding fragment thereof, chemically linked to one or more chemical drug(s) (also referred to herein as agent(s), warhead(s), or payload(s)) that may optionally be therapeutic or cytotoxic agents. In a preferred embodiment, an ADC includes an antibody, a cytotoxic or therapeutic drug, and a linker that enables attachment or conjugation of the drug to the antibody. An ADC typically has anywhere from 1 to 8 drugs conjugated to the antibody, including drug loaded species of 2, 4, 6, or 8. In a preferred embodiment, the ADC of the invention comprises an anti-EGFR antibody conjugated via a linker to a Bcl-xL inhibitor.

The terms "anti-Epidermal Growth Factor antibody drug conjugate," "anti-EGFR antibody drug conjugate," or "anti-EGFR ADC", used interchangeably herein, refer to an ADC comprising an antibody that specifically binds to EGFR, whereby the antibody is conjugated to one or more chemical agent(s). In one embodiment, an anti-EGFR ADC comprises antibody AbA conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbB conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbK conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbG conjugated to a Bcl-xL inhibitor.

The term "drug-to-antibody ratio" or "DAR" refers to the number of drugs, *e.g.*, a Bcl-xL inhibitor, attached to the antibody of the ADC. The DAR of an ADC can range from 1 to 8, although higher loads, *e.g.*, 10, are also possible depending on the number of linkage site on an

antibody. The term DAR may be used in reference to the number of drugs loaded onto an individual antibody, or, alternatively, may be used in reference to the average or mean DAR of a group of ADCs.

The term "undesired ADC species", as used herein, refers to any drug loaded species which is to be separated from an ADC species having a different drug load. In one embodiment, the term undesired ADC species may refer to drug loaded species of 6 or more, *i.e..,* ADCs with a DAR of 6 or more, including DAR6, DAR7, DAR8, and DAR greater than 8 (*i.e.,* drug loaded species of 6, 7, 8, or greater than 8). In a separate embodiment, the term undesired ADC species may refer to drug loaded species of 8 or more, *i.e.,* ADCs with a DAR of 8 or more, including DAR8, and DAR greater than 8 (*i.e.,* drug loaded species of 8, or greater than 8).

The term "ADC mixture", as used herein, refers to a composition containing a heterogeneous DAR distribution of ADCs. In one embodiment, an ADC mixture contains ADCs having a distribution of DARs of 1 to 8, *e.g.,* 2, 4, 6, and 8 (*i.e.,* drug loaded species of 2, 4, 6, and 8). Notably, degradation products may result such that DARs of 1, 3, 5, and 7 may also be included in the mixture. Further, ADCs within the mixture may also have DARs greater than 8. The ADC mixture results from interchain disulfide reduction followed by conjugation. In one embodiment, the ADC mixture comprises both ADCs with a DAR of 4 or less (*i.e.,* a drug loaded species of 4 or less) and ADCs with a DAR of 6 or more (*i.e.,* a drug loaded species of 6 or more).

The term "cancer" is meant to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, colorectal cancer, head and neck cancer, breast cancer (e.g., triple negative breast cancer), pancreatic cancer, squamous cell tumors, squamous cell carcinoma (e.g., squamous cell lung cancer or squamous cell head and neck cancer), anal cancer, skin cancer, and vulvar cancer. In one embodiment, the ADCs of the invention are administered to a patient having a tumor(s) containing amplifications of the EGFR gene, whereby the tumor expresses the truncated version of the EGFR, EGFRvIII. In one embodiment, the ADCs of the invention are administered to a patient having a solid tumor which is likely to over-express EGFR. In one embodiment, the ADCs of the invention are administered to a patient having squamous cell Non-Small Cell Lung Cancer (NSCLC). In one embodiment, the ADCs of the invention are administered to a patient having solid tumors, including advanced solid tumors.

The term "EGFR expressing tumor," as used herein, refers to a tumor which expresses EGFR protein. In one embodiment, EGFR expression in a tumor is determined using immunohistochemical staining of tumor cell membranes, where any immunohistochemical

staining above background level in a tumor sample indicates that the tumor is an EGFR expressing tumor. Methods for detecting expression of EGFR in a tumor are known in the art, *e.g.,* the EGFR pharmDx™ Kit (Dako). In contrast, an "EGFR negative tumor" is defined as a tumor having an absence of EGFR membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

The term "EGFRvIII positive tumor," as used herein, refers to a tumor which expresses EGFRvIII protein. In one embodiment, EGFRvIII expression in a tumor is determined using immunohistochemical staining of tumor cell membranes, where any immunohistochemical staining above background level in a tumor sample indicates that the tumor is an EGFRvIII expressing tumor. Methods for detecting expression of EGFR in a tumor are known in the art, and include immunohistochemical assays. In contrast, an "EGFRvIII negative tumor" is defined as a tumor having an absence of EGFRvIII membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

The terms "overexpress," "overexpression," or "overexpressed" interchangeably refer to a gene that is transcribed or translated at a detectably greater level, usually in a cancer cell, in comparison to a normal cell. Overexpression therefore refers to both overexpression of protein and RNA (due to increased transcription, post transcriptional processing, translation, post translational processing, altered stability, and altered protein degradation), as well as local overexpression due to altered protein traffic patterns (increased nuclear localization), and augmented functional activity, *e.g.*, as in an increased enzyme hydrolysis of substrate. Thus, overexpression refers to either protein or RNA levels. Overexpression can also be by 50%, 60%, 70%, 80%, 90% or more in comparison to a normal cell or comparison cell. In certain embodiments, the anti-EGFR ADCs of the invention are used to treat solid tumors likely to overexpress EGFR.

The term "administering" as used herein is meant to refer to the delivery of a substance (*e.g.*, an anti-EGFR ADC) to achieve a therapeutic objective (e.g., the treatment of an EGFR-associated disorder). Modes of administration may be parenteral, enteral and topical. Parenteral administration is usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The term "combination therapy", as used herein, refers to the administration of two or more therapeutic substances, *e.g.*, an anti-EGFR ADC and an additional therapeutic agent. The additional therapeutic agent may be administered concomitant with, prior to, or following the administration of the anti-EGFR ADC.

As used herein, the term "effective amount" or "therapeutically effective amount" refers to the amount of a drug, *e.g.,* an antibody or ADC, which is sufficient to reduce or ameliorate the severity and/or duration of a disorder, *e.g.,* cancer, or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or therapeutic agent). The effective amount of an antibody or ADC may, for example, inhibit tumor growth (*e.g.,* inhibit an increase in tumor volume), decrease tumor growth (*e.g.,* decrease tumor volume), reduce the number of cancer cells, and/or relieve to some extent one or more of the symptoms associated with the cancer. The effective amount may, for example, improve disease free survival (DFS), improve overall survival (OS), or decrease likelihood of recurrence.

Various aspects of the invention are described in further detail in the following subsections.

2. Anti-EGFR Antibody Drug Conjugates (ADCs): Anti-EGFR Antibodies

One aspect of the invention features an anti-human Epidermal Growth Factor Receptor (anti-hEGFR) Antibody Drug Conjugate (ADC) comprising an anti-hEGFR antibody conjugated to a drug via a linker, wherein the drug is a Bcl-xL inhibitor. Exemplary anti-EGFR antibodies (and sequences thereof) that can be used in the ADCs set forth herein are described below, as well as in US 2015-0337042, incorporated by reference in its entirety herein.

The anti-EGFR antibodies described herein provide the ADCs of the invention with the ability to bind to EGFR such that the cytotoxic Bcl-xL drug attached to the antibody may be delivered to the EGFR-expressing cell.

While the term "antibody" is used throughout, it should be noted that antibody fragments (*i.e.,* antigen-binding portions of an anti-EGFR antibody) may also be conjugated to the Bcl-xL inhibitors described herein. Thus, it is within the scope of the invention that in certain embodiments, antibody fragments of the anti-EGFR antibodies described herein are conjugated to Bcl-xL inhibitors via linkers. In certain embodiments, the anti-EGFR antibody binding portion is a Fab, a Fab', a F(ab')2, a Fv, a disulfide linked Fv, an scFv, a single domain antibody, or a diabody.

Anti-EGFR antibodies that may be used in the ADCs of the invention have characteristics making them advantageous for use in an ADC. In one embodiment, an anti-EGFR antibody has characteristics including, but not limited to, binding to tumor cells expressing EGFRvIII, binding to wild type EGFR on tumor cells expressing EGFR, recognizing the epitope CGADSYEMEEDGVRKC (SEQ ID NO: 45) on EGFR, binding to EGFR on normal human epithelial keratinocytes, and decreasing or inhibiting xenograft tumor growth in a mouse model.

In one embodiment, an anti-EGFR antibody which may be used in the ADC of the invention is capable of binding an epitope of human EGFR defined by SEQ ID NO: 45 and/or is able to compete with any antibody disclosed herein (*e.g.,* Ab1, AbA, AbB, AbC, AbD, AbE, AbF, AbG, AbH, AbJ, AbK) for binding to human EGFR. Binding of the antibody to EGFR may be assessed according to, e.*g.* competition assay analysis, as described in US 2015-0337042 A1, incorporated by reference in its entirety herein. In one embodiment of the invention, an anti-EGFR antibody that may be used in an ADC of the invention has a dissociation constant (K_d) of between about 1 x 10^{-6} M and about 1 x 10^{-10} M, as determined by surface plasmon resonance, to 1-525 of EGFR (SEQ ID NO: 47). In other embodiments of the foregoing aspects, the ADC of the invention comprises an anti-EGFR antibody that binds EGFRvIII, binds EGFR on cells overexpressing EGFR, and recognizes the epitope CGADSYEMEEDGVRKC (SEQ ID NO: 45) on EGFR. In a further embodiment, the anti-EGFR antibody binds EGFRvIII at an epitope which is distinct from the EGFRvIII junctional peptide. In additional embodiments of the foregoing aspects, the anti-EGFR antibody used in an ADC of the invention, does not compete with cetuximab for binding to human EGFR.

In one embodiment, an ADC of the invention comprises an anti-EGFR antibody that binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K_d) of about 1 x 10^{-6} M or less, as determined by surface plasmon resonance. Alternatively, an anti-EGFR antibody may bind to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-10} M, as determined by surface plasmon resonance. In a further alternative, an anti-EGFR antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-7} M, as determined by surface plasmon resonance. Alternatively, antibodies used in the invention may bind to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 5 x 10^{-10} M; a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-9} M; a K_d of between about 1 x 10^{-6} M and about 5 x 10^{-9} M; a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-8} M; a K_d of between about 1 x 10^{-6} M and about 5 x 10^{-8} M; a K_d of between about 5.9 x 10^{-7} M and about 1.7 x 10^{-9} M; a K_d of between about 5.9 x 10^{-7} M and about 2.2 x 10^{-7} M, as determined by surface plasmon resonance. In certain embodiments, the dissociation constant (K_d) of the anti-hEGFR antibody used in the ADC of the invention is lower than the dissociation constant for Ab1 but higher than the dissociation constant of anti-EGFR antibody cetuximab (*i.e.,* the antibody binds to EGFR more tightly than Ab1 but not as tightly as cetuximab).

One advantage of the anti-EGFR antibodies described herein, is that the antibodies are capable of binding to tumor cells expressing EGFRvIII, thus making the ADCs of the invention specific for malignant cells. While EGFRvIII is associated with certain types of cancer, many anti-EGFR antibodies known in the art, *e.g.,* cetuximab, are not effective at inhibiting or decreasing tumor growth in EGFRvIII expressing tumors. Thus, in one embodiment, an antibody

used in an ADC of the invention binds to EGFRvIII (SEQ ID NO: 33) with a K_d of about 8.2 x 10^{-9} M or less, as determined by surface plasmon resonance. Alternatively, an antibody used in an ADC of the invention binds to EGFRvIII (SEQ ID NO: 33) with a K_d of between about 8.2 x 10^{-9} M and about 6.3 x 10^{-10} M; a K_d of between about 8.2 x 10^{-9} M and about 2.0 x 10^{-9} M; a K_d of between about 2.3 x 10^{-9} M and about 1.5 x 10^{-10} M, as determined by surface plasmon resonance.

The term a "xenograft assay", as used herein, refers to a human tumor xenograft assay, wherein human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immunocompromised mice that do not reject human cells.

It should be noted that anti-EGFR antibodies having combinations of the aforementioned characteristics are also considered to be embodiments of the invention. For example, an anti-EGFR antibody may bind to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K_d) of about 1 x 10^{-6} M or less, as determined by surface plasmon resonance, and bind to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) and compete with Ab1 (or an anti-EGFR antibody comprising a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5) for binding to EGFRvIII (SEQ ID NO: 33) in a competitive binding assay. In certain embodiments, an anti-EGFR ADC of the invention comprises an anti-EGFR antibody that binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) and competes with Ab1 (or an anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5) for binding to EGFRvIII (SEQ ID NO: 33) in a competitive binding assay; and bind to EGFRvIII (SEQ ID NO: 33) with a K_d of about 8.2 x 10^{-9} M or less, as determined by surface plasmon resonance.

In one embodiment, anti-EGFR antibodies used in an ADC of the invention exhibits a high capacity to reduce or to neutralize EGFR activity, *e.g.*, as assessed by any one of several *in vitro* and *in vivo* assays known in the art. For example, inhibition of phosphorylation of EGFR in an EGFR expressing cell line, *e.g.,* the h292 cell line, can be measured. In certain embodiments, an anti-EGFR antibody binds human EGFR, wherein the antibody dissociates from human EGFR (EGFR 1-525) with a K_D rate constant of about 5.9 x 10^{-7} M or less, as determined by surface plasmon resonance. In a further embodiment, the antibody may dissociate from human EGFR (1-525) with a K_D rate constant of about 4.2 x 10^{-7} M, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFR (1-525) with a k_{off} rate constant of about K_D rate constant of about 2.5 x 10^{-7} M, as determined by surface plasmon resonance. In certain embodiments, the anti-EGFR antibodies of the invention have a K_D rate constant of

between 5.9 x 10^{-7} M and 5 x 10^{-9} M. Alternatively, the antibody may dissociate from human EGFRvIII with a K_D rate constant of about 6.1 x 10^{-9} M or less, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFRvIII with a K_D rate constant of about 3.9 x 10^{-9} M or less, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFRvIII with a K_D rate constant of about 2.3 x 10^{-9}M or less, as determined by surface plasmon resonance.

Exemplary anti-EGFR antibodies that may be used in the ADCs described herein include, but are not limited to, Antibody 1 (Ab1), Antibody A (AbA), Antibody B (AbB), Antibody C (AbC), Antibody D (AbD), Antibody E (AbE), Antibody F (AbF), Antibody G (AbG), Antibody H (AbH), Antibody J (AbJ), Antibody K (AbK), Antibody L (AbL), Antibody M (AbM), Antibody N (AbN), Antibody O (AbO), Antibody P (AbP), and Antibody Q (AbQ).

In one embodiment, the invention features an anti-EGFR ADC comprising Ab1 conjugated via a linker to a Bcl-xL inhibitor. Ab1 is a humanized anti-EGFR antibody. The light and heavy chain sequences of Ab1 are described in SEQ ID NO: 13 and SEQ ID NO: 14, respectively (see also US Patent Application Publication No. 20120183471, incorporated by reference herein). The light chain variable region of Ab1 is described in SEQ ID NO: 5, and comprises a CDR1 amino acid sequence set forth in SEQ ID NO: 6, a CDR2 amino acid sequence set forth in SEQ ID NO: 7, and a CDR3 amino acid sequence set forth in SEQ ID NO: 8. The heavy chain variable region of Ab1 is described in SEQ ID NO: 1, and comprises a CDR1 amino acid sequence set forth in SEQ ID NO: 2, a CDR2 amino acid sequence set forth in SEQ ID NO: 3, and a CDR3 amino acid sequence set forth in SEQ ID NO: 4. In one embodiment, an ADC of the invention comprises an anti-EGFR antibody that binds to an epitope within the amino acid sequence set forth in SEQ ID NO: 45 and competes with an anti-EGFR antibody comprising a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5 for binding to EGFRvIII in a competitive binding assay.

In one embodiment, the invention features an anti-hEGFR ADC comprising an anti-hEGFR antibody which is antibody AbA conjugated via a linker to a Bcl-xL inhibitor. The term "AbA" is meant to include an IgG antibody having at least the six CDRs of AbA. The AbA antibody has the same light chain as that of Ab1, but has a heavy chain containing six amino acid sequence changes relative to parental antibody Ab1 (four amino acid changes in the variable region and two changes in the constant region of the heavy chain). The AbA antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a

CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. The heavy chain variable region of AbA is defined by the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 5. The full length heavy chain of antibody AbA is set forth in the amino acid sequence described in SEQ ID NO: 15, while the full length light chain of antibody AbA is set forth in the amino acid sequence described in SEQ ID NO: 13 (see Figure 3). The nucleic acid sequence of the heavy chain of AbA is provided below:

gaggtgcaactccaagagagcgggcccggcctcgtgaagccctctcagactctgtccctgacttg
cactgtgagcgggtattccatcagcagagacttcgcatggaactggatccgccagcctcccggta
agggactggagtggatggggtacatcagctacaacggtaatacacgctatcagccctccctgaag
tctcgcattaccattagtcgcgatacctccaagaaccagttctttctgaaactcaacagcgtgac
agccgctgacaccgccacctactactgcgtgaccgccagcaggggttcccttactggggccagg
gcactctggtcaccgtttcttctgcgtcgaccaagggcccatcggtcttcccccctggcaccctcc
tccaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaacc
ggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctac
agtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccag
acctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaa
atcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcag
tcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgc
gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgga
ggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcg
tcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaa
gccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt
gtacaccctgcccccatcccgcgaggagatgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac
aagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgga
caagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacacgcagaagagcctctccctgtctccgggtaaa (SEQ ID NO: 86)

The nucleic acid sequence of the light chain of AbA is provided below:

Gacatccagatgacccagtccccctccagtatgtctgtgtctgtgggcgaccgtgtgaccattac
ctgccactcctcccaggacatcaatagcaatatcggttggttgcaacagaagccaggcaagtcct
tcaaagggctgatttaccatggtaccaacctggacgacggggttcctagtcgtttcagcggctcc
gggtccggaaccgattacactctgaccatcagcagtttgcagcctgaggactttgctacctatta
ttgtgtgcagtacgctcagttcccatggactttcggcgggggcaccaaactggagatcaaacgta
cggtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcc
tctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataa
cgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctaca
gcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaa
gtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgt (SEQ ID
NO: 87)

The amino acid sequence of the heavy chain of AbA is provided below:

EVQLQESGPGLVKPSQTLSLTCTVSGYSISRDFAWNWIRQPPGKGLEWMGYISYNGNTRYQPSLK
SRITISRDTSKNQFFLKLNSVTAADTATYYCVTASRGFPYWGQGTLVTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 15)

In another embodiment, the amino acid sequence of the heavy chain of AbA is provided below:

EVQLQESGPGLVKPSQTLSLTCTVSGYSISRDFAWNWIRQPPGKGLEWMGYISYNGNTRYQPSLK
SRITISRDTSKNQFFLKLNSVTAADTATYYCVTASRGFPYWGQGTLVTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 102)

The amino acid sequence of the light chain of AbA is provided below:

DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFSGS
GSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 13)

Figures 2 and 3 provide an alignment of the amino acid sequences of the VH and VL regions (Figure 2) and the complete heavy and light chains (Figure 3) of Ab1 and AbA. The light chain amino acid sequences of Ab1 and AbA are the same (SEQ ID NO: 13). The heavy chain amino acid sequences of Ab1 and AbA, however, have six amino acid differences between the two sequences, three of which are in the CDRs. Differences between the Ab1 VH amino acid sequence and the AbA VH amino acid sequence are shaded in Figure 2 and are found in each of the VH CDRs. The CDR1 domain of the variable heavy chain of AbA included an amino acid change from a serine (Ab1) to an arginine. The CDR2 domain of the variable heavy chain included an amino acid change from a serine in Ab1 to an asparagine in AbA. Finally, the CDR3 domain of the variable heavy chain included an amino acid change from a glycine in Ab1 to a serine in AbA. Two of the amino acid changes within AbA are in the constant region of the heavy chain (D354E and L356M). The Fc region amino acid mutations in AbA represent human IgG allotype changes from a z, a allotype to a z, non-a allotype. In addition to the other changes, the first amino acid was changed from a glutamine (Q) to a glutamic acid (E), as described, for example, in Figure 3.

Thus, in one embodiment, the invention features an ADC comprising an anti-hEGFR antibody conjugated via a linker to a Bcl-xL inhibitor wherein the antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In one embodiment, the invention features an ADC comprising an anti-hEGFR antibody conjugated via a linker to a Bcl-xL inhibitor, wherein the

antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 5.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbB conjugated via a linker to a Bcl-xL inhibitor. The AbB antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 19, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 17, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 64 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 65. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbB. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbB.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbC conjugated via a linker to a Bcl-xL inhibitor. The AbC antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 84, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 66 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 67. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbC. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbC.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbD conjugated via a linker to a Bcl-xL inhibitor. The AbD antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region

comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 83, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 82. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 68 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 69. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbD. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbD.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbE conjugated via a linker to a Bcl-xL inhibitor. The AbE antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 85, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 82. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 51. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbE. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbE.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbF conjugated via a linker to a Bcl-xL inhibitor. The AbF antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 52 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbF. In a separate embodiment, the ADC of the invention

comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbF.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbG conjugated via a linker to a Bcl-xL inhibitor. The AbG antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 17, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 72 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 73. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbG. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbG.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbH conjugated via a linker to a Bcl-xL inhibitor. The AbH antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbH. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbH.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbJ conjugated via a linker to a Bcl-xL inhibitor. The AbJ antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2

domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 57. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbJ. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbJ.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbK conjugated via a linker to a Bcl-xL inhibitor. The AbK antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 19, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 74 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 75. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbK. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbK.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbL conjugated via a linker to a Bcl-xL inhibitor. The AbL antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 59. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbL. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbL.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbM conjugated via a linker to a Bcl-xL inhibitor. The AbM antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 20, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 76 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbM. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbM.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbN conjugated via a linker to a Bcl-xL inhibitor. The AbN antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 20, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 60 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 61. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbN. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbN.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbO conjugated via a linker to a Bcl-xL inhibitor. The AbO antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an

antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 62 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbO. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbO.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbP conjugated via a linker to a Bcl-xL inhibitor. The AbP antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 22, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 21, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 29. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 78 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 79. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbP. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbP.

In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbQ conjugated via a linker to a Bcl-xL inhibitor. The AbQ antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 22, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 81, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 29. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 70 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 71. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbQ. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbQ.

As described in Table 2, shown below, the antibody sequences disclosed herein provide amino acid consensus sequences that represent CDR domains resulting in improved binding to the Ab1 EGFR epitope. Thus, in one embodiment, the invention features an anti-EGFR antibody comprising a light chain variable region comprising a CDR3 domain comprising the amino acid sequence set forth as SEQ ID NO: 40, a CDR2 domain comprising the amino acid sequence set forth as SEQ ID NO: 39, and a CDR1 domain comprising the amino acid sequence set forth as SEQ ID NO: 38; and a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence set forth as SEQ ID NO: 37, a CDR2 domain comprising the amino acid sequence set forth as SEQ ID NO: 36, and a CDR1 domain comprising the amino acid sequence set forth as SEQ ID NO: 35. In a further embodiment, the anti-EGFR antibody of the invention comprises a heavy chain variable region comprising a CDR3 domain comprising an amino acid sequence as set forth in SEQ ID NO: 12, 18, 19, and 22; a CDR2 domain comprising an amino acid sequence as set forth in SEQ ID NO: 11 or 17; and a CDR1 domain comprising an amino acid sequence as set forth in SEQ ID NO: 10, 16, 20, and 21; and a light chain variable region comprising a CDR3 domain comprising an amino acid sequence as set forth in SEQ ID NO: 8, 25, 28, and 31; a CDR2 domain comprising an amino acid sequence as set forth in SEQ ID NO: 7, 24, 27, and 30; and a CDR1 domain comprising an amino acid sequence as set forth in SEQ ID NO: 6, 23, 26, and 29.

Table 2: Heavy and Light Chain CDR Sequence Comparison of Ab1 vs. AbA, AbG, AbK, AbM, and AbP Variants

HEAVY CHAIN CDRS

	Variable Heavy Chain (VH) CDR1											SEQ ID NO:	VH CDR2																SEQ ID NO:	VH CDR3							SEQ ID NO:
	G	Y	S	I	S	S	D	F	A	W	N	2	Y	I	S	Y	S	G	N	T	R	Y	Q	P	S	L	K	S	3	A	G	R	G	F	P	Y	4
						R						10					N												11		S						12
AbG						N						16					K												17		S			L			18
AbK						R						10					N												11		S					W	19
AbM					G	R						20					N												11		S						12
AbP					H							21																	3		S	W		L		W	22

LIGHT CHAIN CDRS

	Variable Light Chain (VL) CDR1											SEQ ID NO:	VL CDR2							SEQ ID NO:	VL CDR3									SEQ ID NO:
Ab1	H	S	S	Q	D	I	N	S	N	I	G	6	H	G	T	N	L	D	D	7	V	Q	Y	A	Q	F	P	W	T	8
AbA												6								7										8
AbG							T	Y				23			A					24				D	E					25
AbK							T	Y		V		26			S				H	27				D	D					28
AbM							T	Y		V		26			S				H	27				D	D					28
AbP								M		V		29			A	I				30					E					31

In one embodiment, the ADC of the invention includes an anti-hEGFR antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 50, 52, 53, 56, 58, 60, 62, 64, 66, and 68; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, and 69.

The foregoing anti-EGFR antibody CDR sequences establish a novel family of EGFR binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences listed in Tables 2-4.

Table 2, above, provides an alignment of the amino acid sequences of the heavy and light chain CDRs for Ab1 variant antibodies AbA, AbG, AbK, AbM, and AbP in comparison to Ab1.

As described in Table 3, below, the Ab1 variant antibodies AbA, AbG, AbK, AbM, AbP each has a serine residue in the variable heavy chain of CDR3 in place of a glycine (shown in bold/underlined in Table 3).

Table 3: CDR Consensus Sequences for Ab1 Variants from Table 2

CDR region	SEQ ID NO:	CDR Consensus Sequences for Ab1 Variants
VH CDR1	SEQ ID NO:35	G Y S I(S/G/H)(S/R/N)D F A W N
VH CDR2	SEQ ID NO:36	Y I S Y(S/N/K)G N T R Y Q P S L K S
VH CDR3	SEQ ID NO:37	A **S**(R/W)G(F/L)P(Y/W)
VL CDR1	SEQ ID NO:38	H S S Q D I(N/T)(Y/M/S)N(I/V)G
VL CDR2	SEQ ID NO:39	H G(T/A/S)(N/I)L D(D/H)
VL CDR3	SEQ ID NO:40	V Q Y(A/D)(Q/E/D)F P W T

A comparison of the VH and VL CDR sequences of Ab1 versus antibodies AbB, AbC, AbD, AbE, AbF, AbH, AbJ, AbL, AbN, AbO, and AbQ is described in Table 4. In addition to the CDR changes described in Table 4, below, AbG has an amino acid residue change within the framework 2 regions of the VH.

In one embodiment, the invention includes an anti-hEGFR antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

In one embodiment, the invention includes an anti-hEGFR antibody comprising an HC CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11,

and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and an LC light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85, wherein the antibody, or antigen binding portion thereof, does not comprise both the HC CDR set of SEQ ID NOs: 2, 3, and 4, and the LC CDR set of SEQ ID NOs: 6, 7, and 8. In one embodiment, the invention includes an anti-hEGFR antibody comprising an LC CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, an LC CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and an LC CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and an HC CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, an HC CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and an HC CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

Table 4. Heavy and Light Chain CDR Sequence Comparison of Ab1 vs. Certain Ab1 Variants

HEAVY CHAIN CDRS																																						
	Variable Heavy Chain (VH) CDR1											SEQ ID NO:	VH CDR2																SEQ ID NO:	VH CDR3									SEQ ID NO:
Ab1	G	Y	S	I	S	S	D	F	A	W	N	2	Y	I	S	Y	S	G	N	T	R	Y	Q	P	S	L	K	S	3			A	G	R	G	F	P	Y	4
AbB						N						16					K												17				S					W	19
AbC												2																	3										4
AbD												2																	3										4
AbE												2																	3										4
AbF						R						10																	3				S						12
AbH					G	K						80					N												11				S			L			18
AbJ					G	K						80																	3				S			L			18
AbL					G	K						80					N												11				S			L			18
AbN					G	R						20																	3				S						12
AbO					G	K						80					N												11				S						12
AbQ						H						81					N												11				S	W		L		W	22

Table 4 (continued)

LIGHT CHAIN CDRS																													
	Variable Light Chain (VL) CDR1											SEQ ID NO:	VL CDR2							SEQ ID NO:	VL CDR3									SEQ ID NO:
Ab1	H	S	S	Q	D	I	N	S	N	I	G	6	H	G	T	N	L	D	D	7	V	Q	Y	A	Q	F	P	W	T	8
AbB												6								7										8
AbC												6								7				E						84
AbD										L		82			A			H		83					E					31
AbE										L		82			S				H	27				D						85
AbF												6								7										8
AbH							T	Y				23			A					24				D	E					25
AbJ							T	Y				23			A					24				D	E					25
AbL							T	Y		V		26			S				H	27				D	D					28
AbN							T	Y		V		26			S				H	27				D	D					28
AbO							T	Y		V		26			S				H	27				D	D					28
AbQ								M		V		29			A	I				30					E					31

The full length heavy and light chain sequences of AbB are provided below:

AbB
Heavy chain
EVQLQESGPGLVKPSQTLSLTCTVSGYSIS
NDFAWNWIRQPPGKGLEWMGYISYKGNTRY
QPSLKSRITISRDTSKNQFFLKLNSVTAAD
TATYYCVTASRGFPWWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTHTCPPCPAPE**LL**GGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 90)

In one embodiment, the above AbB heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 91).

AbB Light chain
DIQMTQSPSSMSVSVGDRVTITCHSSQDIN
SNIGWLQQKPGKSFKGLIYHGTNLDDGVPS
RFSGSGSGTDYTLTISSLQPEDFATYYCVQ
YAQFPWTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 92)

The full length heavy and light chain sequences of AbG are provided below:

AbG
Heavy chain
EVQLQESGPGLVKPSQTLSLTCTVSGYSIS
NDFAWNWIRQLPGKGLEWMGYISYKGNTRY
QPSLKSRITISRDTSKNQFFLKLNSVTAAD
TATYYCVTASRGLPYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTHTCPPCPAPE**LL**GGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 93)

In one embodiment, the above AbG heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 94).

Light chain
DIQMTQSPSSMSVSVGDRVTITCHSSQDIT
YNIGWLQQKPGKSFKGLIYHGANLDDGVPS
RFSGSGSGTDYTLTISSLQPEDFATYYCVQ
YDEFPWTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 95)

The full length heavy and light chain sequences of AbK are provided below:

AbK
Heavy chain
EVQLQESGPGLVKPSQTLSLTCTVSGYSIS
RDFAWNWIRQPPGKGLEWMGYISYNGNTRY
QPSLKSRITISRDTSKNQFFLKLNSVTAAD
TATYYCVTASRGFPWWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTHTCPPCPAPE**LL**GGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 96)

In one embodiment, the above AbK heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 97).

Light chain
DIQMTQSPSSMSVSVGDRVTITCHSSQDIT
YNVGWLQQKPGKSFKGLIYHGSNLDHGVPS
RFSGSGSGTDYTLTISSLQPEDFATYYCVQ
YDDFPWTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 98)

To generate and to select CDRs having preferred EGFR binding and/or neutralizing activity with respect to hEGFR, standard methods known in the art for generating antibodies, or antigen binding portions thereof, and assessing the EGFR binding and/or neutralizing characteristics of those antibodies, or antigen binding portions thereof, may be used, including but not limited to those specifically described herein.

In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, or IgD constant region. In certain embodiments, the anti-EGFR antibody comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgG constant domain, a human IgM constant domain, a human IgE constant domain, and a human IgA constant domain. In further embodiments, the antibody, or antigen binding portion thereof, has an IgG1 heavy chain constant region, an IgG2 heavy chain constant region, an IgG3 constant region, or an IgG4 heavy chain constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. In one embodiment, the antibody comprises a kappa light chain constant region.

In certain embodiments, the anti-EGFR antibody is a multispecific antibody, *e.g.* a bispecific antibody.

In certain embodiments, the anti-EGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43.

Replacements of amino acid residues in the Fc portion to alter antibody effector function have been described (Winter, *et al.* US Patent Nos. 5,648,260 and 5,624,821, incorporated by reference herein). The Fc portion of an antibody mediates several important effector functions *e.g.* cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

One embodiment of the invention includes a labeled anti-EGFR antibody where the antibody is derivatized or linked to one or more functional molecule(s) (*e.g.*, another peptide or protein) in addition to the Bcl-xL inhibitors described below. For example, a labeled antibody can be derived by functionally linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a pharmaceutical agent, a protein or peptide that can mediate the association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a

polyhistidine tag), and/or a cytotoxic or therapeutic agent selected from the group consisting of a mitotic inhibitor, an antitumor antibiotic, an immunomodulating agent, a vector for gene therapy, an alkylating agent, an antiangiogenic agent, an antimetabolite, a boron-containing agent, a chemoprotective agent, a hormone, an antihormone agent, a corticosteroid, a photoactive therapeutic agent, an oligonucleotide, a radionuclide agent, a topoisomerase inhibitor, a kinase inhibitor, a radiosensitizer, and a combination thereof.

Useful detectable agents with which an antibody or antibody portion thereof, may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

In one embodiment, the antibody of the invention is conjugated to an imaging agent. Examples of imaging agents that may be used in the compositions and methods described herein include, but are not limited to, a radiolabel (*e.g.,* indium), an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

In one embodiment, the antibodies are linked to a radiolabel, such as, but not limited to, indium (^{111}In). ^{111}Indium may be used to label the antibodies and ADCs described herein for use in identifying EGFR positive tumors. In a certain embodiment, anti-EGFR antibodies (or ADCs) described herein are labeled with ^{111}I via a bifunctional chelator which is a bifunctional cyclohexyl diethylenetriaminepentaacetic acid (DTPA) chelate (see US Patent Nos. 5,124,471; 5,434,287; and 5,286,850, each of which is incorporated herein by reference).

Another embodiment of the invention provides a glycosylated binding protein wherein the anti-EGFR antibody comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* 21 (2005), pp.

11–16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., *et al.*, *Mol. Immunol.* (1993) 30:1361- 1367), or result in increased affinity for the antigen (Wallick, S.C., *et al.*, *Exp. Med.* (1988) 168:1099-1109; Wright, A., *et al.*, *EMBO J.* (1991) 10:2717-2723).

One aspect of the invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity, but have increased or decreased binding activity, are another object of the invention.

In still another embodiment, the glycosylation of the anti-EGFR antibody is modified. For example, an aglycosylated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, a modified anti-EGFR antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740; Umana *et al.* (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80, each of which is incorporated herein by reference in its entirety.

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (*e.g.*, glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the

particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

Differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using recombinant techniques, a practitioner may generate antibodies or antigen binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S. patent Publication Nos. 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

Antibodies may be produced by any of a number of techniques. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell in a suitable culture medium until a recombinant antibody is synthesized. Recombinant antibodies of the invention may be produced

using nucleic acid molecules corresponding to the amino acid sequences disclosed herein. In one embodiment, the nucleic acid molecules set forth in SEQ ID NOs: 86 and/or 87 are used in the production of a recombinant antibody. The method can further comprise isolating the recombinant antibody from the culture medium.

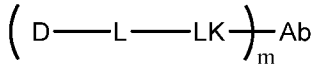
The antibodies and the sequences of the antibodies recited herein are also described in US Patent No. 9,493,568 (AbbVie Inc.), which is incorporated by reference herein.

3. Anti-EGFR Antibody Drug Conjugates (ADCs): Bcl-xL Inhibitors and Linkers

Dysregulated apoptotic pathways have also been implicated in the pathology of cancer. The implication that down-regulated apoptosis (and more particularly the Bcl-2 family of proteins) is involved in the onset of cancerous malignancy has revealed a novel way of targeting this still elusive disease. Research has shown, for example, the anti-apoptotic proteins, Bcl 2 and Bcl-xL, are over-expressed in many cancer cell types. See, Zhang, 2002, Nature Reviews/Drug Discovery 1:101; Kirkin et al., 2004, Biochimica Biophysica Acta 1644:229-249; and Amundson et al., 2000, Cancer Research 60:6101-6110. The effect of this deregulation is the survival of altered cells which would otherwise have undergone apoptosis in normal conditions. The repetition of these defects associated with unregulated proliferation is thought to be the starting point of cancerous evolution.

Aspects of the disclosure concern anti-hEGFR ADCs comprising an anti-hEGFR antibody conjugated to a drug via a linker, wherein the drug is a Bcl-xL inhibitor. In specific embodiments, the ADCs are compounds according to structural formula (I) below, or a pharmaceutically acceptable salt thereof, wherein Ab represents the anti-hEGFR antibody, D represents a Bcl-xL inhibitor drug (i.e., a compound of formula IIa as shown below), L represents a linker, LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab) and m represents the number of D-L-LK units linked to the antibody, which is an integer ranging from 1 to 20. In certain embodiments, m is 2, 3 or 4. In some embodiments, m ranges from 1 to 8, 1 to 7, 1 to 6, 2 to 6, 1 to 5, 1 to 4, or 2 to 4.

In some embodiments, the ADC has the following formula (formula I):

(I) 

wherein Ab is the antibody, *e.g.*, anti-EGFR antibody AbA, AbB, AbG, or AbK, and (D-L-LK) is a Drug-Linker-Covalent Linkage. The Drug-Linker moiety is made of L- which is a Linker, and –D, which is a drug moiety having, for example, cytostatic, cytotoxic, or otherwise therapeutic activity against a target cell, *e.g.*, a cell expressing EGFR; and m is an integer from 1 to 20. In

some embodiments, m ranges from 1 to 8, 1 to 7, 1 to 6, 2 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, 1.5 to 8, 1.5 to 7, 1.5 to 6, 1.5 to 5, 1.5 to 4, 2 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 2 to 4.. The DAR of an ADC is equivalent to the "m" referred to in Formula I. In one embodiment, the ADC has a formula of Ab-(LK-L-D)$_m$, wherein Ab is an anti-EGFR antibody, *e.g.* AbA, AbB, AbG, or AbK, L is a linker, D is a drug, *e.g.,* a Bcl-xL inhibitor, LK is a covalent linker, e.g. –S-, and m is 1 to 8 (*e.g.* a DAR of 2-4, a DAR of 1.5-4 , a DAR of 1.5-8). Additional details regarding drugs (D of Formula I) and linkers (L of Formula I) that may be used in the ADCs of the invention, as well as alternative ADC structures, are described below.

Specific embodiments of the various Bcl-xL inhibitors (D), linkers (L) and anti-EGFR antibodies (Ab) that can comprise the ADCs described herein, as well as the number of Bcl-xL inhibitors linked to the ADCs, are described in more detail below.

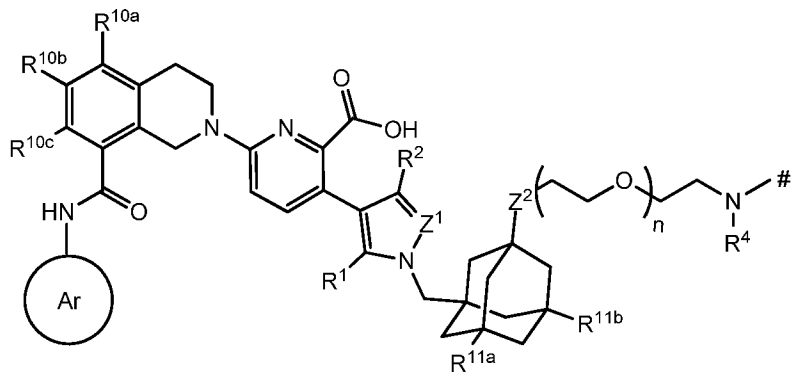
Examples of Bcl-xL inhibitors that may be used in the anti-EGFR ADC of the invention are provided below, as are linkers that may be used to conjugate the antibody and the one or more Bcl-xL inhibitor(s). The terms "linked" and "conjugated" are also used interchangeably herein and indicate that the antibody and moiety are covalently linked.

Bcl-xL inhibitors and linkers that may be used in the ADCs described herein and methods of making the same, are described in WO 2016/094505 (AbbVie Inc.), which is incorporated by reference herein.

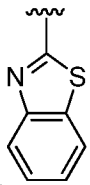
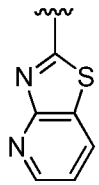
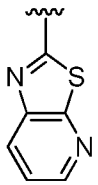
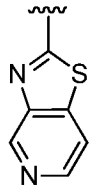
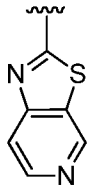
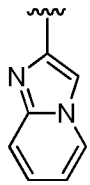
3.1. Bcl-xL Inhibitors

The ADCs comprise one or more Bcl-xL inhibitors, which may be the same or different, but are typically the same. In some embodiments, the Bcl-xL inhibitors comprising the ADCs, and in certain specific embodiments D of structural formula (I), above, are compounds according to structural formula (IIa). In the present invention, when the Bcl-xL inhibitors are included as part of an ADC, # shown in structural formula (IIa) below represents a point of attachment to a linker, which indicates that they are represented in a monoradical form.

(IIa)



or a pharmaceutically acceptable salt thereof, wherein:

Ar is selected from  ,  ,  ,  ,  , and  , which is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

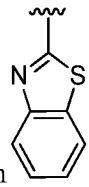
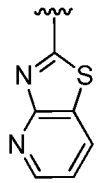
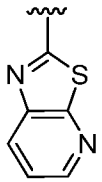
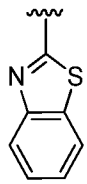
R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents the point of attachment to linker L.

In certain embodiments, Ar of formula (IIa) is unsubstituted.

In certain embodiments, Ar of formula (IIa) is selected from  ,  , and  and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl. In particular embodiments, Ar is  .

In certain embodiments, Z^1 of formula (IIa) is N.

In certain embodiments, Z^1 of formula (IIa) is CH.

In certain embodiments, Z^2 of formula (IIa) is CH_2 or O.

In certain embodiments, Z^2 of formula (IIa) is O.

In certain embodiments, R^1 of formula (IIa) is selected from methyl and chloro.

In certain embodiments, R^2 of formula (IIa) is selected from hydrogen and methyl. In particular embodiments, R^2 is hydrogen.

In certain embodiments, R^1 in formula (IIa) is methyl, R^2 is hydrogen and Z^1 is N.

In certain embodiments, R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with OCH_3.

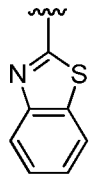
In certain embodiments, R^{10a} in formula (IIa) is halo and R^{10b} and R^{10c} are each hydrogen. In particular embodiments, R^{10a} is fluoro.

In certain embodiments, R^{10b} in formula (IIa) is halo and R^{10a} and R^{10c} are each hydrogen. In particular embodiments, R^{10b} is fluoro.

In certain embodiments, R^{10c} in formula (IIa) is halo and R^{10a} and R^{10b} are each hydrogen. In particular embodiments, R^{10c} is fluoro.

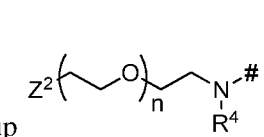
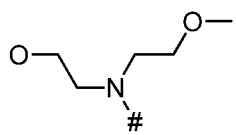
In certain embodiments, R^{10a}, R^{10b} and R^{10c} in formula (IIa) are each hydrogen.

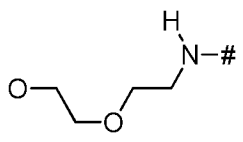
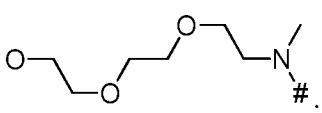
In certain embodiments, R^{11a} and R^{11b} in formula (IIa) are the same. In particular embodiments, R^{11a} and R^{11b} are each methyl.

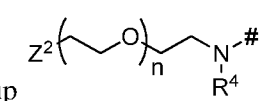
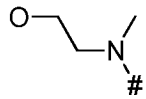
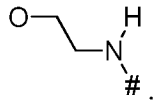
In certain embodiments, Z^1 is N; R^1 is methyl; R^2 is hydrogen; R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with OCH_3; one of R^{10a}, R^{10b} and R^{10c} is hydrogen or halo, and the others are hydrogen; R^{11a} and R^{11b} are each methyl, and Ar is  .

In certain embodiments, Z^2 oxygen, R^4 is hydrogen or C_{1-4} alkanyl optionally substituted withOCH_3, and n is 0, 1 or 2.

In certain embodiments, *n* of formula (IIa) is 0, 1 or 2. In particular embodiments, *n* of formula (IIa) is 0 or 1.

In certain embodiments, the group  is  ,

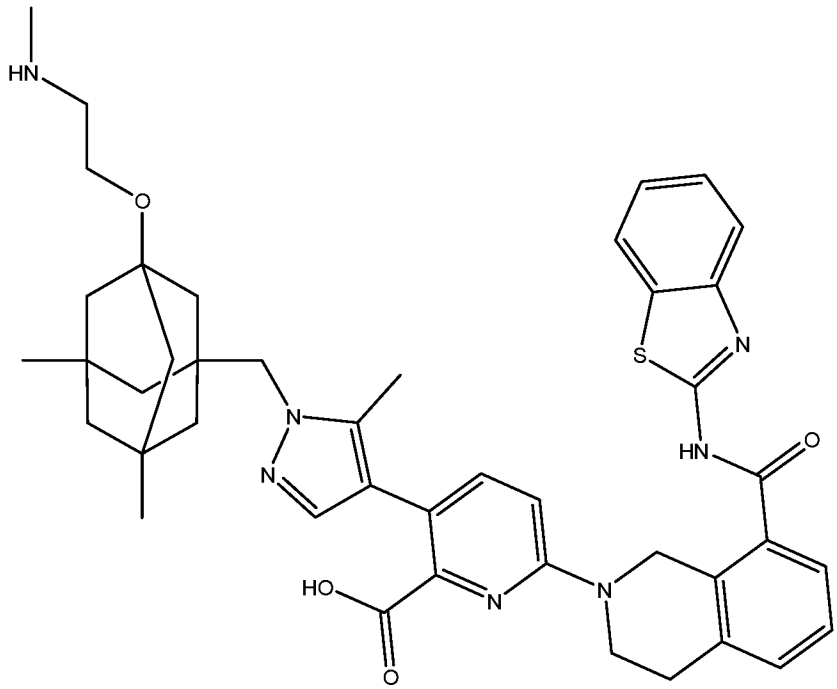
 , or  .

In certain embodiments, the group  is  or  .

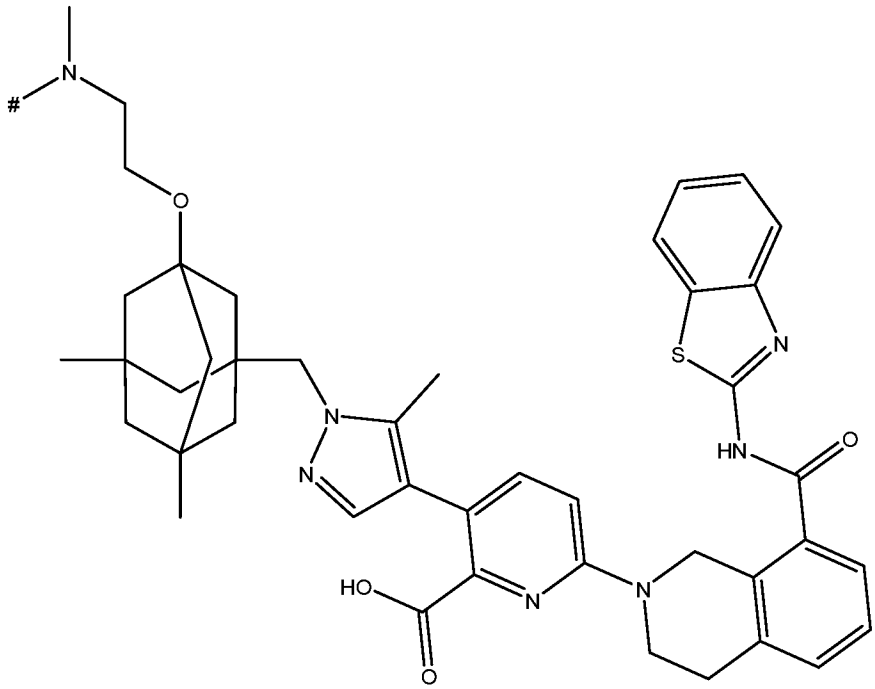
Exemplary Bcl-xL inhibitors and/or salts thereof that may be used in the methods described herein in unconjugated form and/or included in the ADCs described herein include compounds W1.01-W1.08, described in Examples 1.1-1.8, respectively.

Notably, when the Bcl-xL inhibitor of the present application is in conjugated form, the hydrogen corresponding to the # position of structural formula (IIa) is not present, forming a monoradical. For example, compound W1.01 (Example 1.1) is 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid.

When it is in unconjugated form, it has the following structure:



When the same compound is included in the ADCs as shown in structural formula (IIa) or (IIb), the hydrogen corresponding to the # position is not present, forming a monoradical.



In certain embodiments, the Bcl-xL inhibitor is according to structural formula (IIa), wherein the # is replaced with a hydrogen to form a compound as follows:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

and a pharmaceutically acceptable salt thereof.

The Bcl-xL inhibitors comprising the ADCs, when not included in an ADC, bind to and inhibit anti-apoptotic Bcl-xL proteins, inducing apoptosis. The ability of a specific Bcl-xL inhibitor according to structural formula (IIa) to bind and inhibit Bcl-xL activity when not included in an ADC (*i.e.*, a compound or salt according to structural formula (IIa) in which # represents a hydrogen atom), may be confirmed in standard binding and activity assays, including, for example, the TR-FRET Bcl-xL binding assays described in Tao *et al.*, 2014, ACS Med. Chem. Lett., 5:1088-1093. A specific TR-FRET Bcl-xL binding assay that can be used to confirm Bcl-xL binding is provided in Example 4, below. Typically, Bcl-xL inhibitors useful in the ADCs described herein will exhibit a K_i in the binding assay of Example 4 of less than about 10 nM, but may exhibit a significantly lower K_i, for example a K_i of less than about 1, 0.1, or even 0.01 nM.

Bcl-xL inhibitory activity may also be confirmed in standard cell-based cytotoxicity assays, such as the FL5.12 cellular and Molt-4 cytotoxicity assays described in Tao *et al.*, 2014, *ACS Med. Chem. Lett.*, 5:1088-1093. A specific Molt-4 cellular cytoxicity assay that may be used to confirm Bcl-xL inhibitory activity of specific Bcl-xL inhibitors is provided in Example 5, below. Typically, Bcl-xL inhibitors useful in the ADCs described herein will exhibit an EC_{50} of less than about 500 nM in the Molt-4 cytotoxicity assay of Example 5, but may exhibit a significantly lower EC_{50}, for example an EC_{50} of less than about 250, 100, 50, 20, 10 or even 5 nM.

Although the Bcl-xL inhibitors defined by structural formula (IIa) are expected to be cell permeable and penetrate cells when not included in an ADC, the Bcl-xL inhibitory activity of compounds that do not freely traverse cell membranes may be confirmed in cellular assays with permeabilized cells. As discussed in the Background section, the process of mitochondrial outer-membrane permeabilization (MOMP) is controlled by the Bcl-2 family proteins. Specifically, MOMP is promoted by the pro-apoptotic Bcl-2 family proteins Bax and Bak which, upon activation oligomerize on the outer mitochondrial membrane and form pores, leading to release of

cytochrome c (cyt c). The release of cyt c triggers formulation of the apoptosome which, in turn, results in caspase activation and other events that commit the cell to undergo programmed cell death (*see*, Goldstein *et al.*, 2005, *Cell Death and Differentiation* 12:453-462). The oligomerization action of Bax and Bak is antagonized by the anti-apoptotic Bcl-2 family members, including Bcl-2 and Bcl-xL. Bcl-xL inhibitors, in cells that depend upon Bcl-xL for survival, can cause activation of Bax and/or Bak, MOMP, release of cyt c and downstream events leading to apoptosis. The process of cyt c release can be assessed via western blot of both mitochondrial and cytosolic fractions of cytochrome c in cells and used as a proxy measurement of apoptosis in cells.

As a means of detecting Bcl-xL inhibitory activity and consequent release of cyt c for molecules with low cell permeability, the cells can be treated with an agent that causes selective pore formation in the plasma, but not mitochondrial, membrane. Specifically, the cholesterol/phospholipid ratio is much higher in the plasma membrane than the mitochondrial membrane. As a result, short incubation with low concentrations of the cholesterol-directed detergent digitonin selectively permeabilizes the plasma membrane without significantly affecting the mitochondrial membrane. This agent forms insoluble complexes with cholesterol leading to the segregation of cholesterol from its normal phospholipid binding sites. This action, in turn, leads to the formation of holes about 40-50 Å wide in the lipid bilayer. Once the plasma membrane is permeabilized, cytosolic components able to pass over digitonin-formed holes can be washed out, including the cytochrome C that was released from mitochondria to cytosol in the apoptotic cells (Campos, 2006, *Cytometry A* 69(6):515-523).

Typically, Bcl-xL inhibitors will yield an EC_{50} of less than about 10 nM in the Molt-4 cell permeabilized cyt c assay of Example 5, although the compounds may exhibit significantly lower EC_{50}s, for example, less than about 5, 1, or even 0.5 nM.

Although many of the Bcl-xL inhibitors of structural formula (IIa) selectively or specifically inhibit Bcl-xL over other anti-apoptotic Bcl-2 family proteins, selective and/or specific inhibition of Bcl-xL is not necessary. The Bcl-xL inhibitors comprising the ADCs may also, in addition to inhibiting Bcl-xL, inhibit one or more other anti-apoptotic Bcl-2 family proteins, such as, for example, Bcl-2. In some embodiments, the Bcl-xL inhibitors comprising the ADC are selective and/or specific for Bcl-xL. By specific or selective is meant that the particular Bcl-xL inhibitor binds or inhibits Bcl-xL to a greater extent than Bcl-2 under equivalent assay conditions. In specific embodiments, the Bcl-xL inhibitors comprising the ADCs exhibit in the range of 10-fold, 100-fold, or even greater specificity for Bcl-xL than Bcl-2 in a Bcl-xL binding assay.

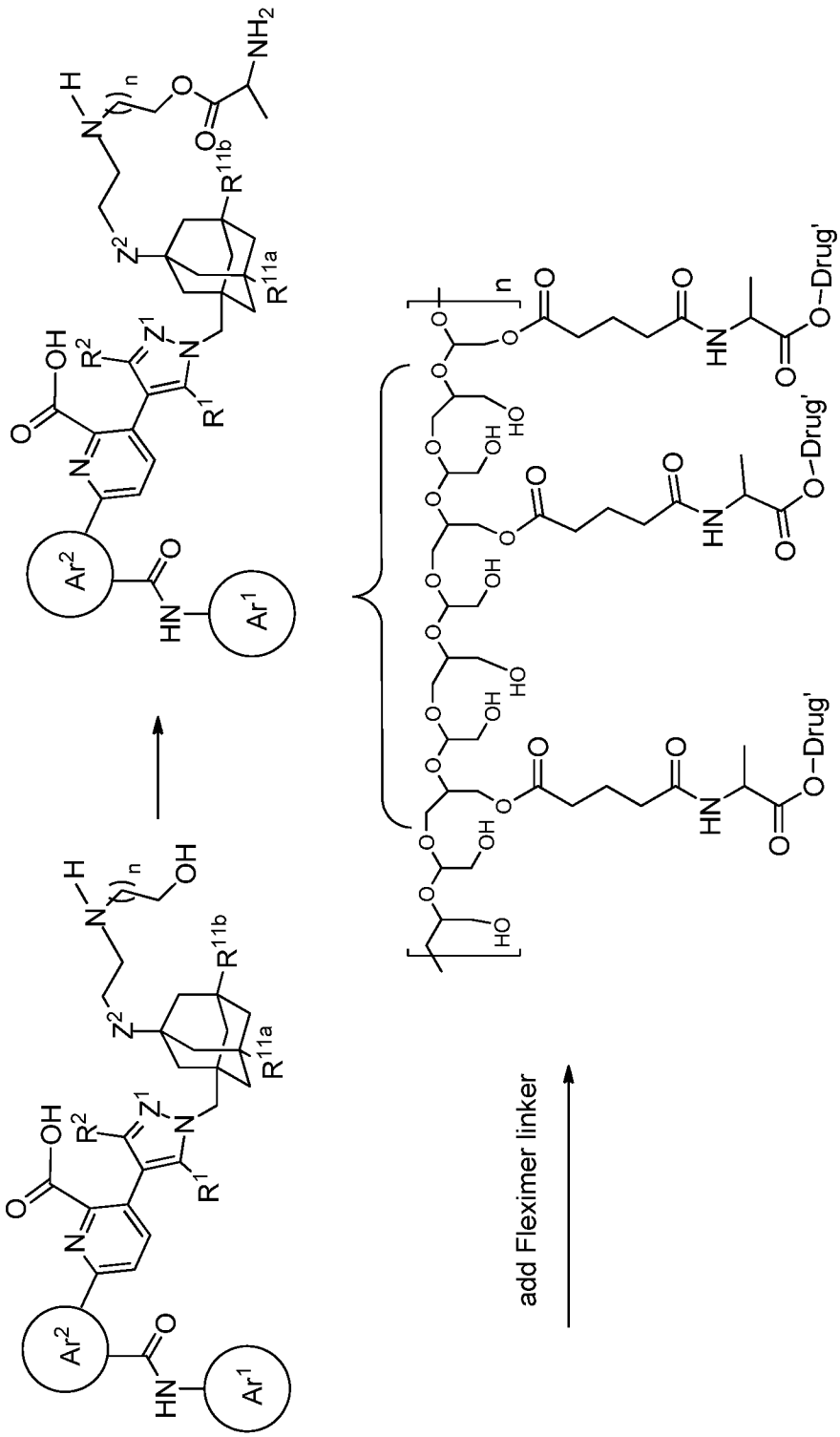
3.2. Linkers

In the ADCs described herein, the Bcl-xL inhibitors are linked to the antibody by way of linkers. The linker linking a Bcl-xL inhibitor to the antibody of an ADC may be short, long, hydrophobic, hydrophilic, flexible or rigid, or may be composed of segments that each independently has one or more of the above-mentioned properties such that the linker may include segments having different properties. The linkers may be polyvalent such that they covalently link more than one Bcl-xL inhibitor to a single site on the antibody, or monovalent such that covalently they link a single Bcl-xL inhibitor to a single site on the antibody.

As will be appreciated by skilled artisans, the linkers link the Bcl-xL inhibitors to the antibody by forming a covalent linkage to the Bcl-xL inhibitor at one location and a covalent linkage to antibody at another. The covalent linkages are formed by reaction between functional groups on the linker and functional groups on the inhibitors and antibody. As used herein, the expression "linker" is intended to include (i) unconjugated forms of the linker that include a functional group capable of covalently linking the linker to a Bcl-xL inhibitor and a functional group capable of covalently linking the linker to an antibody; (ii) partially conjugated forms of the linker that include a functional group capable of covalently linking the linker to an antibody and that is covalently linked to a Bcl-xL inhibitor, or *vice versa*; and (iii) fully conjugated forms of the linker that are covalently linked to both a Bcl-xL inhibitor and an antibody. In some specific embodiments of intermediate synthons and ADCs described herein, moieties comprising the functional groups on the linker and covalent linkages formed between the linker and antibody are specifically illustrated as R^x and LK, respectively. One embodiment pertains to an ADC formed by contacting an antibody that binds a cell surface receptor or tumor associated antigen expressed on a tumor cell with a synthon described herein under conditions in which the synthon covalently links to the antibody. One embodiment pertains to a method of making an ADC formed by contacting a synthon described herein under conditions in which the synthon covalently links to the antibody. One embodiment pertains to a method of inhibiting Bcl-xL activity in a cell that expresses Bcl-xL, comprising contacting the cell with an ADC described herein that is capable of binding the cell, under conditions in which the ADC binds the cell.

The linkers are preferably, but need not be, chemically stable to conditions outside the cell, and may be designed to cleave, immolate and/or otherwise specifically degrade inside the cell. Alternatively, linkers that are not designed to specifically cleave or degrade inside the cell may be used. A wide variety of linkers useful for linking drugs to antibodies in the context of ADCs are known in the art. Any of these linkers, as well as other linkers, may be used to link the Bcl-xL inhibitors to the antibody of the ADCs described herein. Exemplary polyvalent linkers that may be used to link many Bcl-xL inhibitors to an antibody are described, for example, in U.S. Patent No 8,399,512; U.S. Published Application No. 2010/0152725; U.S. Patent No.

8,524,214; U.S. Patent No. 8,349,308; U.S. Published Application No. 2013/189218; U.S. Published Application No. 2014/017265; WO 2014/093379; WO 2014/093394; WO 2014/093640, the contents of which are incorporated herein by reference in their entireties. For example, the Fleximer® linker technology developed by Mersana *et al.* has the potential to enable high-DAR ADCs with good physicochemical properties. As shown below, the Fleximer® linker technology is based on incorporating drug molecules into a solubilizing poly-acetal backbone via a sequence of ester bonds. The methodology renders highly-loaded ADCs (DAR up to 20) whilst maintaining good physicochemical properties. This methodology could be utilized with Bcl-xL inhibitors as shown in the Scheme below.


add Fleximer linker

To utilize the Fleximer® linker technology depicted in the scheme above, an aliphatic alcohol must be present or introduced into the Bcl-xL inhibitor. The alcohol moiety is then conjugated to an alanine moiety, which is then synthetically incorporated into the Fleximer® linker. Liposomal processing of the ADC *in vitro* releases the parent alcohol–containing drug.

Additional examples of dendritic type linkers can be found in US 2006/116422; US 2005/271615; de Groot *et al.*, (2003) *Angew. Chem. Int. Ed.* 42:4490-4494; Amir *et al.*, (2003) *Angew. Chem. Int. Ed.* 42:4494-4499; Shamis *et al.*, (2004) *J. Am. Chem. Soc.* 126:1726-1731 ; Sun *et al.*, (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun *et al.*, (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768; and King *et al.*, (2002) *Tetrahedron Letters* 43:1987-1990.

Exemplary monovalent linkers that may be used are described, for example, in Nolting, 2013, *Antibody-Drug Conjugates, Methods in Molecular Biology* 1045:71-100; Kitson *et al.*, 2013, *CROs/CMOs - Chemica Oggi – Chemistry Today* 31(4): 30-36; Ducry *et al.*, 2010, *Bioconjugate Chem.* 21:5-13; Zhao *et al.*, 2011, *J. Med. Chem.* 54:3606-3623; U.S. Patent No. 7,223,837; U.S. Patent No. 8,568,728; U.S. Patent No. 8,535,678; and WO2004010957, the content of each of which is incorporated herein by reference in their entireties.

By way of example and not limitation, some cleavable and noncleavable linkers that may be included in the ADCs described herein are described below.

3.2.1 Cleavable Linkers

In certain embodiments, the linker selected is cleavable *in vitro* and *in vivo*. Cleavable linkers may include chemically or enzymatically unstable or degradable linkages. Cleavable linkers generally rely on processes inside the cell to liberate the drug, such as reduction in the cytoplasm, exposure to acidic conditions in the lysosome, or cleavage by specific proteases or other enzymes within the cell. Cleavable linkers generally incorporate one or more chemical bonds that are either chemically or enzymatically cleavable while the remainder of the linker is noncleavable.

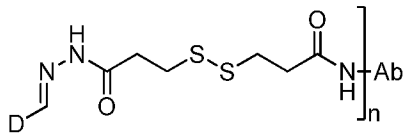
In certain embodiments, a linker comprises a chemically labile group such as hydrazone and/or disulfide groups. Linkers comprising chemically labile groups exploit differential properties between the plasma and some cytoplasmic compartments. The intracellular conditions to facilitate drug release for hydrazone containing linkers are the acidic environment of endosomes and lysosomes, while the disulfide containing linkers are reduced in the cytosol, which contains high thiol concentrations, *e.g.*, glutathione. In certain embodiments, the plasma stability of a linker comprising a chemically labile group may be increased by introducing steric hindrance using substituents near the chemically labile group.

Acid-labile groups, such as hydrazone, remain intact during systemic circulation in the blood's neutral pH environment (pH 7.3-7.5) and undergo hydrolysis and release the drug once

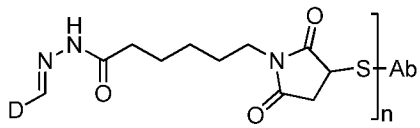
the ADC is internalized into mildly acidic endosomal (pH 5.0-6.5) and lysosomal (pH 4.5-5.0) compartments of the cell. This pH dependent release mechanism has been associated with nonspecific release of the drug. To increase the stability of the hydrazone group of the linker, the linker may be varied by chemical modification, *e.g.*, substitution, allowing tuning to achieve more efficient release in the lysosome with a minimized loss in circulation.

Hydrazone-containing linkers may contain additional cleavage sites, such as additional acid-labile cleavage sites and/or enzymatically labile cleavage sites. ADCs including exemplary hydrazone-containing linkers include the following structures:

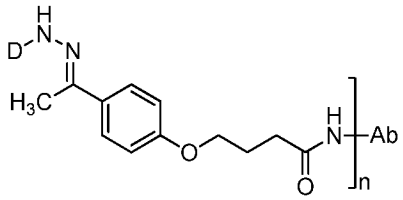
(Id)



(Ie)



(If)



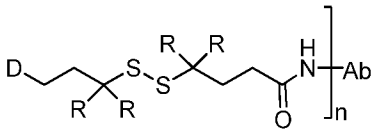
wherein D and Ab represent the drug and Ab, respectively, and n represents the number of drug-linkers linked to the antibody. In certain linkers such as linker (Id), the linker comprises two cleavable groups – a disulfide and a hydrazone moiety. For such linkers, effective release of the unmodified free drug requires acidic pH or disulfide reduction and acidic pH. Linkers such as (Ie) and (If) have been shown to be effective with a single hydrazone cleavage site.

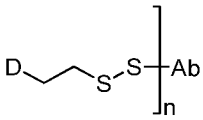
Other acid-labile groups that may be included in linkers include *cis*-aconityl-containing linkers. *cis*-Aconityl chemistry uses a carboxylic acid juxtaposed to an amide bond to accelerate amide hydrolysis under acidic conditions.

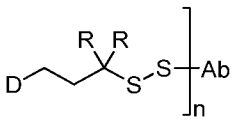
Cleavable linkers may also include a disulfide group. Disulfides are thermodynamically stable at physiological pH and are designed to release the drug upon internalization inside cells, wherein the cytosol provides a significantly more reducing environment compared to the extracellular environment. Scission of disulfide bonds generally requires the presence of a cytoplasmic thiol cofactor, such as (reduced) glutathione (GSH), such that disulfide-containing linkers are reasonable stable in circulation, selectively releasing the drug in the cytosol. The

intracellular enzyme protein disulfide isomerase, or similar enzymes capable of cleaving disulfide bonds, may also contribute to the preferential cleavage of disulfide bonds inside cells. GSH is reported to be present in cells in the concentration range of 0.5-10 mM compared with a significantly lower concentration of GSH or cysteine, the most abundant low-molecular weight thiol, in circulation at approximately 5 μM. Tumor cells, where irregular blood flow leads to a hypoxic state, result in enhanced activity of reductive enzymes and therefore even higher glutathione concentrations. In certain embodiments, the *in vivo* stability of a disulfide-containing linker may be enhanced by chemical modification of the linker, *e.g.*, use of steric hindrance adjacent to the disulfide bond.

ADCs including exemplary disulfide-containing linkers include the following structures:

(Ig) 

(Ih) 

(Ii) 

wherein D and Ab represent the drug and antibody, respectively, n represents the number of drug-linkers linked to the antibody and R is independently selected at each occurrence from hydrogen or alkyl, for example. In certain embodiments, increasing steric hindrance adjacent to the disulfide bond increases the stability of the linker. Structures such as (Ig) and (Ii) show increased *in vivo* stability when one or more R groups are selected from a lower alkyl such as methyl.

Another type of linker that may be used is a linker that is specifically cleaved by an enzyme. In one embodiment, the linker is cleavable by a lysosomal enzyme. Such linkers are typically peptide-based or include peptidic regions that act as substrates for enzymes. Peptide based linkers tend to be more stable in plasma and extracellular mille than chemically labile linkers. Peptide bonds generally have good serum stability, as lysosomal proteolytic enzymes have very low activity in blood due to endogenous inhibitors and the unfavorably high pH value of blood compared to lysosomes. Release of a drug from an antibody occurs specifically due to the action of lysosomal proteases, *e.g.*, cathepsin and plasmin. These proteases may be present at

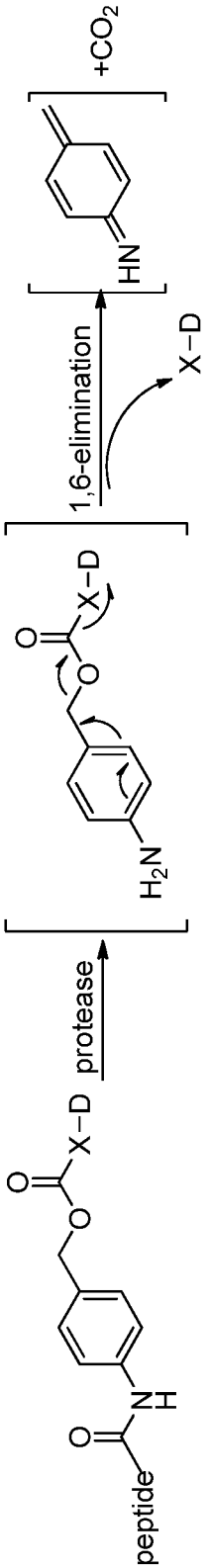
elevated levels in certain tumor tissues. In one embodiment, the linker is cleavable by the lysosomal enzyme is Cathepsin B. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β-glucuronidase or β-galactosidase. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β-glucuronidase. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β-galactosidase.

In exemplary embodiments, the cleavable peptide is selected from tetrapeptides such as Gly-Phe-Leu-Gly, Ala-Leu-Ala-Leu or dipeptides such as Val-Cit, Val-Ala, and Phe-Lys. In certain embodiments, dipeptides are preferred over longer polypeptides due to hydrophobicity of the longer peptides.

A variety of dipeptide-based cleavable linkers useful for linking drugs such as doxorubicin, mitomycin, campotothecin, tallysomycin and auristatin/auristatin family members to antibodies have been described (*see*, Dubowchik *et al.*, 1998, *J. Org. Chem.* 67:1866-1872; Dubowchik *et al.*, 1998, *Bioorg. Med. Chem. Lett.* 8:3341-3346; Walker *et al.*, 2002, *Bioorg. Med. Chem. Lett.* 12:217-219; Walker *et al.*, 2004, *Bioorg. Med. Chem. Lett.*14:4323-4327; and Francisco *et al.*, 2003, *Blood* 102:1458-1465, the contents of each of which are incorporated herein by reference). All of these dipeptide linkers, or modified versions of these dipeptide linkers, may be used in the ADCs described herein. Other dipeptide linkers that may be used include those found in ADCs such as Seattle Genetics' Brentuximab Vendotin SGN-35 (Adcetris™), Seattle Genetics SGN-75 (anti-CD-70, MC-monomethyl auristatin F(MMAF), Celldex Therapeutics glembatumumab (CDX-011) (anti-NMB, Val-Cit- monomethyl auristatin E(MMAE), and Cytogen PSMA-ADC (PSMA-ADC-1301) (anti-PSMA, Val-Cit-MMAE).

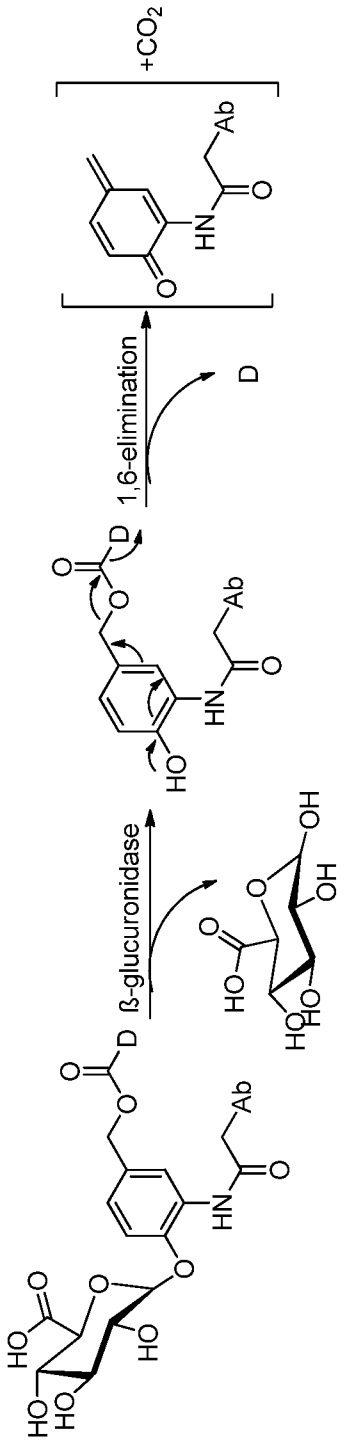
Enzymatically cleavable linkers may include a self-immolative spacer to spatially separate the drug from the site of enzymatic cleavage. The direct attachment of a drug to a peptide linker can result in proteolytic release of an amino acid adduct of the drug, thereby impairing its activity. The use of a self-immolative spacer allows for the elimination of the fully active, chemically unmodified drug upon amide bond hydrolysis.

One self-immolative spacer is the bifunctional *para*-aminobenzyl alcohol group, which is linked to the peptide through the amino group, forming an amide bond, while amine containing drugs may be attached through carbamate functionalities to the benzylic hydroxyl group of the linker (to give a *p*-amidobenzylcarbamate, PABC). The resulting prodrugs are activated upon protease-mediated cleavage, leading to a 1,6-elimination reaction releasing the unmodified drug, carbon dioxide, and remnants of the linker group. The following scheme depicts the fragmentation of *p*-amidobenzyl carbamate and release of the drug:


peptide
NH
O
X–D
O
O
protease
H2N
X–D
O
O
1,6-elimination
X–D
HN
+CO2

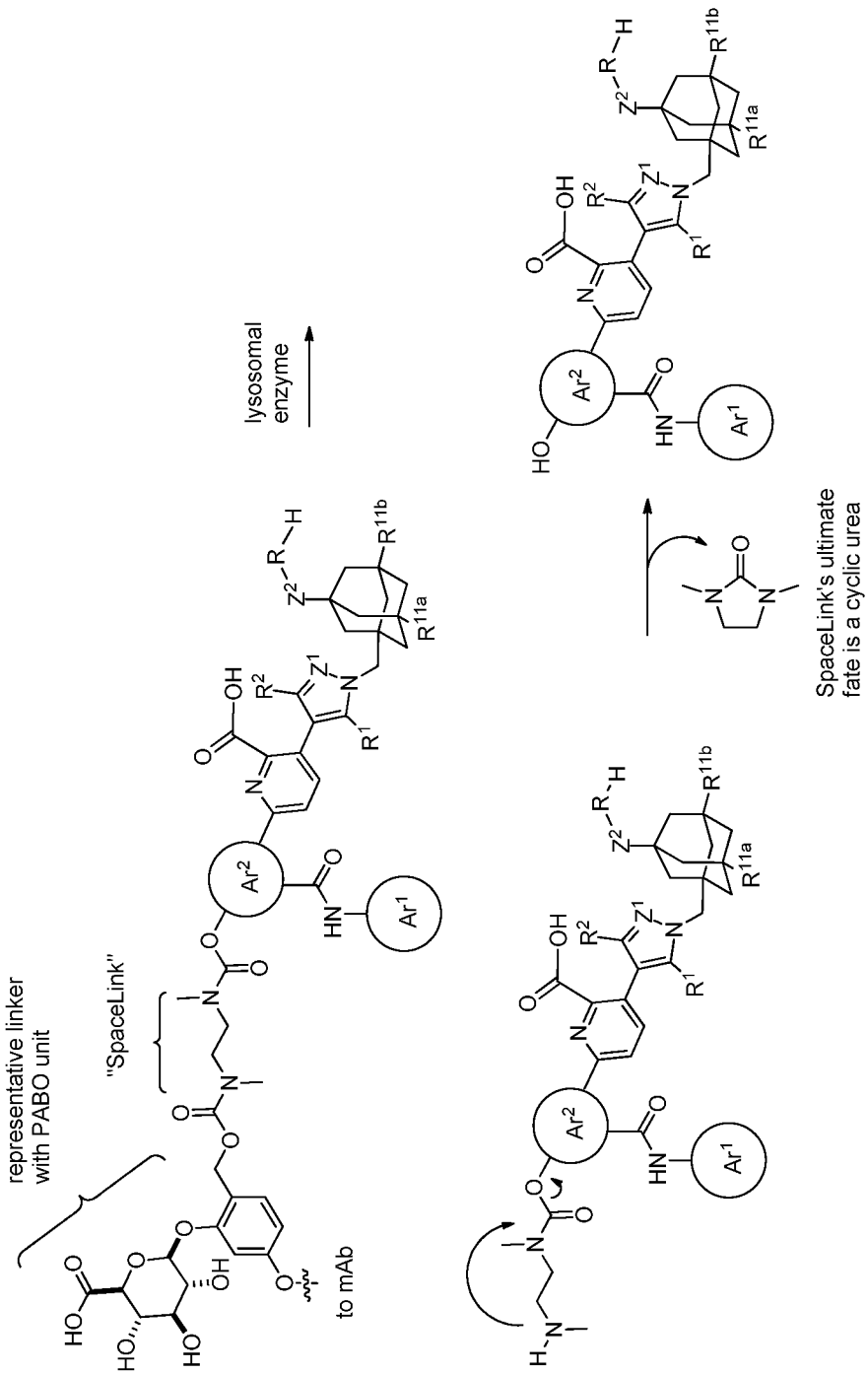
wherein X-D represents the unmodified drug. Heterocyclic variants of this self-immolative group have also been described. *See* U.S. Patent No. 7,989,434.

In certain embodiments, the enzymatically cleavable linker is a ß-glucuronic acid-based linker. Facile release of the drug may be realized through cleavage of the ß-glucuronide glycosidic bond by the lysosomal enzyme ß-glucuronidase. This enzyme is present abundantly within lysosomes and is overexpressed in some tumor types, while the enzyme activity outside cells is low. ß-Glucuronic acid-based linkers may be used to circumvent the tendency of an ADC to undergo aggregation due to the hydrophilic nature of ß-glucuronides. In certain embodiments, ß-glucuronic acid-based linkers are preferred as linkers for ADCs linked to hydrophobic drugs. The following scheme depicts the release of the drug from an ADC containing a ß-glucuronic acid-based linker:


+CO2
Ab
HN
O
1,6-elimination
D
ß-glucuronidase
HO
OH

A variety of cleavable ß-glucuronic acid-based linkers useful for linking drugs such as auristatins, camptothecin and doxorubicin analogues, CBI minor-groove binders, and psymberin to antibodies have been described (*see*, Jeffrey *et al.*, 2006, *Bioconjug. Chem.* 17:831-840; Jeffrey *et al.*, 2007, *Bioorg. Med. Chem. Lett.* 17:2278-2280; and Jiang *et al.*, 2005, *J. Am. Chem. Soc.* 127:11254-11255, the contents of each of which are incorporated herein by reference). All of these ß-glucuronic acid-based linkers may be used in the ADCs described herein. In certain embodiments, the enzymatically cleavable linker is a ß-galactoside-based linker. ß-galactoside is present abundantly within lysosomes, while the enzyme activity outside cells is low.

Additionally, Bcl-xL inhibitors containing a phenol group can be covalently bonded to a linker through the phenolic oxygen. One such linker, described in U.S. Published App. No. 2009/0318668, relies on a methodology in which a diamino-ethane "SpaceLink" is used in conjunction with traditional "PABO"-based self-immolative groups to deliver phenols. The cleavage of the linker is depicted schematically below using a Bcl-xL inhibitor of the disclosure.

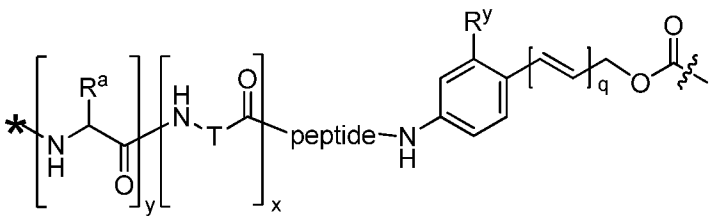

representative linker
with PABO unit
"SpaceLink"
to mAb
lysosomal
enzyme
SpaceLink's ultimate
fate is a cyclic urea

Cleavable linkers may include noncleavable portions or segments, and/or cleavable segments or portions may be included in an otherwise non-cleavable linker to render it cleavable. By way of example only, polyethylene glycol (PEG) and related polymers may include cleavable groups in the polymer backbone. For example, a polyethylene glycol or polymer linker may include one or more cleavable groups such as a disulfide, a hydrazone or a dipeptide.

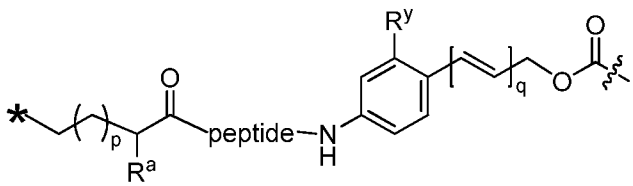
Other degradable linkages that may be included in linkers include ester linkages formed by the reaction of PEG carboxylic acids or activated PEG carboxylic acids with alcohol groups on a biologically active agent, wherein such ester groups generally hydrolyze under physiological conditions to release the biologically active agent. Hydrolytically degradable linkages include, but are not limited to, carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; acetal linkages that are the reaction product of an aldehyde and an alcohol; orthoester linkages that are the reaction product of a formate and an alcohol; and oligonucleotide linkages formed by a phosphoramidite group, including but not limited to, at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVa), (IVb), (IVc) or (IVd):

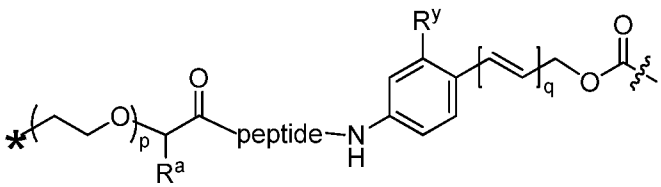
(IVa)

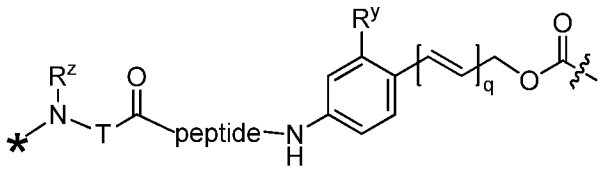


(IVb)



(IVc)



(IVd) 

or a pharmaceutically acceptable salt thereof, wherein:

peptide represents a peptide (illustrated N→C, wherein peptide includes the amino and carboxy "termini") cleavable by a lysosomal enzyme;

T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof;

R^a is selected from hydrogen, C_{1-6} alkyl, SO_3H and CH_2SO_3H;

R^y is hydrogen or C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^1 or C_{1-4} alkyl-(N)-[(C_{1-4} alkylene)-G^1]$_2$;

R^z is C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^2;

G^1 is SO_3H, CO_2H, PEG 4-32, or sugar moiety;

G^2 is SO_3H, CO_2H, or PEG 4-32 moiety;

r is 0 or 1;

s is 0 or 1;

p is an integer ranging from 0 to 5;

q is 0 or 1;

x is 0 or 1;

y is 0 or 1;

 represents the point of attachment of the linker to the Bcl-xL inhibitor; and

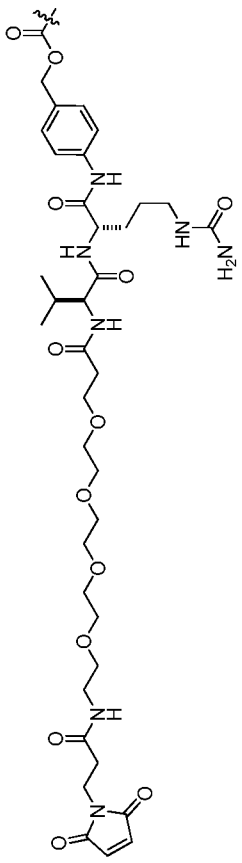
* represents the point of attachment to the remainder of the linker.

In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVa), (IVb), (IVc), or (IVd), or salts thereof.

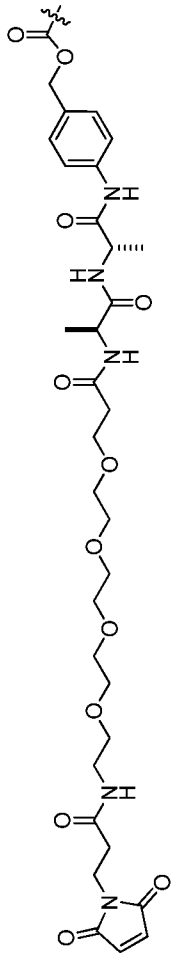
In certain embodiments, linker L comprises a segment according to structural formula IVa or IVb or a pharmaceutically acceptable salt thereof.

In certain embodiments, the peptide is selected from a tripeptide or a dipeptide. In particular embodiments, the dipeptide is selected from: 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; or a pharmaceutically acceptable salt thereof.

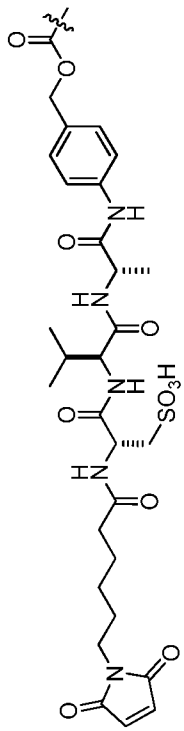
Exemplary embodiments of linkers according to structural formula (IVa) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):



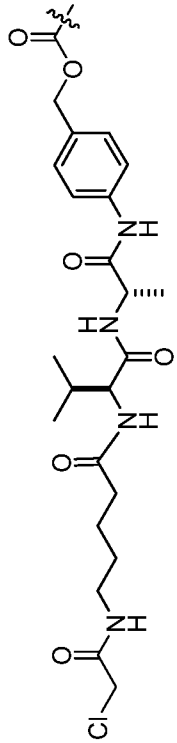
(IVa.1)



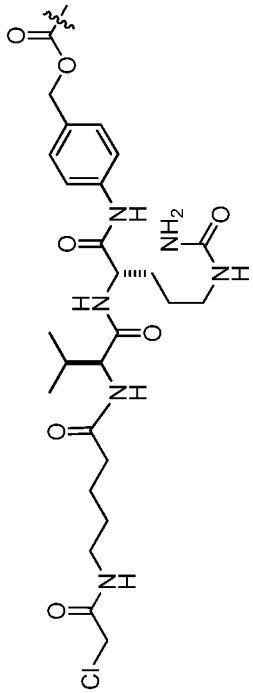
(IVa.2)



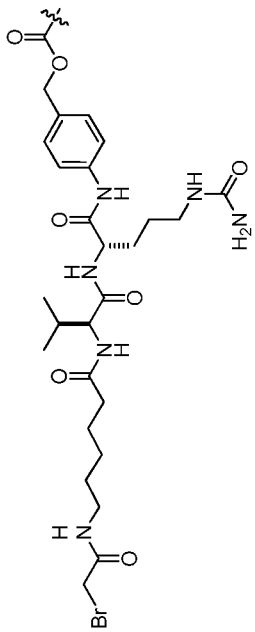
(IVa.3)



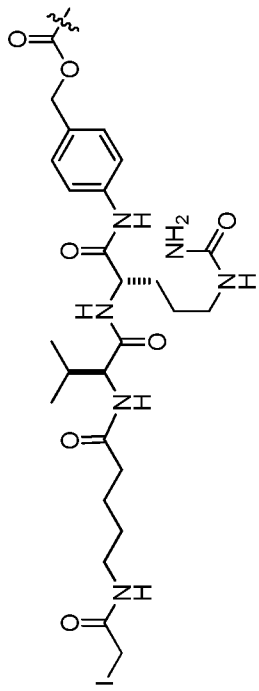
(IVa.4)



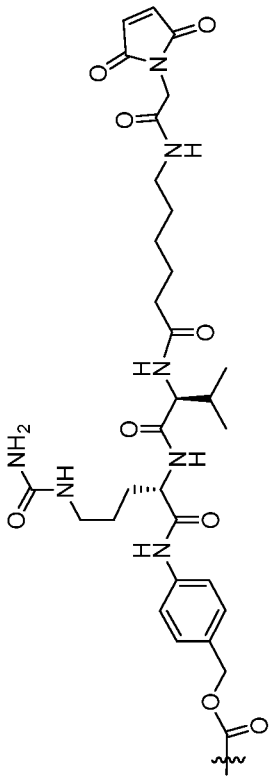
(IVa.5)



(IVa.6)



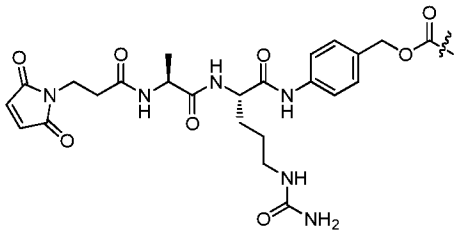
(IVa.7)



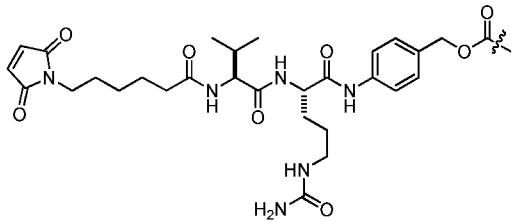
(IVa.8)

Exemplary embodiments of linkers according to structural formula (IVb), (IVc), or (IVd) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

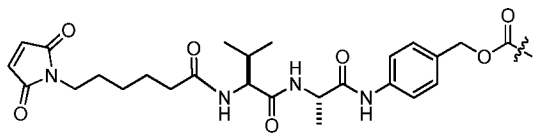
(IVb.1)



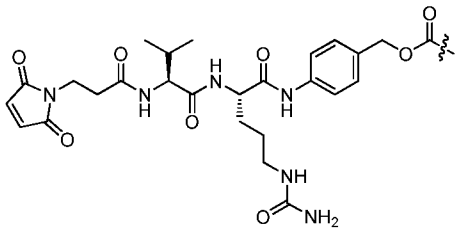
(IVb.2)



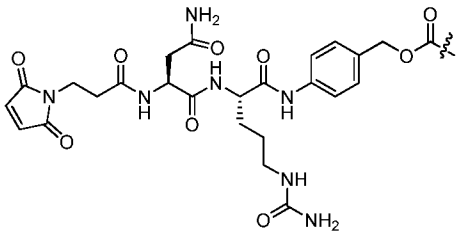
(IVb.3)



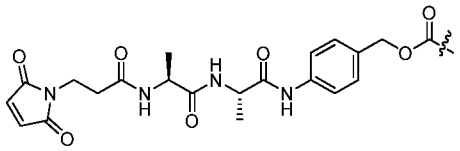
(IVb.4)



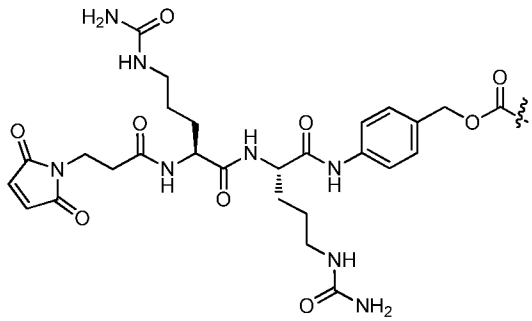
(IVb.5)



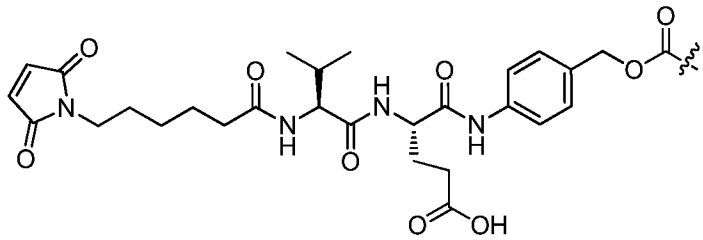
(IVb.6)



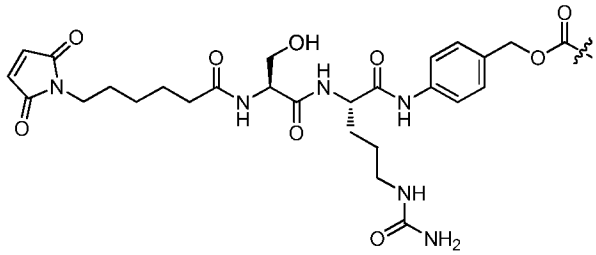
(IVb.7)



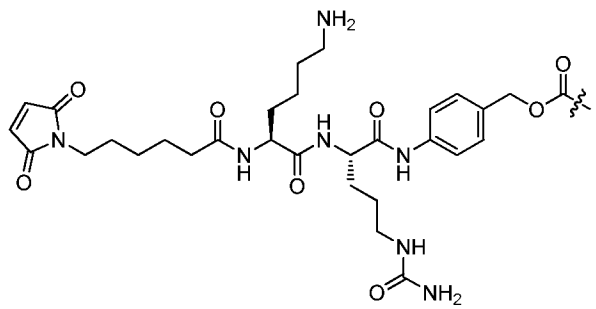
(IVb.8)



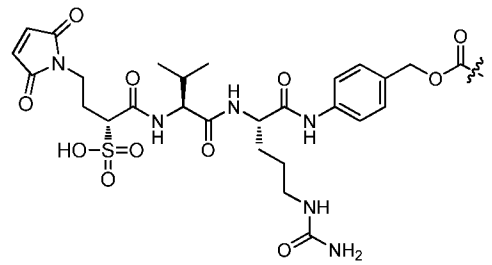
(IVb.9)



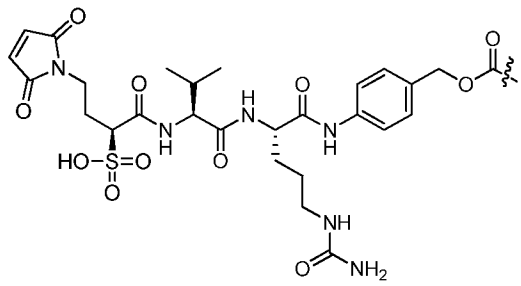
(IVb.10)



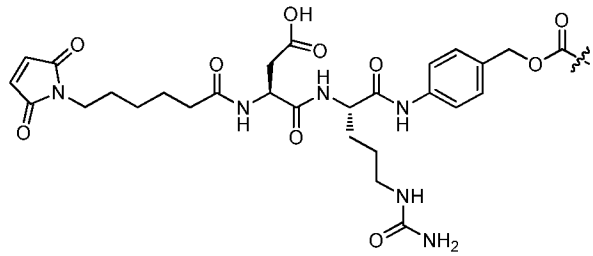
(IVb.11)



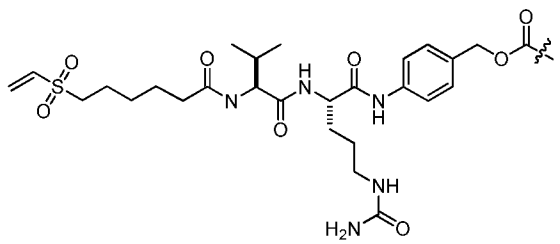
(IVb.12)



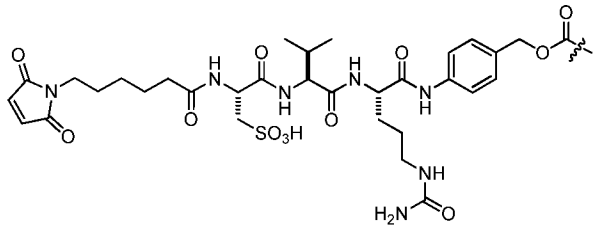
(IVb.13)



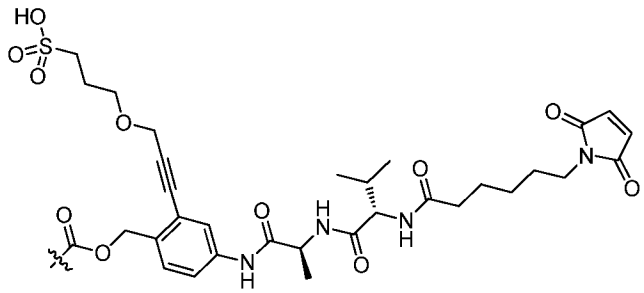
(IVb.14)



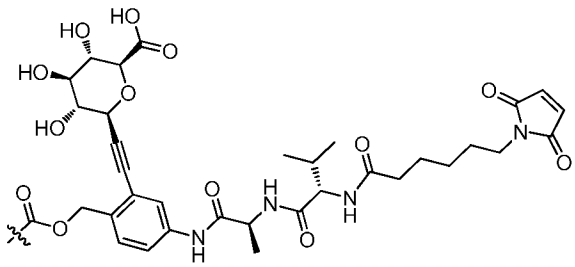
(IVb.15)



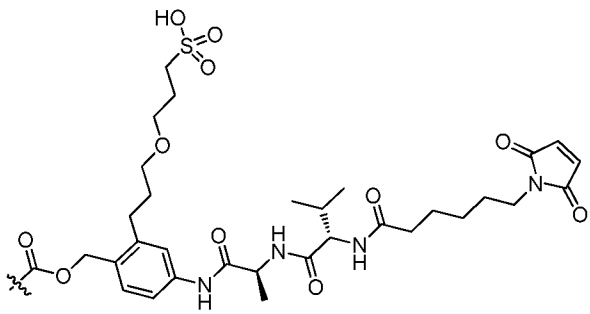
(IVb.16)



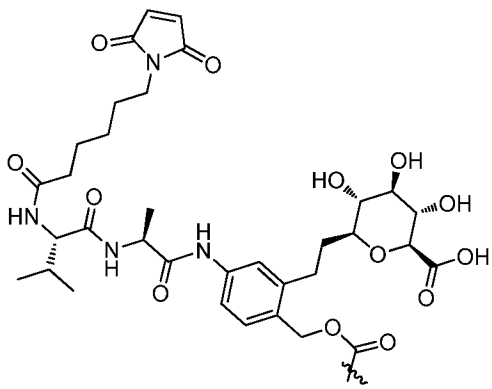
(IVb.17)



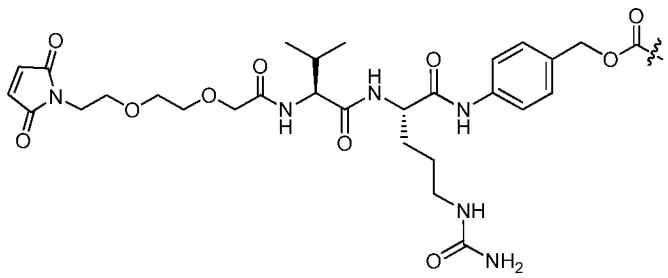
(IVb.18)



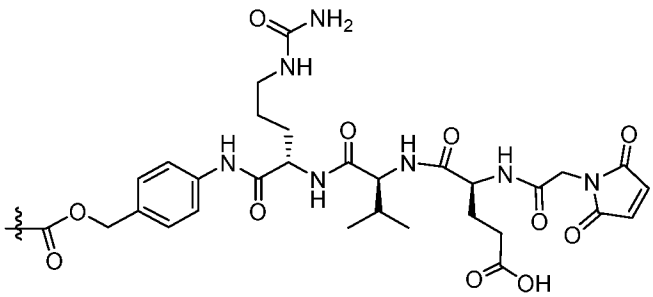
(IVb.19)



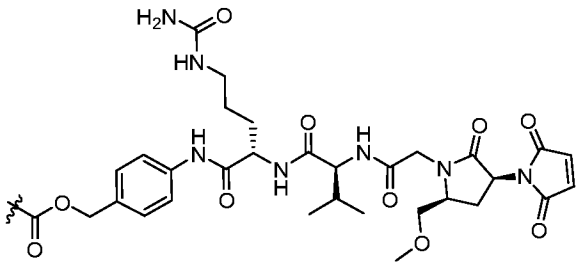
(IVc.1)



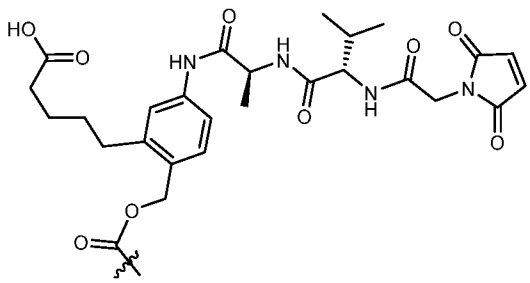
(IVc.2)



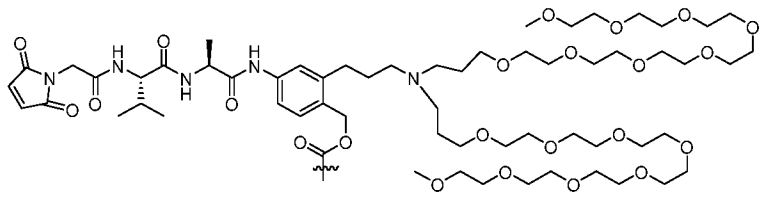
(IVc.3)



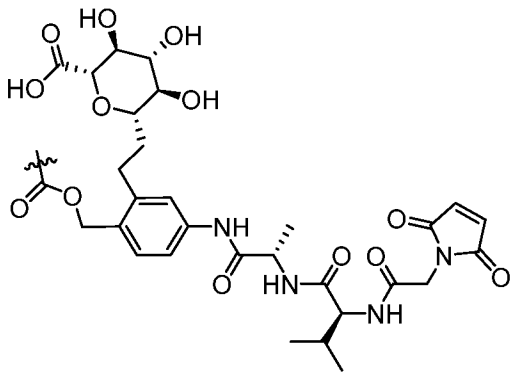
(IVc.4)



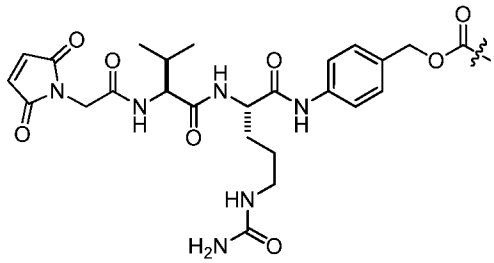
(IVc.5)



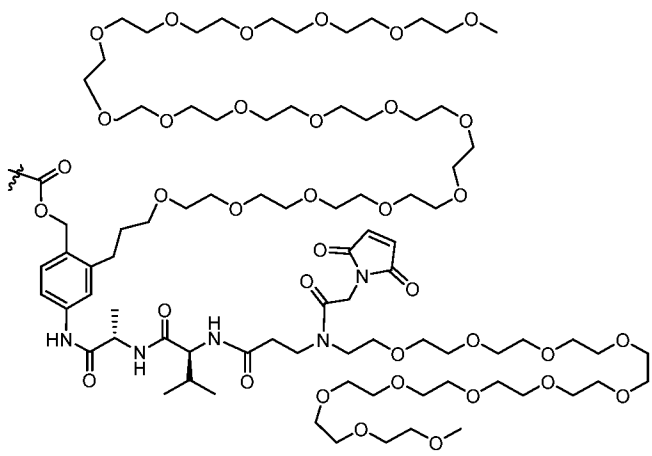
(IVc.6)



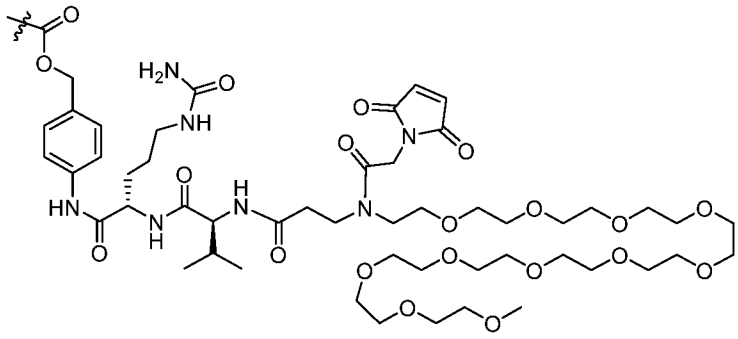
(IVc.7)



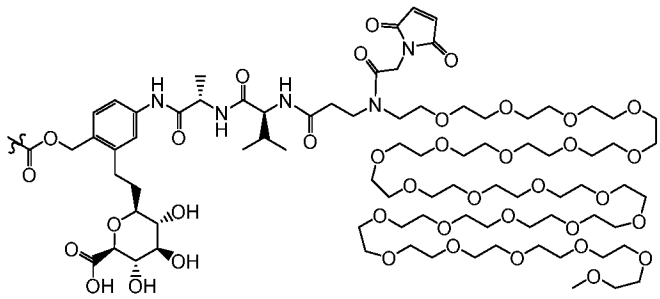
(IVd.1)



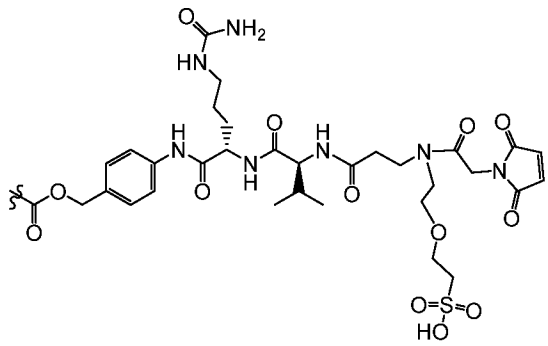
(IVd.2)



(IVd.3)

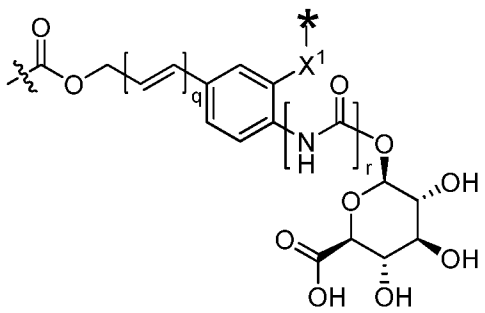


(IVd.4)

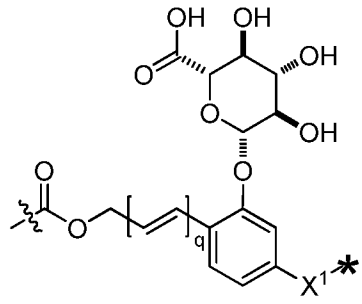


In certain embodiments, the linker comprises an enzymatically cleavable sugar moiety, for example, a linker comprising structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

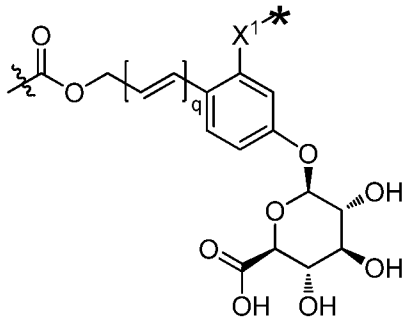
(Va)



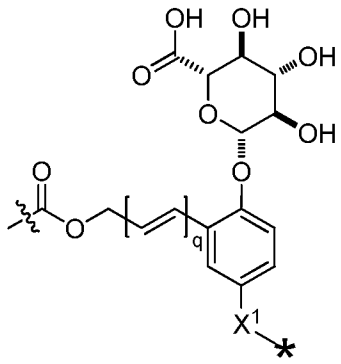
(Vb)



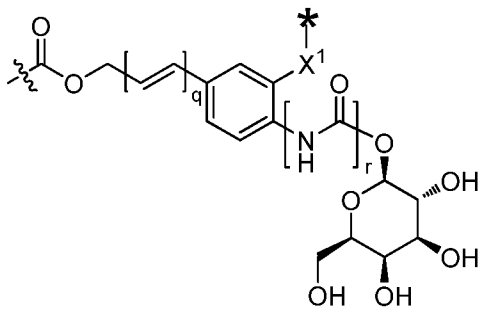
(Vc)



(Vd)



(Ve)



or a pharmaceutically acceptable salt thereof, wherein:

q is 0 or 1;

r is 0 or 1;

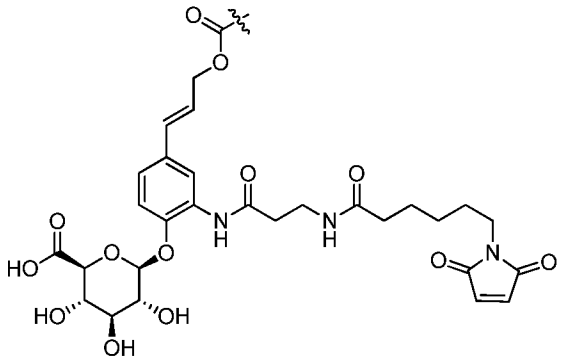
X^1 is CH_2, O or NH;

 represents the point of attachment of the linker to the drug; and

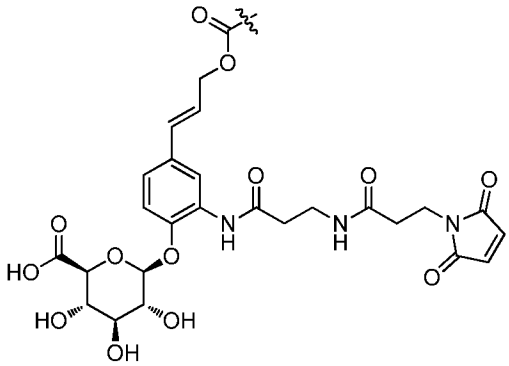
* represents the point of attachment to the remainder of the linker.

Exemplary embodiments of linkers according to structural formula (Va) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

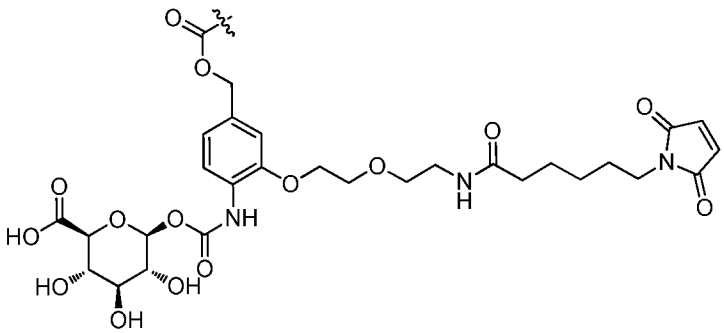
(Va.1)



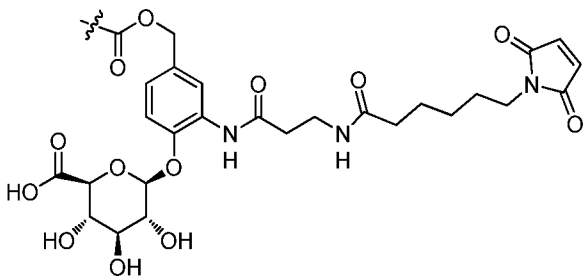
(Va.2)

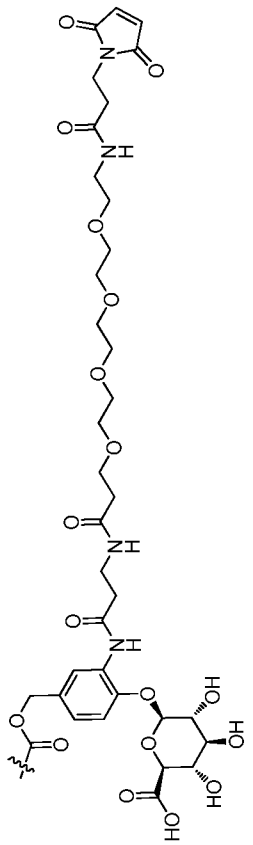


(Va.3)

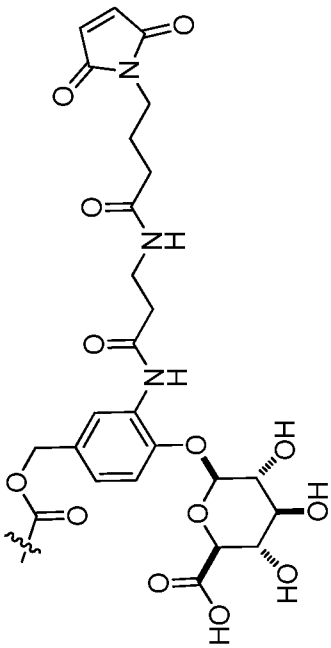


(Va.4)

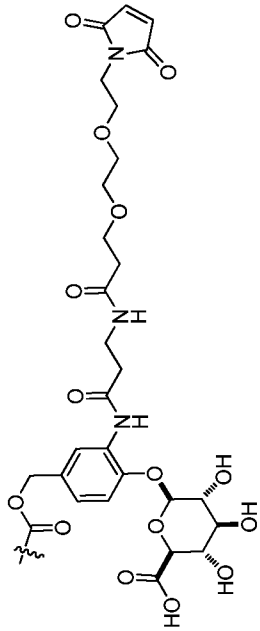




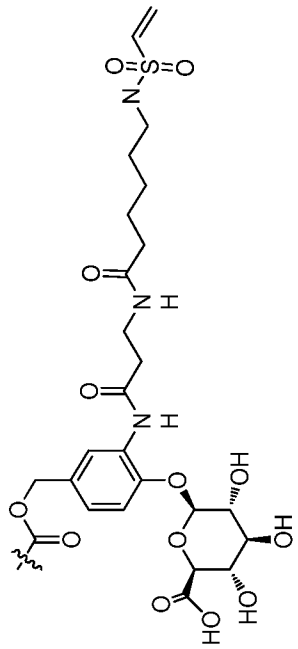
(Va.5)



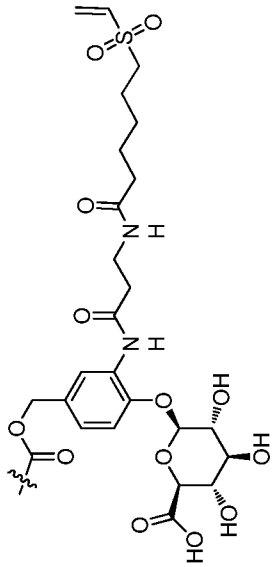
(Va.6)



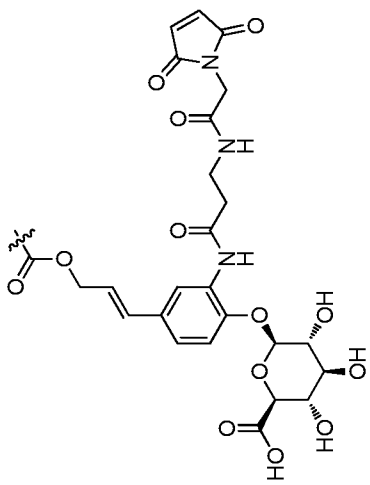
(Va.7)



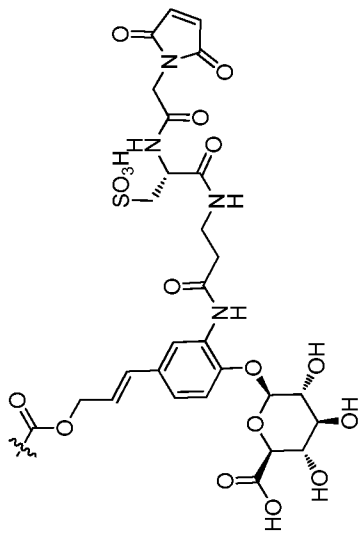
(Va.8)



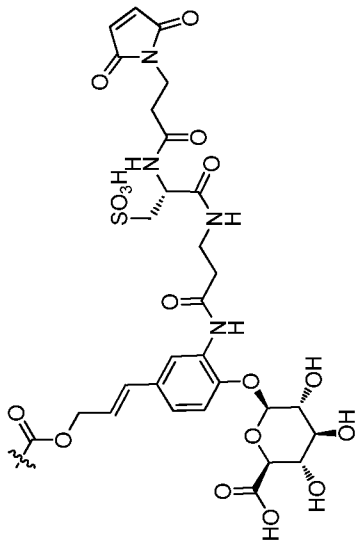
(Va.9)



(Va.10)



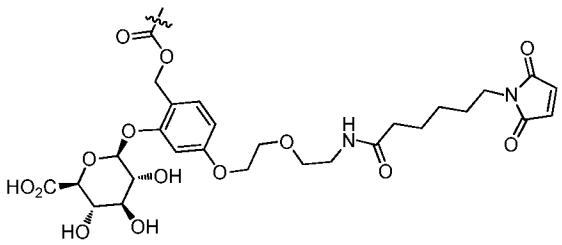
(Va.11)



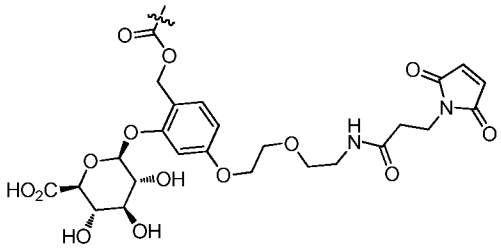
(Va.12)

Exemplary embodiments of linkers according to structural formula (Vb) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

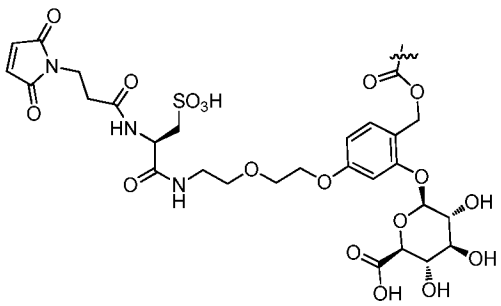
(Vb.1)



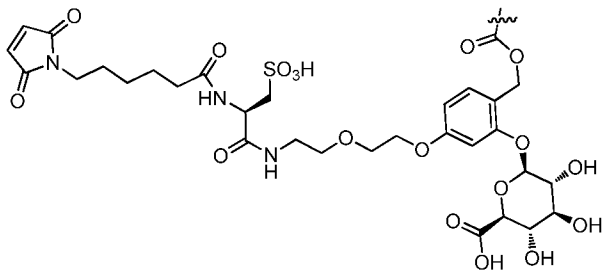
(Vb.2)



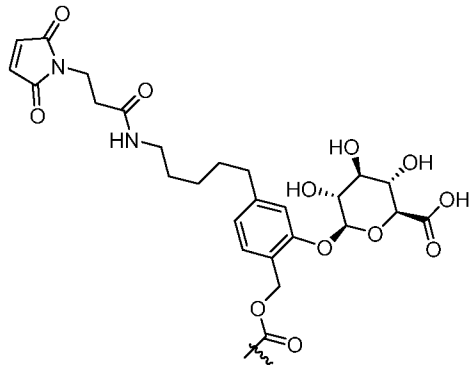
(Vb.3)



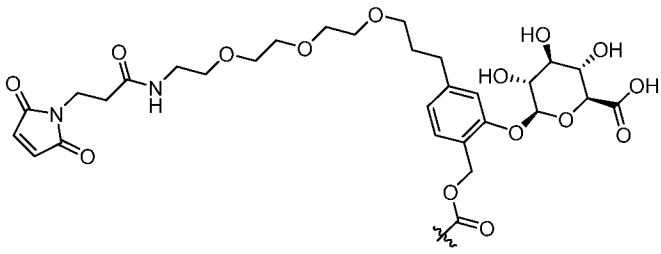
(Vb.4)



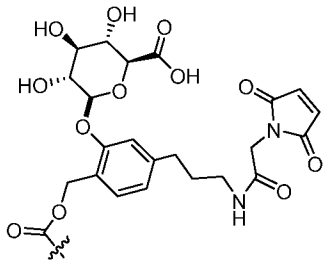
(Vb.5)



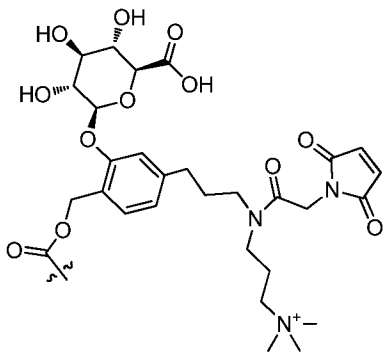
(Vb.6)



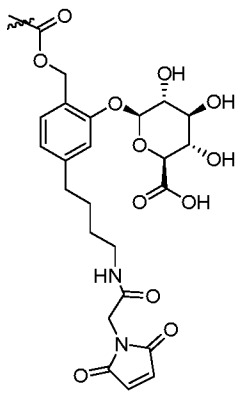
(Vb.7)



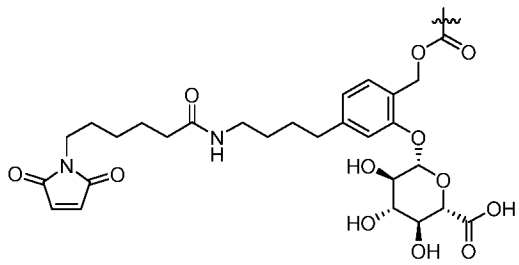
(Vb.8)



(Vb.9)

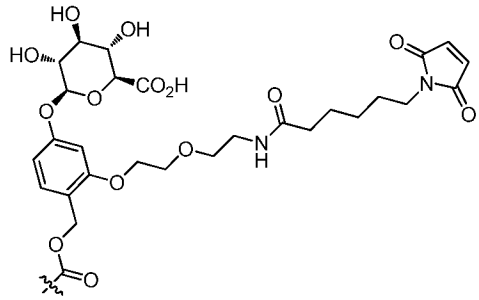


(Vb.10)

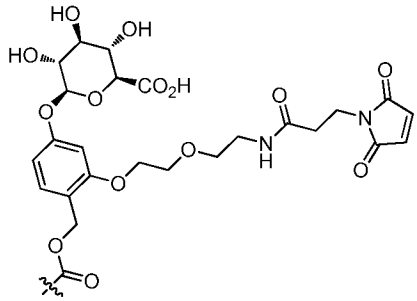


Exemplary embodiments of linkers according to structural formula (Vc) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

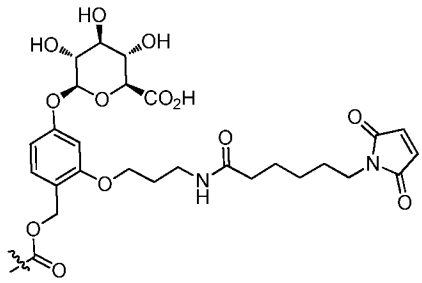
(Vc.1)



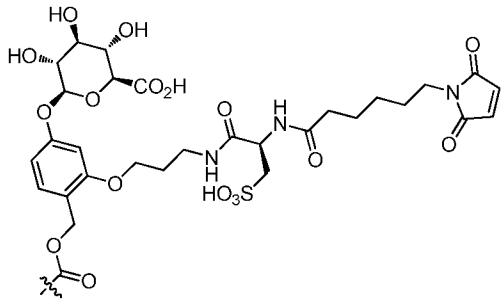
(Vc.2)



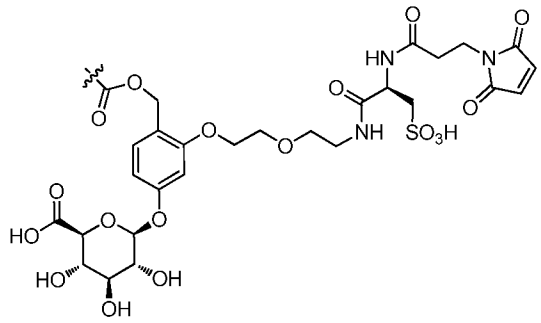
(Vc.3)



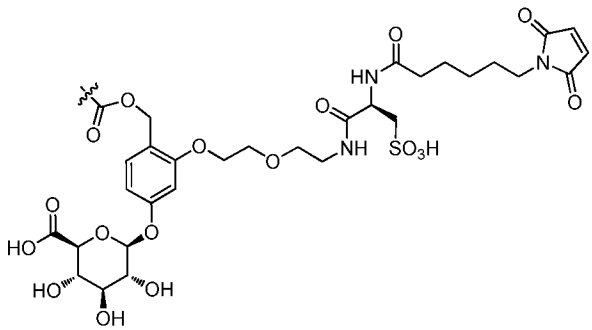
(Vc.4)



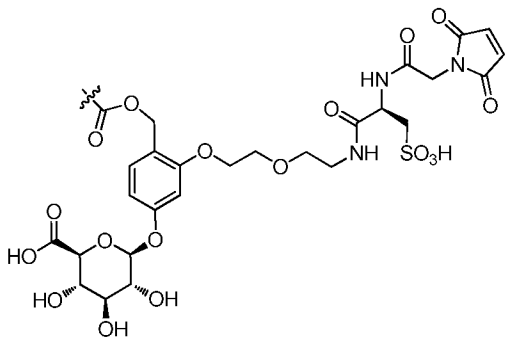
(Vc.5)



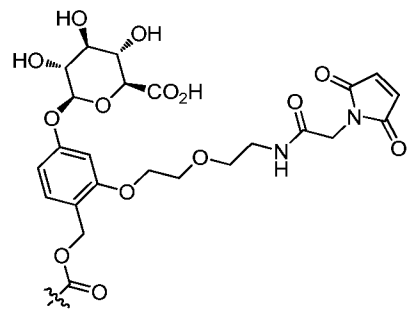
(Vc.6)



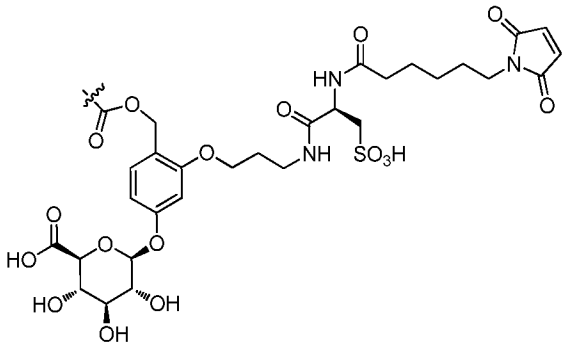
(Vc.7)



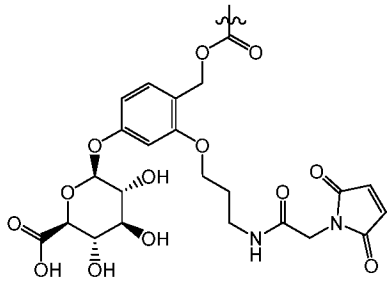
(Vc.8)



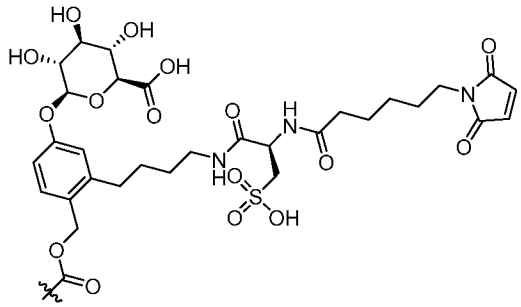
(Vc.9)



(Vc.10)

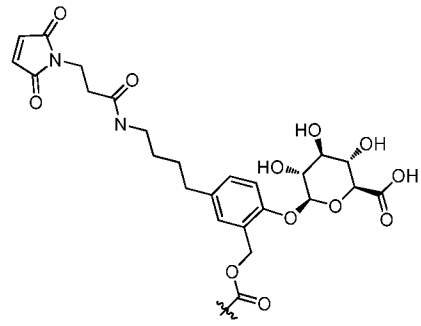


(Vc.11)

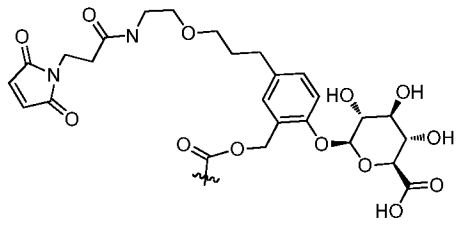


Exemplary embodiments of linkers according to structural formula (Vd) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

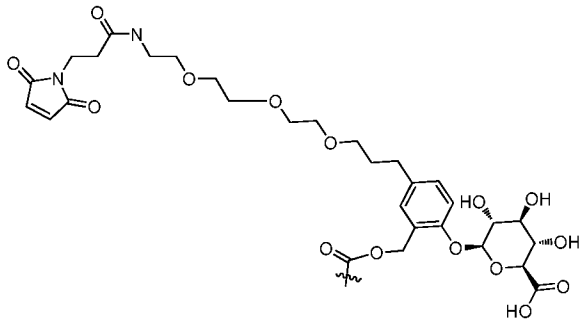
(Vd.1)



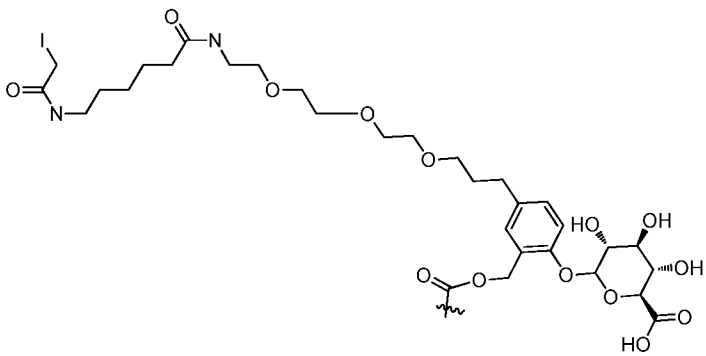
(Vd.2)



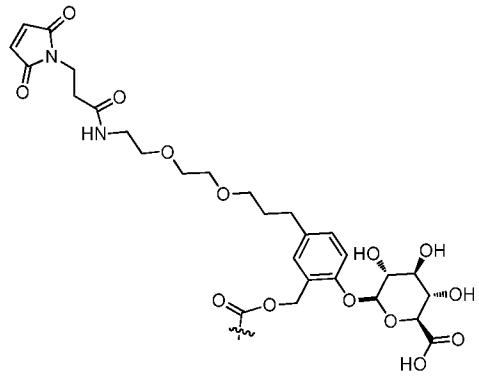
(Vd.3)



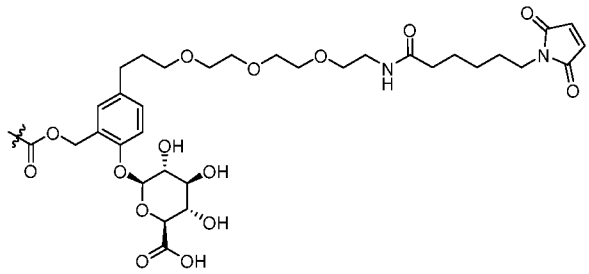
(Vd.4)



(Vd.5)

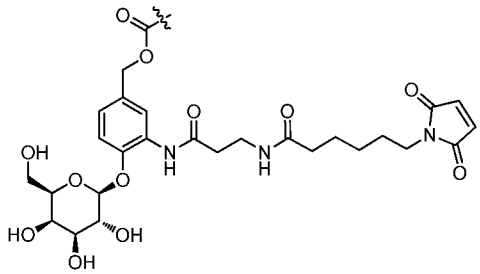


(Vd.6)

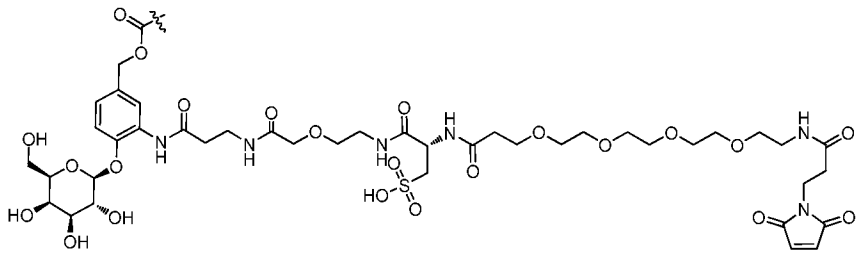


Exemplary embodiments of linkers according to structural formula (Ve) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

(Ve.1)



(Ve.2)



3.2.2 Non-Cleavable Linkers

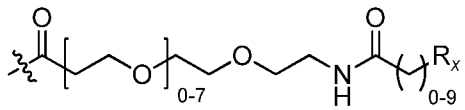
Although cleavable linkers may provide certain advantages, the linkers comprising the ADC described herein need not be cleavable. For noncleavable linkers, the drug release does not

depend on the differential properties between the plasma and some cytoplasmic compartments. The release of the drug is postulated to occur after internalization of the ADC via antigen-mediated endocytosis and delivery to lysosomal compartment, where the antibody is degraded to the level of amino acids through intracellular proteolytic degradation. This process releases a drug derivative, which is formed by the drug, the linker, and the amino acid residue to which the linker was covalently attached. The amino-acid drug metabolites from conjugates with noncleavable linkers are more hydrophilic and generally less membrane permeable, which leads to less bystander effects and less nonspecific toxicities compared to conjugates with a cleavable linker. In general, ADCs with noncleavable linkers have greater stability in circulation than ADCs with cleavable linkers. Non-cleavable linkers may be alkylene chains, or maybe polymeric in natures, such as, for example, based upon polyalkylene glycol polymers, amide polymers, or may include segments of alkylene chains, polyalkylene glycols and/or amide polymers. In certain embodiments, the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

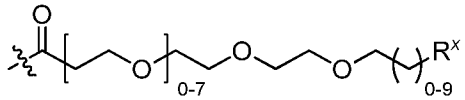
A variety of non-cleavable linkers used to link drugs to antibodies have been described. (*See*, Jeffrey *et al.*, 2006, *Bioconjug. Chem.* 17;831-840; Jeffrey *et al.*, 2007, *Bioorg. Med. Chem. Lett.* 17:2278-2280; and Jiang *et al.*, 2005, *J. Am . Chem. Soc.* 127:11254-11255, the contents of which are incorporated herein by reference). All of these linkers may be included in the ADCs described herein.

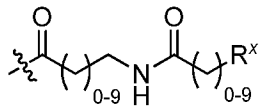
In certain embodiments, the linker is non-cleavable *in vivo*, for example a linker according to structural formula (VIa), (VIb), (VIc) or (VId) (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody:

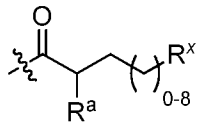
(VIa)



(VIb)



(VIc) 

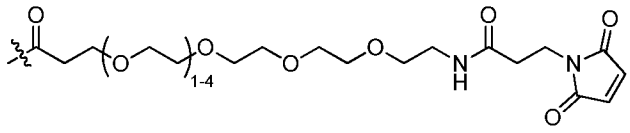
(VId) 

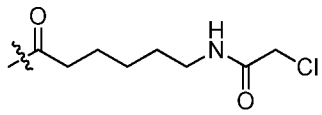
or a pharmaceutically acceptable salt thereof, wherein:

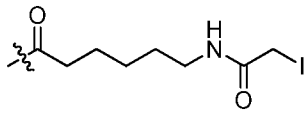
R^a is selected from hydrogen, alkyl, sulfonate and methyl sulfonate;

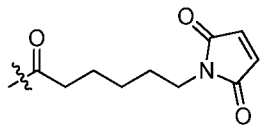
R^x is a moiety including a functional group capable of covalently linking the linker to an antibody; and

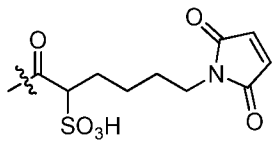
~~~ represents the point of attachment of the linker to the Bcl-xL inhibitor.

Exemplary embodiments of linkers according to structural formula (VIa)-(VId) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody, and "~~~" represents the point of attachment to a Bcl-xL inhibitor):
~~~

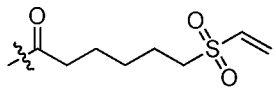
(VIa.1) 

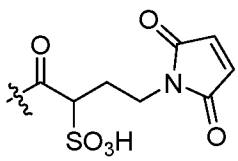
(VIc.1) 

(VIc.2) 

(VId.1) 

(VId.2) 

(VId.3) 

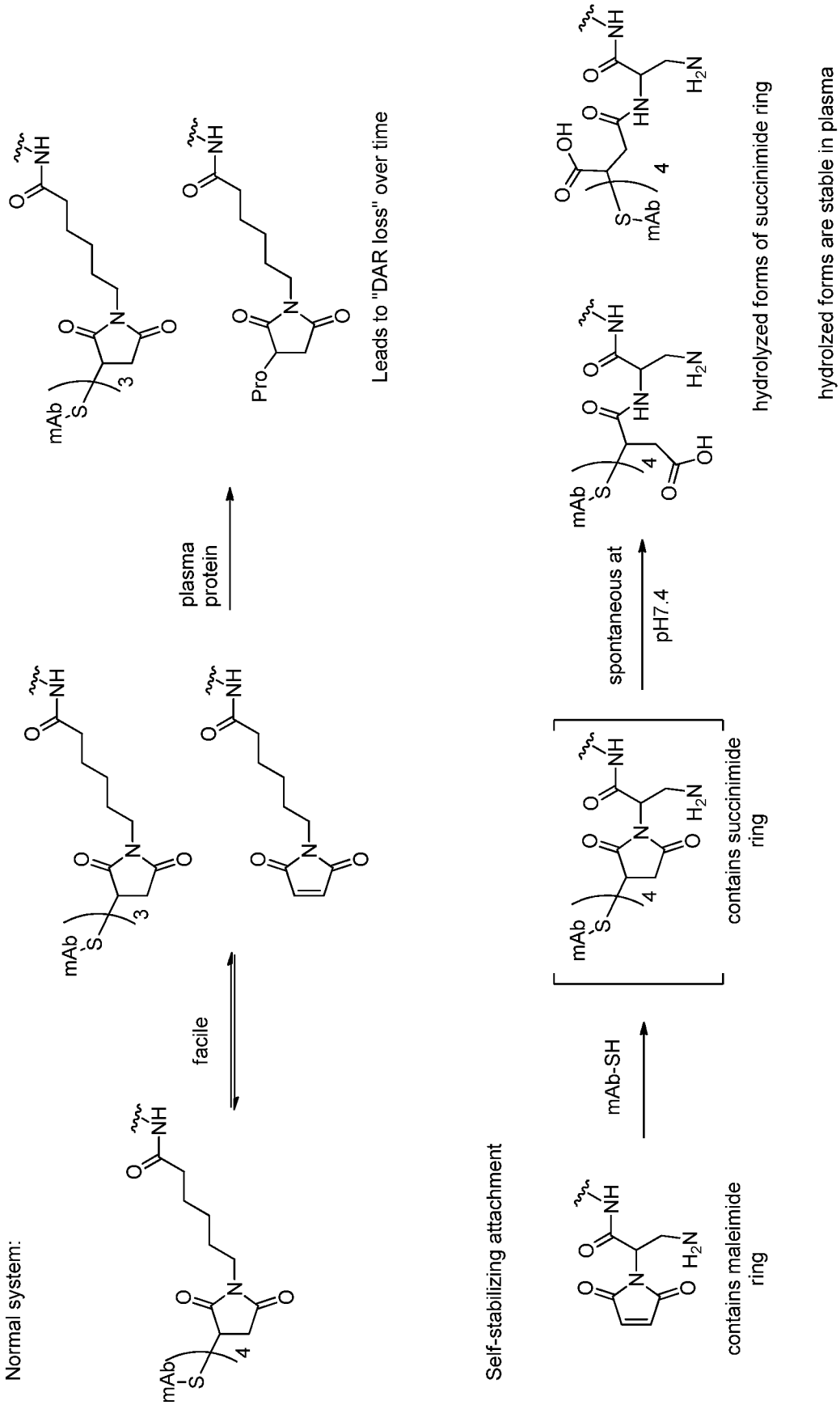
(VId.4) 

3.2.3 Groups Used to Attach Linkers to Anti-EGFR Antibodies

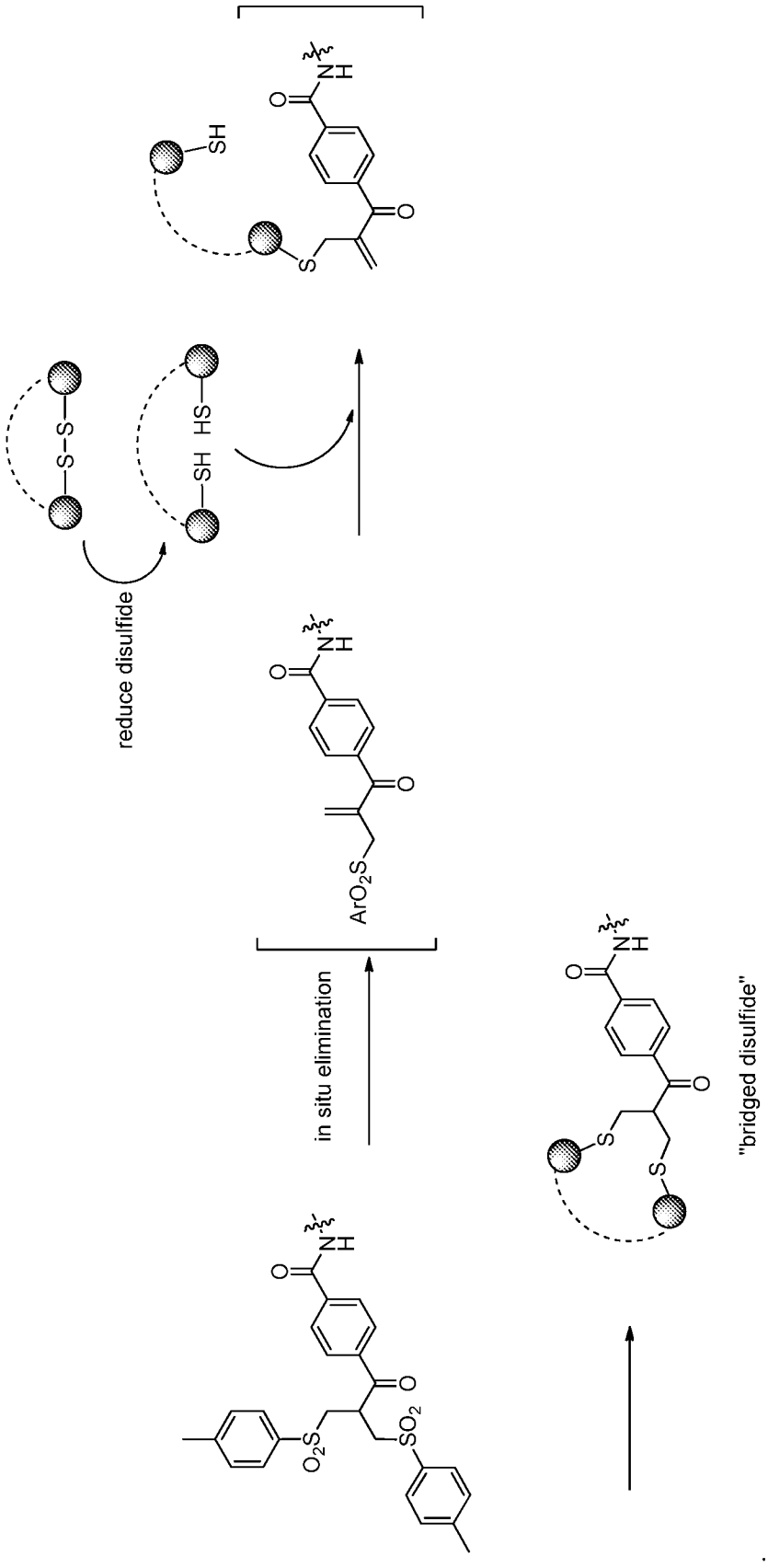
Attachment groups can be electrophilic in nature and include: maleimide groups, activated disulfides, active esters such as NHS esters and HOBt esters, haloformates, acid halides, alkyl and benzyl halides such as haloacetamides. As discussed below, there are also

emerging technologies related to "self-stabilizing" maleimides and "bridging disulfides" that can be used in accordance with the disclosure.

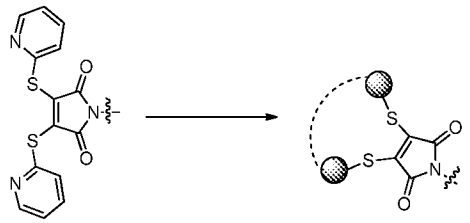
Loss of the drug-linker from the ADC has been observed as a result of a maleimide exchange process with albumin, cysteine or glutathione (Alley *et al.*, 2008, *Bioconjugate Chem.* 19: 759-769). This is particularly prevalent from highly solvent-accessible sites of conjugation while sites that are partially accessible and have a positively charged environment promote maleimide ring hydrolysis (Junutula *et al.*, 2008, *Nat. Biotechnol.* 26: 925-932). A recognized solution is to hydrolyze the succinimide formed from conjugation as this is resistant to deconjugation from the antibody, thereby making the ADC stable in serum. It has been reported previously that the succinimide ring will undergo hydrolysis under alkaline conditions (Kalia *et al.*, 2007, *Bioorg. Med. Chem. Lett. 17*: 6286-6289). One example of a "self-stabilizing" maleimide group that hydrolyzes spontaneously under antibody conjugation conditions to give an ADC species with improved stability is depicted in the schematic below. *See* U.S. Published Application No. 2013/0309256, International Application Publication No. WO 2013/173337, Tumey *et al.*, 2014, Bioconjugate Chem. 25: 1871-1880, and Lyon *et al.*, 2014, *Nat. Biotechnol.* 32: 1059-1062. Thus, the maleimide attachment group is reacted with a sulfhydryl of an antibody to give an intermediate succinimide ring. The hydrolyzed form of the attachment group is resistant to deconjugation in the presence of plasma proteins.


Normal system:
facile
plasma protein
Leads to "DAR loss" over time
Pro
Self-stabilizing attachment
mAb-SH
contains maleimide ring
contains succinimide ring
spontaneous at pH7.4
hydrolyzed forms of succinimide ring
hydrolzed forms are stable in plasma

As shown above, the maleimide ring of a linker may react with an antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form). Polytherics has disclosed a method for bridging a pair of sulfhydryl groups derived from reduction of a native hinge disulfide bond. *See*, Badescu *et al.*, 2014, *Bioconjugate Chem.* 25:1124-1136. The reaction is depicted in the schematic below. An advantage of this methodology is the ability to synthesize homogenous DAR4 ADCs by full reduction of IgGs (to give 4 pairs of sulfhydryls) followed by reaction with 4 equivalents of the alkylating agent. ADCs containing "bridged disulfides" are also claimed to have increased stability.

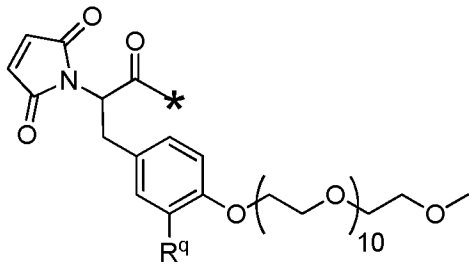

reduce disulfide
in situ elimination
"bridged disulfide"

Similarly, as depicted below, a maleimide derivative that is capable of bridging a pair of sulfhydryl groups has been developed. *See* U.S. Published Application No. 2013/0224228.

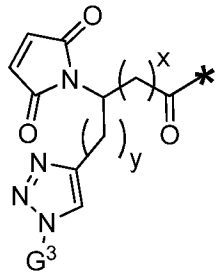


In certain embodiments the attachment moiety comprises the structural formulae (VIIa), (VIIb), or (VIIc):

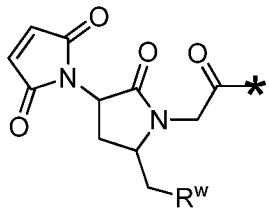
(VIIa)



(VIIb)



(VIIc)



or salts thereof, wherein:

R^q is H or $-O-(CH_2CH_2O)_{11}-CH_3$;

x is 0 or 1;

y is 0 or 1;

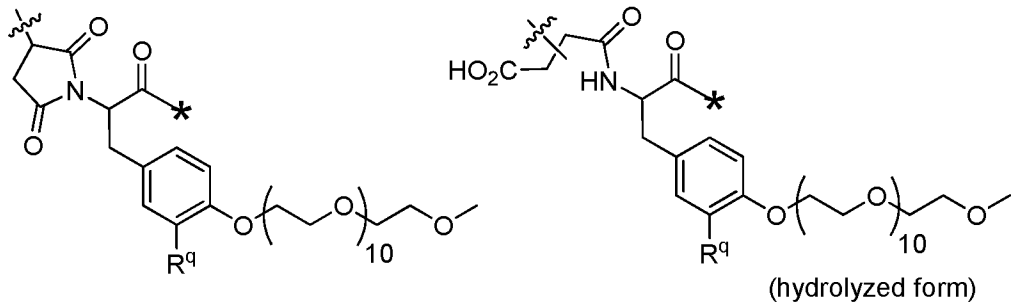
G^3 is $-CH_2CH_2CH_2SO_3H$ or $-CH_2CH_2O-(CH_2CH_2O)_{11}-CH_3$;

R^w is $-O-CH_2CH_2SO_3H$ or $-NH(CO)-CH_2CH_2O-(CH_2CH_2O)_{12}-CH_3$; and

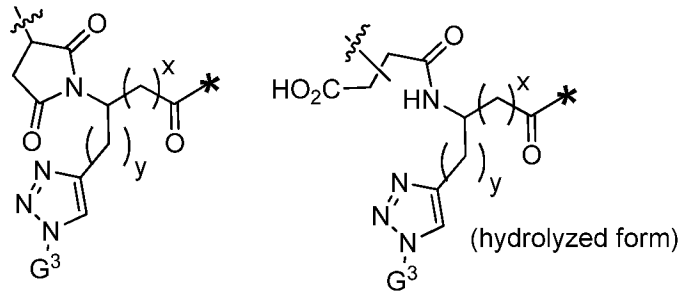
* represents the point of attachment to the remainder of the linker.

In certain embodiments, the linker comprises a segment according to structural formulae (VIIIa), (VIIIb), or (VIIIc):

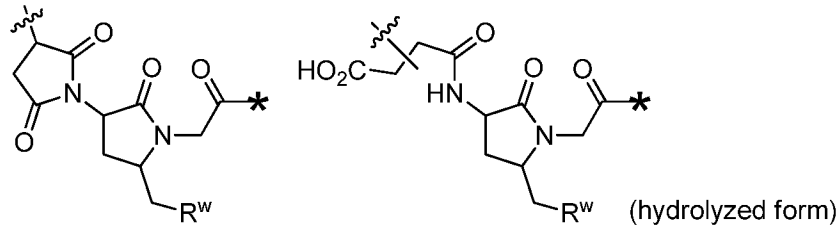
(VIIIa)



(VIIIb)



(VIIIc)



or a hydrolyzed derivative or a pharmaceutically acceptable salt thereof, wherein:

R^q is H or $-O-(CH_2CH_2O)_{11}-CH_3$;

x is 0 or 1;

y is 0 or 1;

G^3 is $-CH_2CH_2CH_2SO_3H$ or $-CH_2CH_2O-(CH_2CH_2O)_{11}-CH_3$;

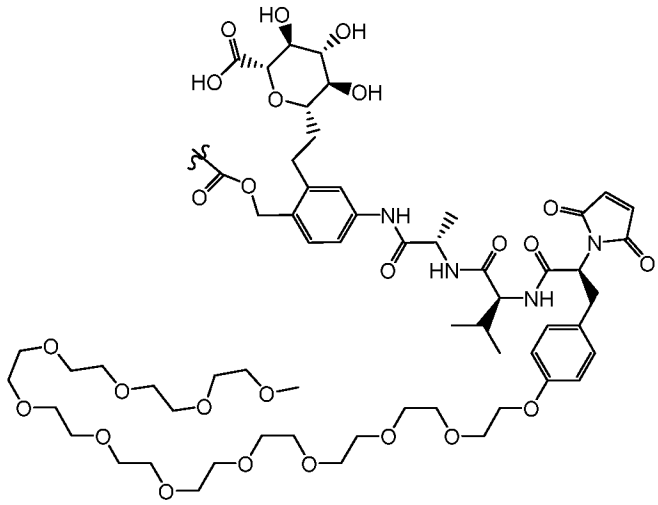
R^w is $-O-CH_2CH_2SO_3H$ or $-NH(CO)-CH_2CH_2O-(CH_2CH_2O)_{12}-CH_3$;

* represents the point of attachment to the remainder of the linker; and

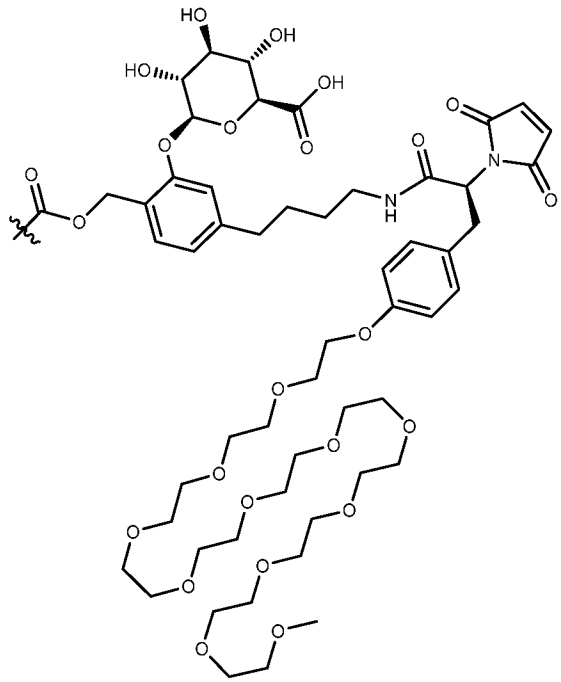
 represents the point of attachment of the linker to the antibody.

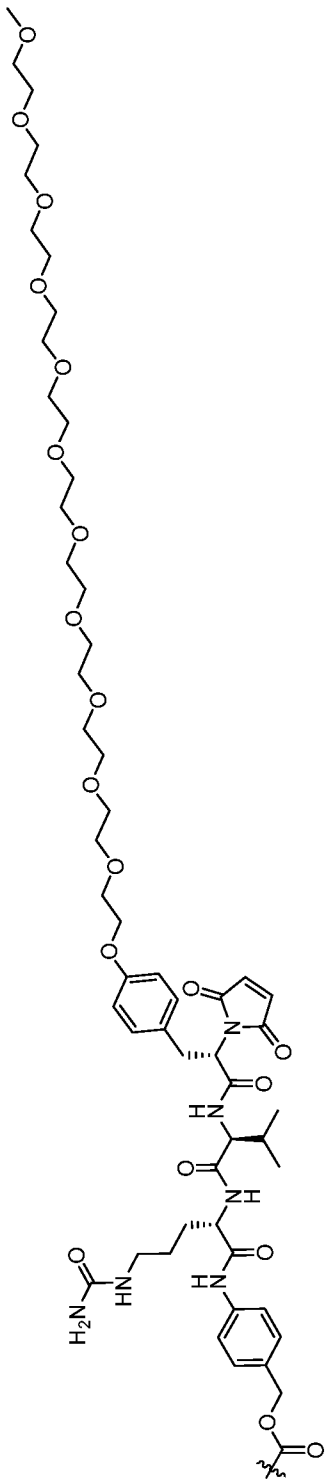
Exemplary embodiments of linkers according to structural formula (VIIa) and (VIIb) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

(VIIa.1)

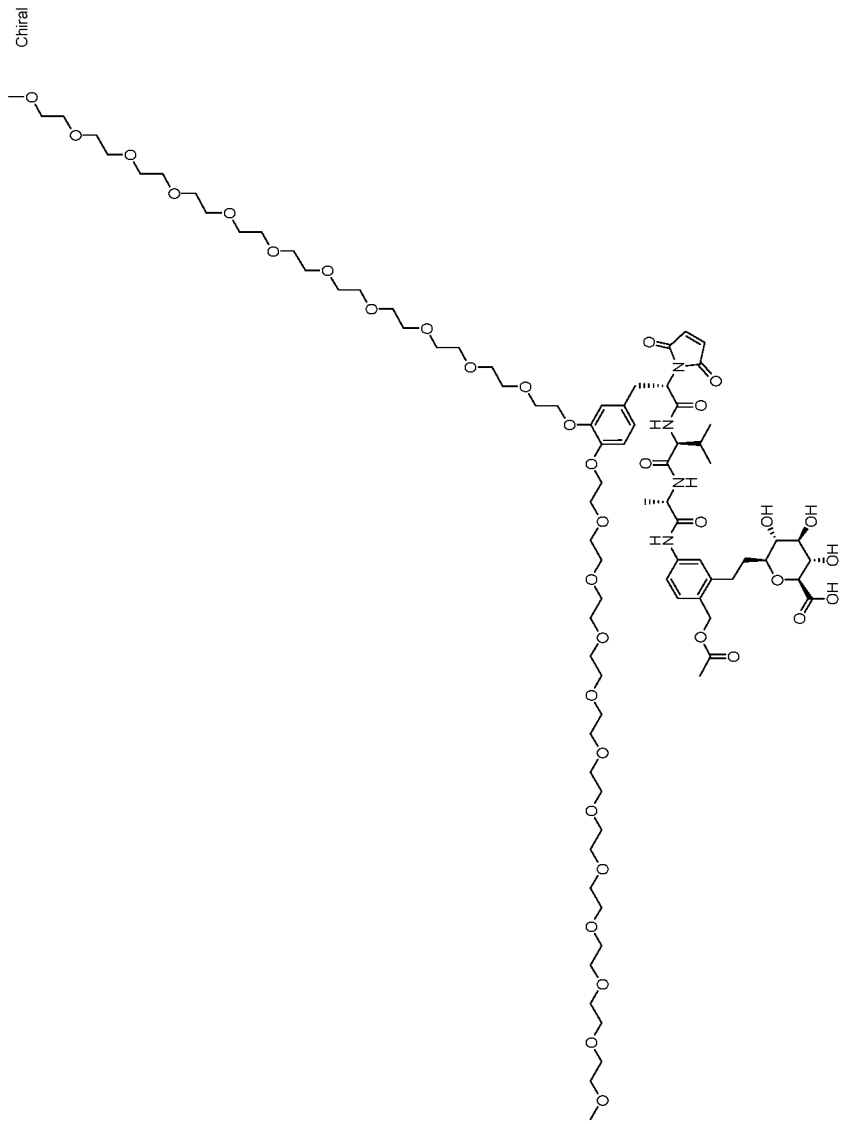


(VIIa.2)

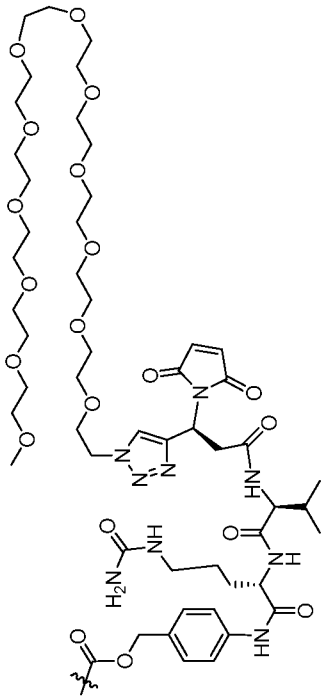




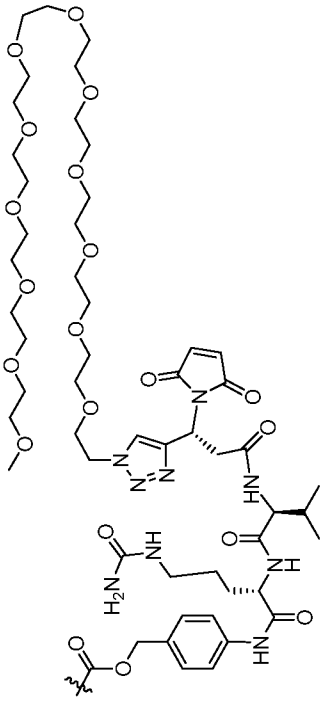
(VIIa.3)



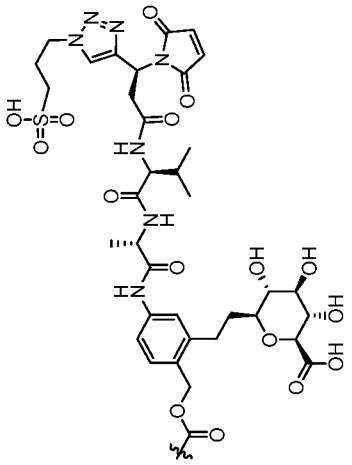
(VIIa.4)



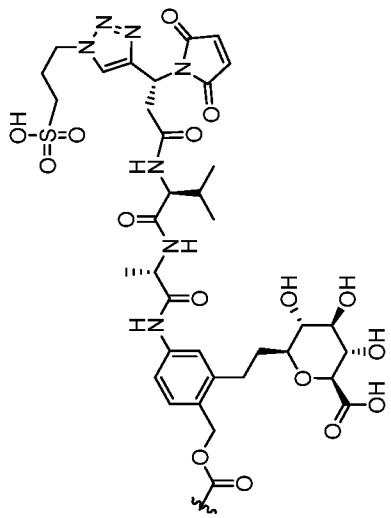
(VIIb.1)



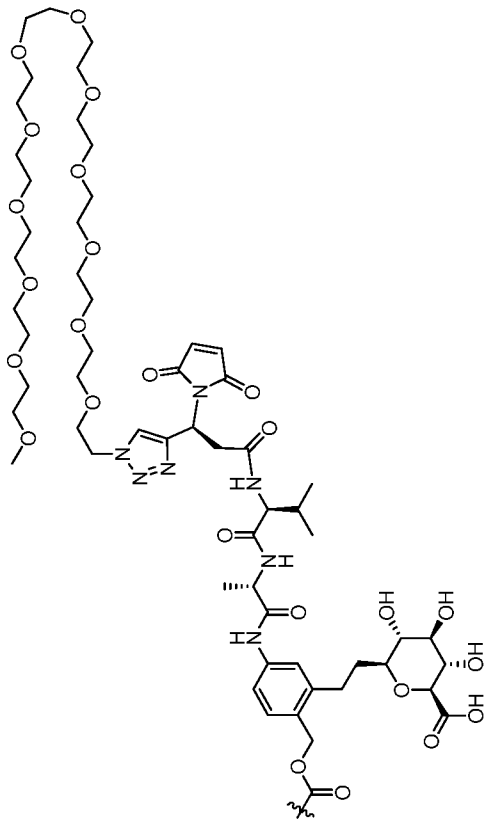
(VIIb.2)



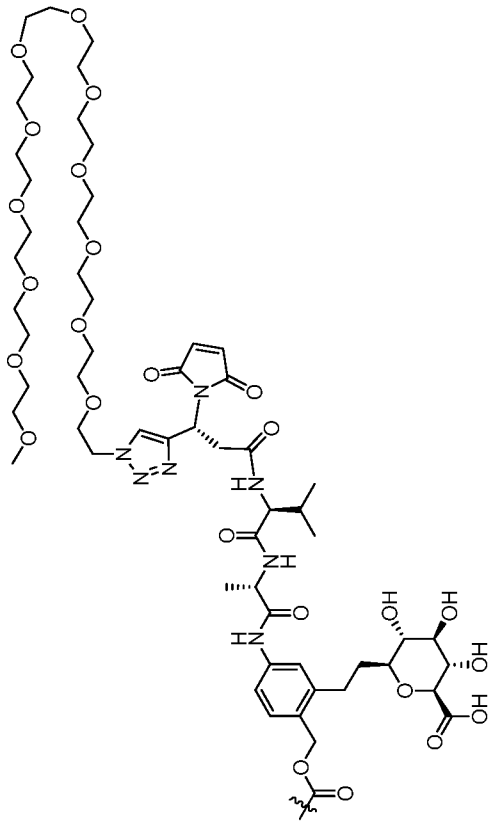
(VIIb.3)



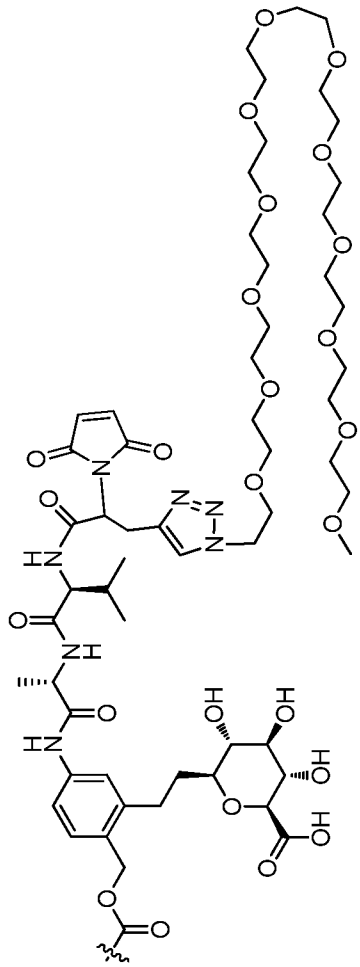
(VIIb.4)



(VIIb.6)



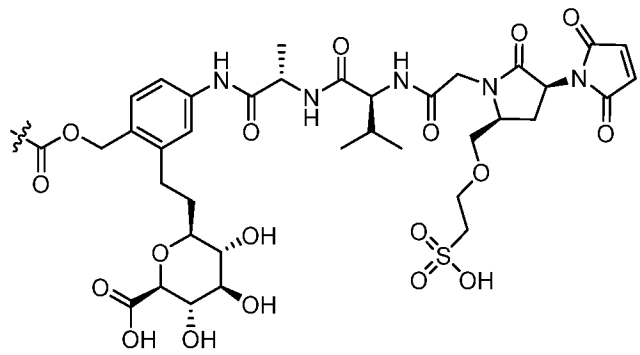
(VIIb.7)



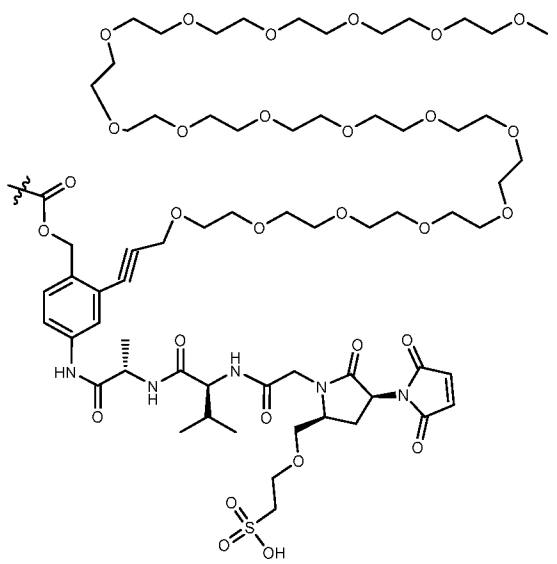
(VIIb.8)

Exemplary embodiments of linkers according to structural formula (VIIc) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

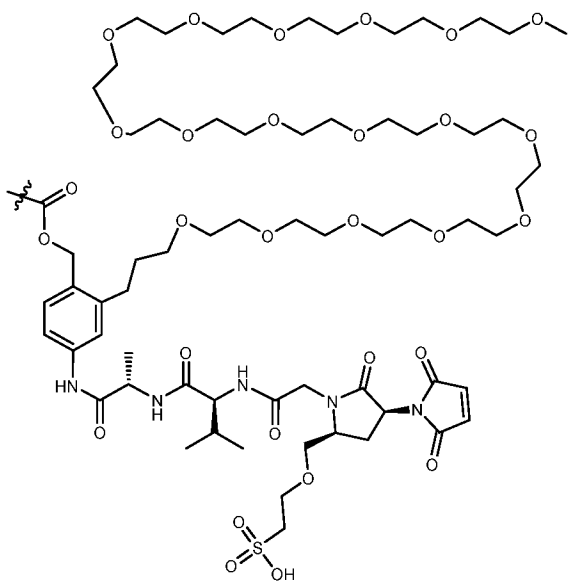
(VIIc.1)



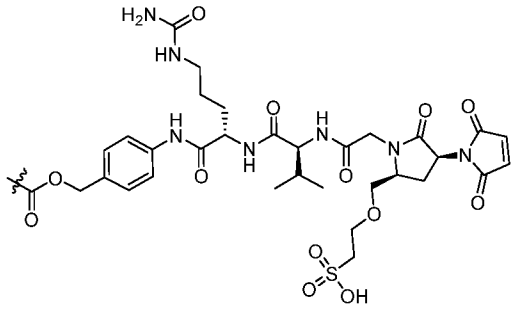
(VIIc.2)



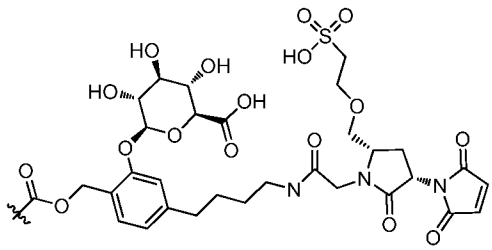
(VIIc.3)



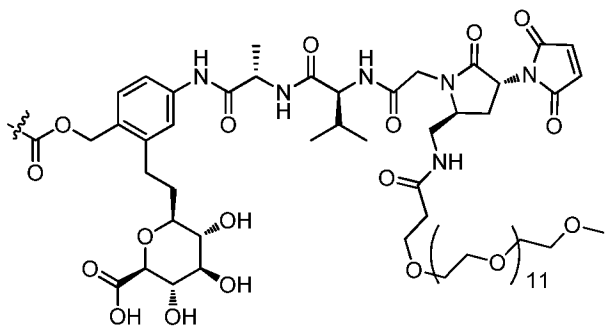
(VIIc.4)



(VIIc.5)



(VIIc.6)

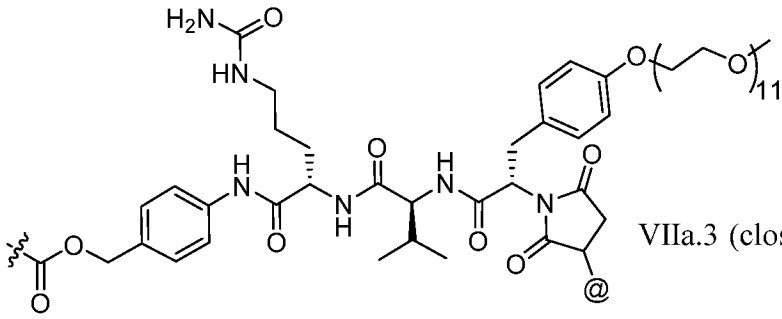


In certain embodiments, L is selected from the group consisting of IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-V1c.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 in the closed or open form, and a pharmaceutically acceptable salt thereof. In certain embodiments, L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

In certain embodiments, L is selected from the group consisting of IVc.5, IVc.6, IVd.4, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has

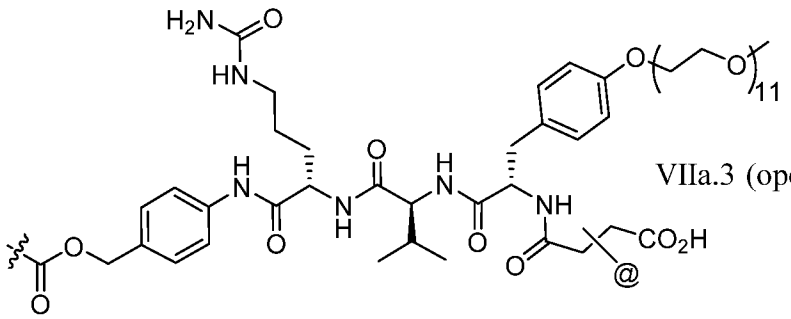
reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

In certain embodiments, L is selected from the group consisting of VIIa.3, IVc.6, VIIc.5, and VIIc.1, wherein ~ is the attachment point to drug D and @ is the attachment point to the LK, wherein when the linker is in the open form as shown below, @ can be either at the α-position or β-position of the carboxylic acid next to it:



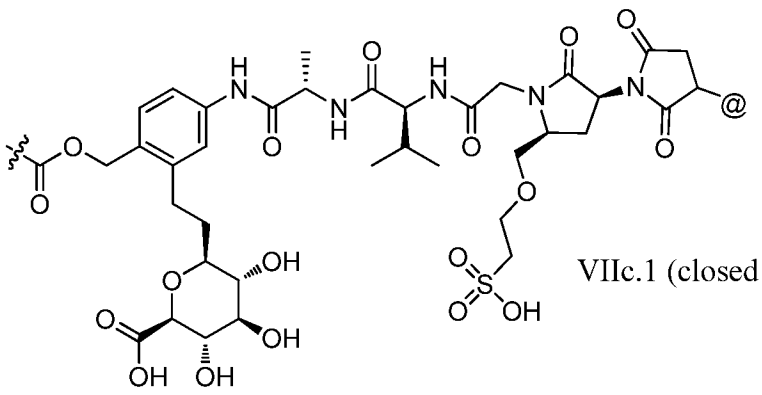
VIIa.3 (closed form)

,



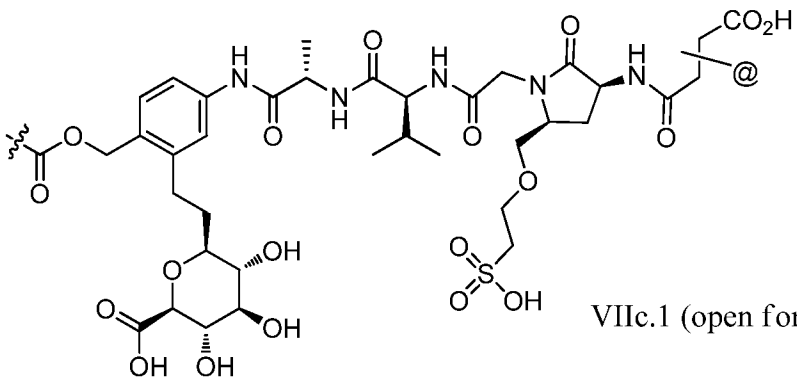
VIIa.3 (open form)

,



VIIc.1 (closed form)

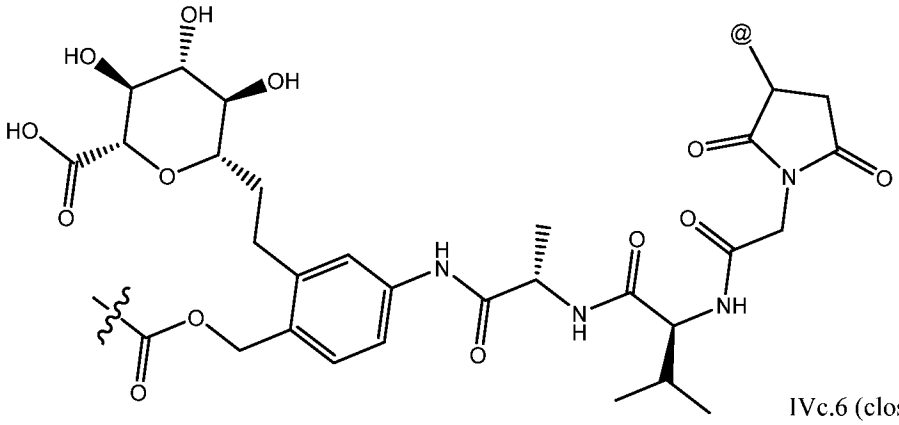
,



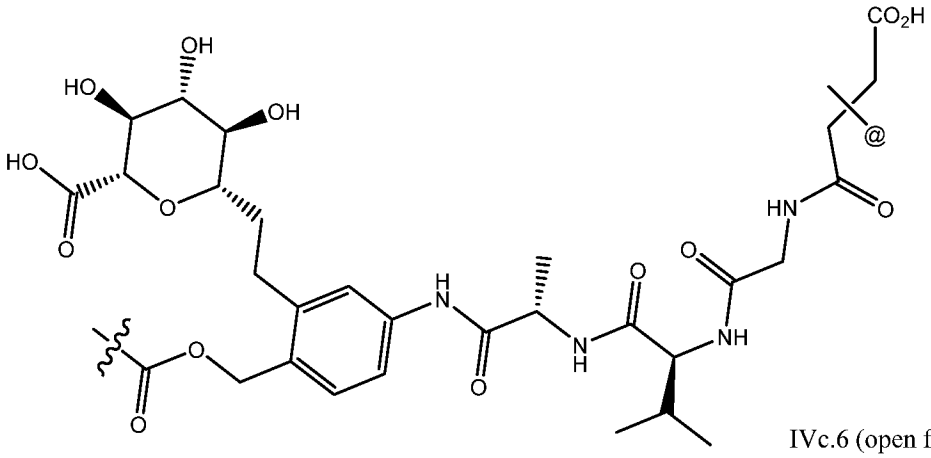
VIIc.1 (open form)

,

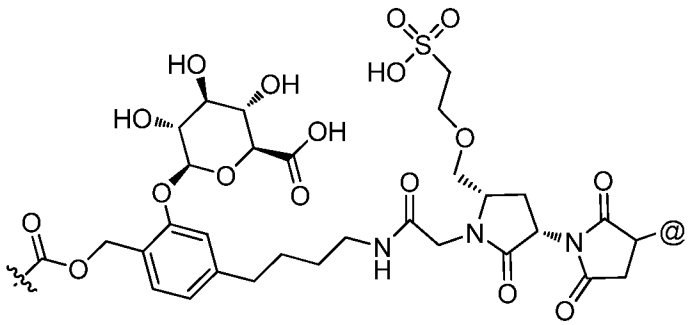
,



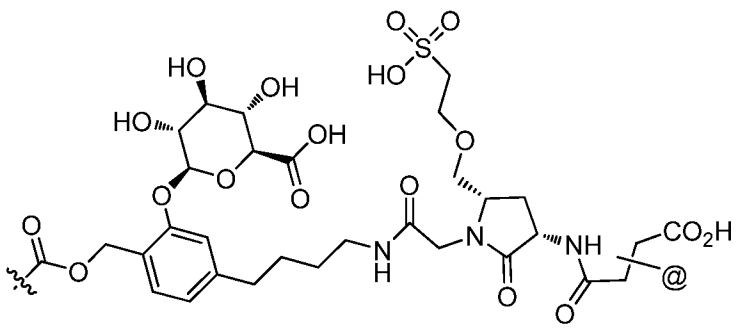
IVc.6 (closed form),



IVc.6 (open form),



VIIc.5 (closed form), and



VIIc.5 (open form)..

3.2.3 Linker Selection Considerations

As is known by skilled artisans, the linker selected for a particular ADC may be influenced by a variety of factors, including but not limited to, the site of attachment to the antibody (*e.g.*, lys, cys or other amino acid residues), structural constraints of the drug pharmacophore and the lipophilicity of the drug. The specific linker selected for an ADC should seek to balance these different factors for the specific antibody/drug combination. For a review of the factors that are influenced by choice of linkers in ADCs, *see* Nolting, Chapter 5 "Linker Technology in Antibody-Drug Conjugates," *In: Antibody-Drug Conjugates: Methods in Molecular Biology*, vol. 1045, pp. 71-100, Laurent Ducry (Ed.), Springer Science & Business Medica, LLC, 2013.

For example, ADCs have been observed to effect killing of bystander antigen-negative cells present in the vicinity of the antigen-positive tumor cells. The mechanism of bystander cell killing by ADCs has indicated that metabolic products formed during intracellular processing of the ADCs may play a role. Neutral cytotoxic metabolites generated by metabolism of the ADCs in antigen-positive cells appear to play a role in bystander cell killing while charged metabolites may be prevented from diffusing across the membrane into the medium and therefore cannot affect bystander killing. In certain embodiments, the linker is selected to attenuate the bystander killing effect caused by cellular metabolites of the ADC. In certain embodiments, the linker is selected to increase the bystander killing effect.

The properties of the linker may also impact aggregation of the ADC under conditions of use and/or storage. Typically, ADCs reported in the literature contain no more than 3-4 drug molecules per antibody molecule (*see*, *e.g.*, Chari, 2008, *Acc Chem Res* 41:98-107). Attempts to obtain higher drug-to-antibody ratios ("DAR") often failed, particularly if both the drug and the linker were hydrophobic, due to aggregation of the ADC (King *et al.*, 2002, *J Med Chem* 45:4336-4343; Hollander *et al.*, 2008, *Bioconjugate Chem* 19:358-361; Burke *et al.*, 2009 *Bioconjugate Chem* 20:1242-1250). In many instances, DARs higher than 3-4 could be beneficial as a means of increasing potency. In instances where the Bcl-xL inhibitor is hydrophobic in nature, it may be desirable to select linkers that are relatively hydrophilic as a means of reducing ADC aggregation, especially in instances where DARS greater than 3-4 are desired. Thus, in certain embodiments, the linker incorporates chemical moieties that reduce aggregation of the ADCs during storage and/or use. A linker may incorporate polar or hydrophilic groups such as charged groups or groups that become charged under physiological pH to reduce the aggregation of the ADCs. For example, a linker may incorporate charged groups such as salts or groups that deprotonate, *e.g.*, carboxylates, or protonate, *e.g.*, amines, at physiological pH.

Exemplary polyvalent linkers that have been reported to yield DARs as high as 20 that may be used to link numerous Bcl-xL inhibitors to an antibody are described in in U.S. Patent No 8,399,512; U.S. Published Application No. 2010/0152725; U.S. Patent No. 8,524,214; U.S. Patent No. 8,349,308; U.S. Published Application No. 2013/189218; U.S. Published Application No. 2014/017265; WO 2014/093379; WO 2014/093394; WO 2014/093640, the content of which are incorporated herein by reference in their entireties.

In particular embodiments, the aggregation of the ADCs during storage or use is less than about 40% as determined by size-exclusion chromatography (SEC). In particular embodiments, the aggregation of the ADCs during storage or use is less than 35%, such as less than about 30%, such as less than about 25%, such as less than about 20%, such as less than about 15%, such as less than about 10%, such as less than about 5%, such as less than about 4%, or even less, as determined by size-exclusion chromatography (SEC).

One embodiment pertains to ADCs or synthons in which linker **L** is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, VIa.1, Ve.1-Ve.2, VIa.1, V1c.1-V1c.2, V1d.1-V1d.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 and salts thereof.

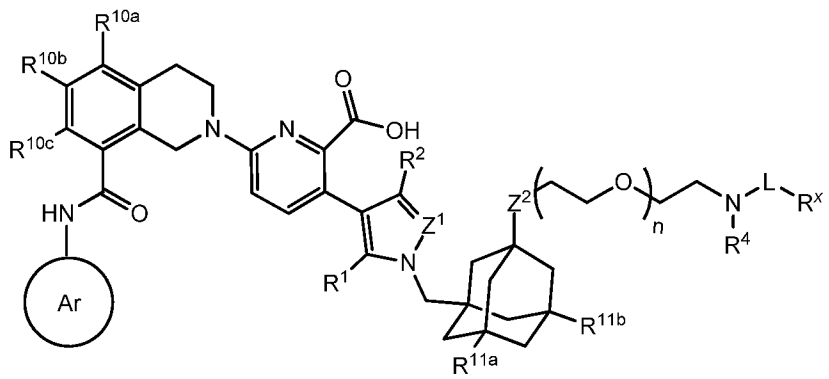
4. ADC Synthons

Antibody-Drug Conjugate synthons are synthetic intermediates used to form ADCs. The synthons are generally compounds according to structural formula (III):

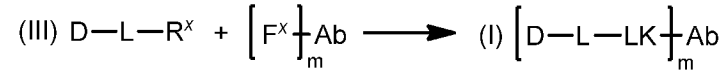
(III) D-L-R^x

or salts thereof, wherein D is a Bcl-xL inhibitor as previously described, L is a linker as previously described, and R^x is a moiety that comprises a functional group suitable for covalently linking the synthon to an antibody. In specific embodiments, the synthons are compounds according to structural formula (IIIa) or salts thereof, where Ar, R^1, R^2, R^4, R^{10a}, R^{10b}, R^{10c}, R^{11a}, R^{11b}, Z^1, Z^2, and *n* are as previously defined for structural formula (IIa), and L and R^x are as defined for structural formula (III):

(IIIa)



To synthesize an ADC, an intermediate synthon according to structural formula (III), or a salt thereof, is contacted with an antibody of interest under conditions in which functional group R^x reacts with a "complementary" functional group on the antibody, F^x, to form a covalent linkage.



The identities of groups R^x and F^x will depend upon the chemistry used to link the synthon to the antibody. Generally, the chemistry used should not alter the integrity of the antibody, for example its ability to bind its target. Preferably, the binding properties of the conjugated antibody will closely resemble those of the unconjugated antibody. A variety of chemistries and techniques for conjugating molecules to biological molecules such as antibodies are known in the art and in particular to antibodies, are well-known. *See, e.g.*, Amon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* eds., Alan R. Liss, Inc., 1985; Hellstrom *et al.*, "Antibodies For Drug Delivery," in *Controlled Drug Delivery*(Robinson et al. eds., Marcel Dekker, Inc., 2nd ed. 1987; Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological And Clinical Applications,* Pinchera *et al.*, eds., 1985; "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in *Monoclonal Antibodies For Cancer Detection And Therapy,* Baldwin *et al.*, eds., *Academic Press*, 1985; and Thorpe *et al.*, 1982, *Immunol. Rev.* 62:119-58; and WO 89/12624. Any of these chemistries may be used to link the synthons to an antibody.

In one embodiment, R^x comprises a functional group capable of linking the synthon to an amino group on an antibody. In another embodiment, R^x comprises an NHS-ester or an isothiocyanate. In another embodiment, R^x comprises a functional group capable of linking the synthon to a sulfhydryl group on an antibody. In another embodiment, R^x comprises a haloacetyl or a maleimide.

Typically the synthons are linked to the side chains of amino acid residues of the antibody, including, for example, the primary amino group of accessible lysine residues or the sulfhydryl group of accessible cysteine residues. Free sulfhydryl groups may be obtained by reducing interchain disulfide bonds.

In one embodiment, LK is a linkage formed with an amino group on the anti-hEGFR antibody Ab (*e.g.,* AbA, AbB, AbG, or AbK). In another embodiment, LK is an amide or a thiourea. In another embodiment, LK is a linkage formed with a sulfhydryl group on the anti-hEGFR antibody Ab. In another embodiment, LK is a thioether.

In one embodiment, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

and a pharmaceutically acceptable salt thereof;

L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4,Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, VIa.1, Ve.1-Ve.2, VIa.1, V1c.1-V1c.2, V1d.1-V1d.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6, wherein each linker has reacted with the anti-hEGFR antibody, Ab, forming a covalent attachment; LK is thioether; and m is an integer ranging from 1 to 8.

In one embodiment, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

and a pharmaceutically acceptable salt thereof;

L is selected from the group consisting of linkers Vc.5, IVc.6, IVd.4, VIIa.1, VIIc.1, VIIc.3, VIIc.4, and VIIc.5 in either closed or open forms and a pharmaceutically acceptable salt thereof;

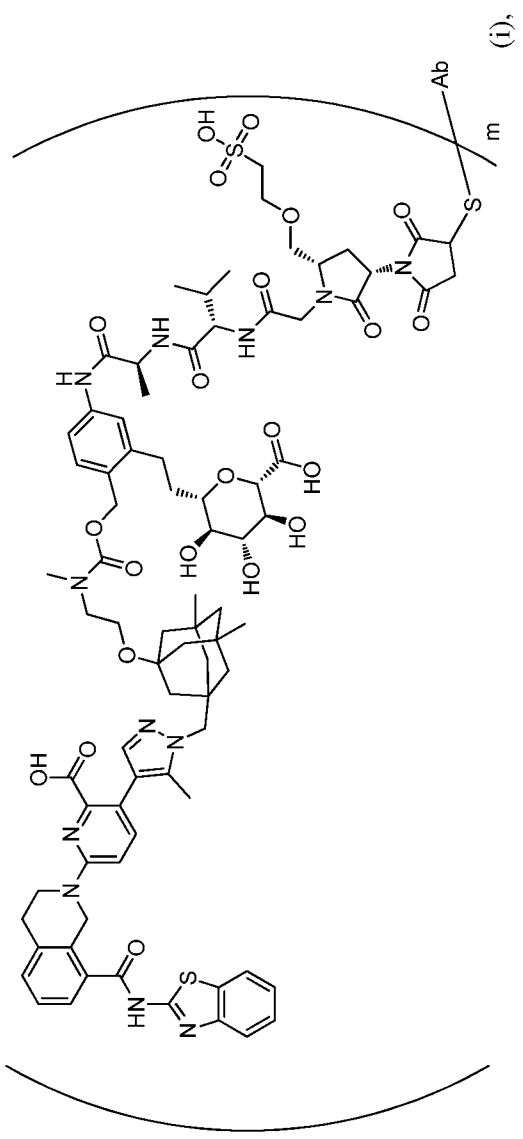
LK is thioether; and

m is an integer ranging from 2 to 4.

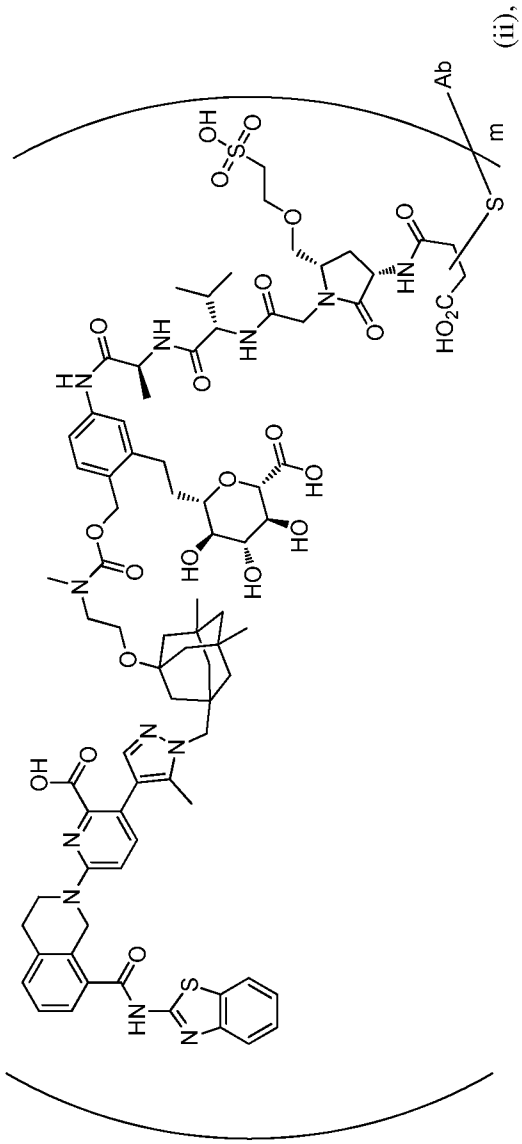
To form an ADC, the maleimide ring of a synthon (for example, the synthons listed in Table 5) may react with an antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of AbA-WD, AbA-LB, AbA-VD, AbB-WD, AbB-LB, AbB-VD, AbG-WD, AbG-LB, AbG-VD, AbK-WD, AbK-LB, and AbK-VD, wherein WD, LB, and VD are synthons disclosed in Table 5, and where in the synthons are either in open or closed form.

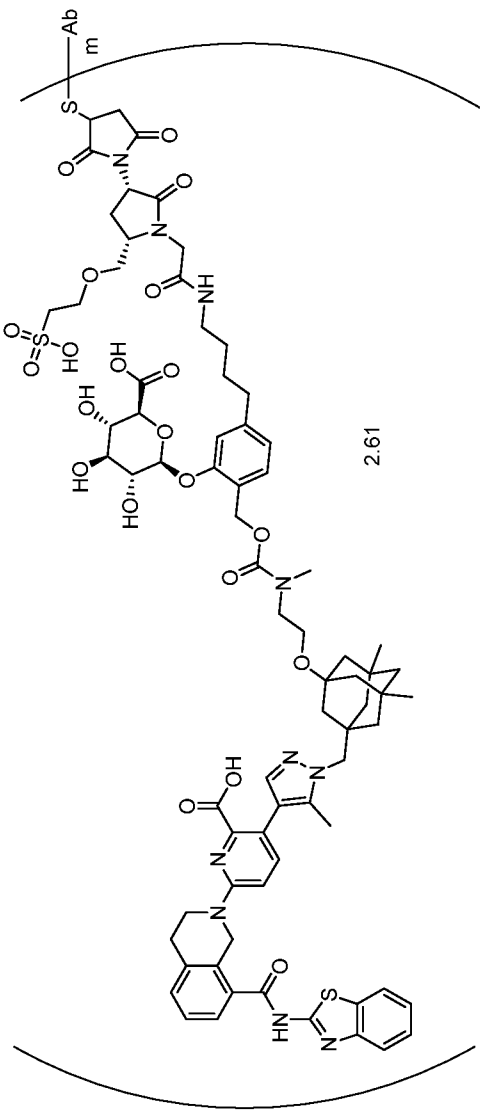
In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of formulas i-vi:



(i),

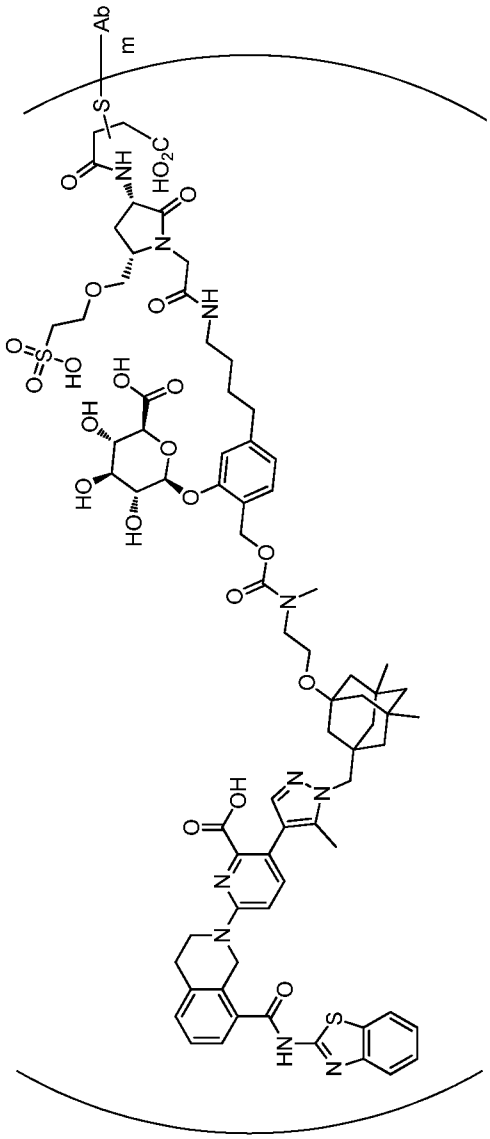


(ii),

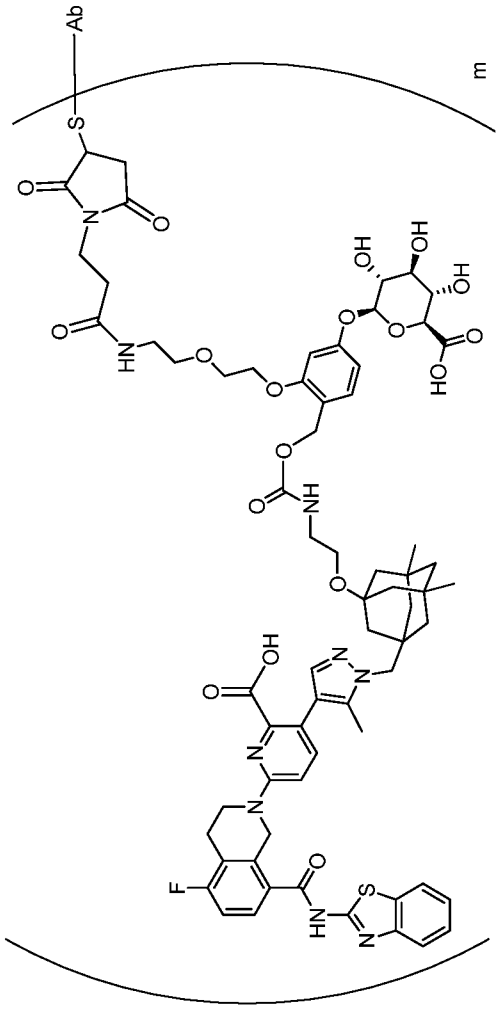


(iii),

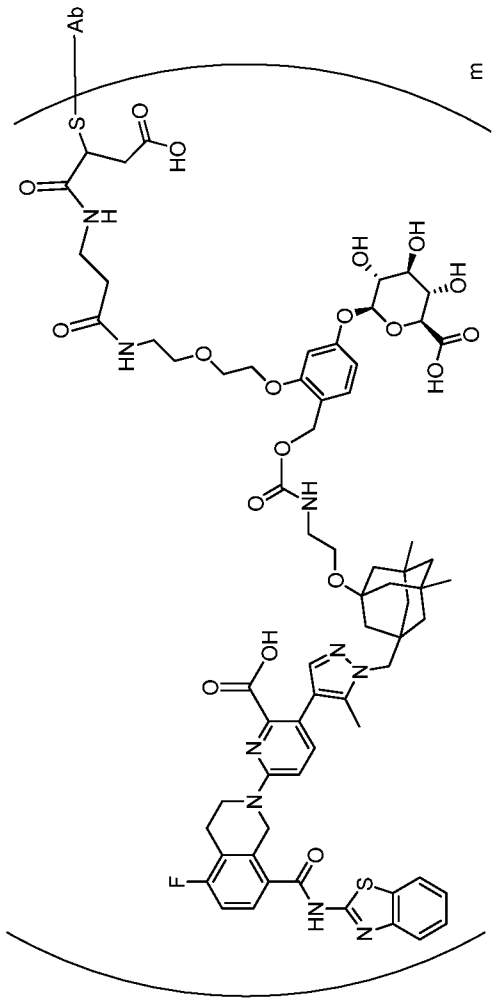
2.61



(iv),



m (v), and



m (vi),

wherein m is an integer from 1 to 6. In a specific embodiment, m is an integer from 1 to 4.

A number of functional groups R^x and chemistries useful for linking synthons to accessible lysine residues are known, and include by way of example and not limitation NHS-esters and isothiocyanates.

A number of functional groups R^x and chemistries useful for linking synthons to accessible free sulfhydryl groups of cysteine residues are known, and include by way of example and not limitation haloacetyls and maleimides.

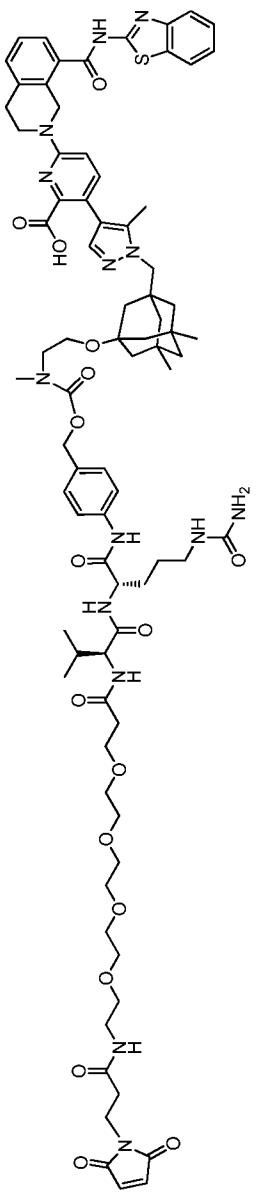
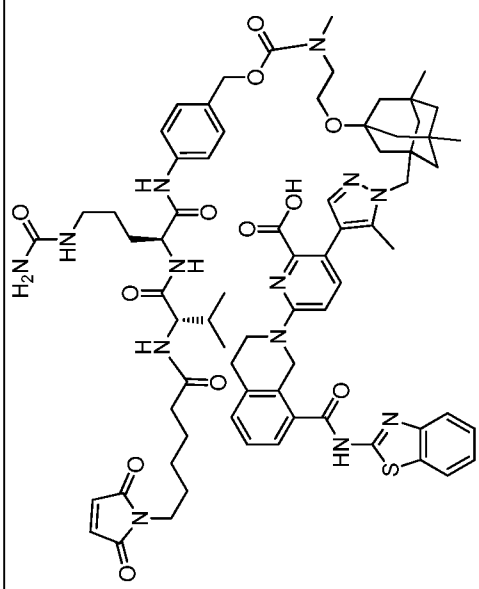
In one embodiment, D is selected from the group consisting of W1.01, W1.02, W1.03, W1.04, W1.05, W1.06, W1.07, and W1.08 and salts thereof; L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, VIa.1, Ve.1-Ve.2, VIa.1, V1c.1-V1c.2, V1d.1-V1d.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6, and salts thereof; R^x comprises a functional group selected from the group consisting of NHS-ester, isothiocyanate, haloacetyl and maleimide.

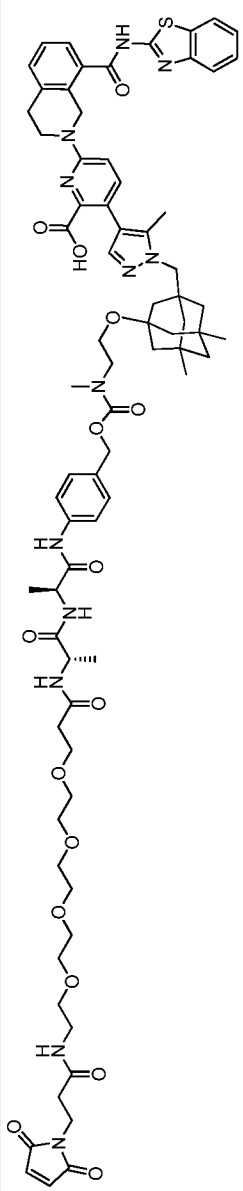
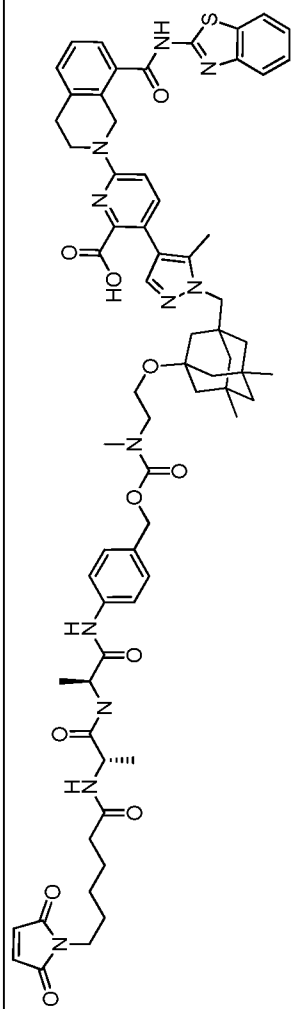
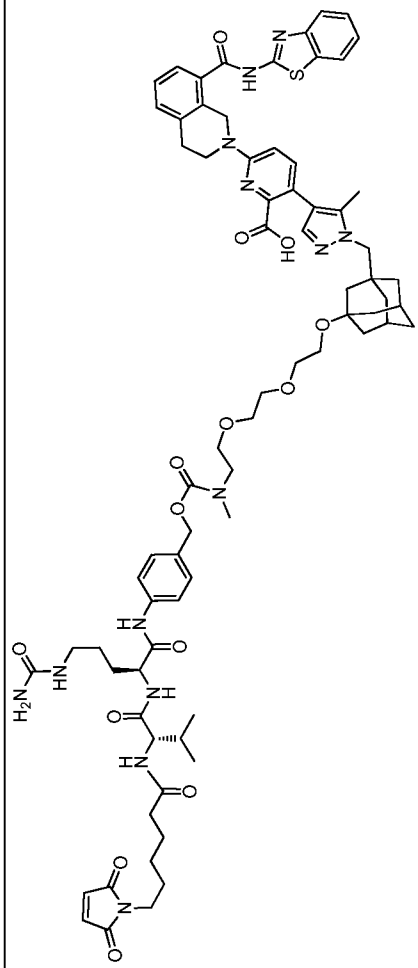
However, conjugation chemistries are not limited to available side chain groups. Side chains such as amines may be converted to other useful groups, such as hydroxyls, by linking an appropriate small molecule to the amine. This strategy can be used to increase the number of available linking sites in the antibody by conjugating multifunctional small molecules to side chains of accessible amino acid residues of the antibody.

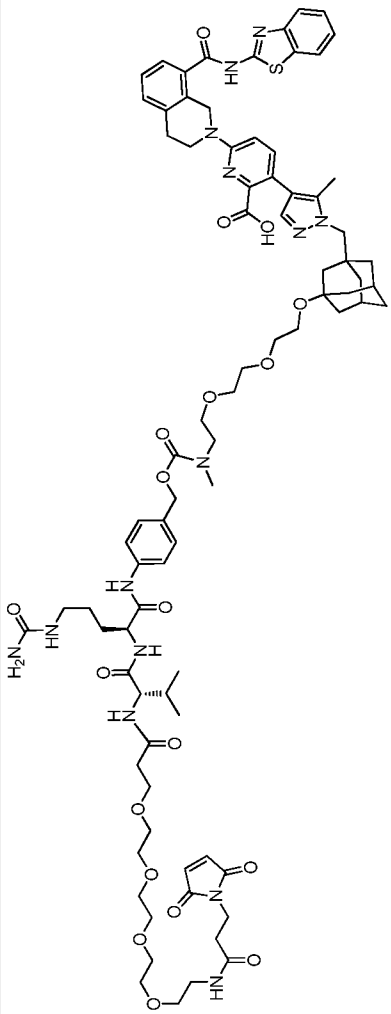
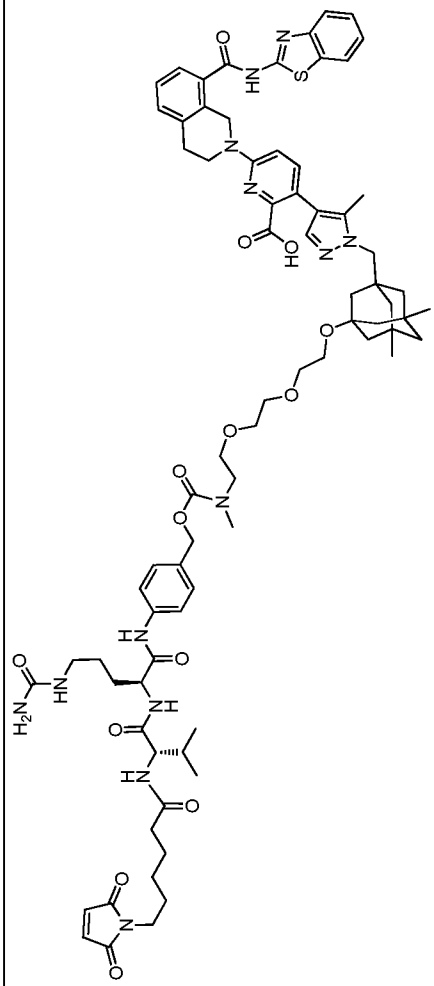
The antibody may also be engineered to include amino acid residues for conjugation. An approach for engineering antibodies to include non-genetically encoded amino acid residues useful for conjugating drugs in the context of ADCs is described in Axup *et al.*, 2003, *Proc Natl Acad Sci* 109:16101-16106 and Tian *et al.*, 2014, *Proc Natl Acad Sci* 111:1776-1771.

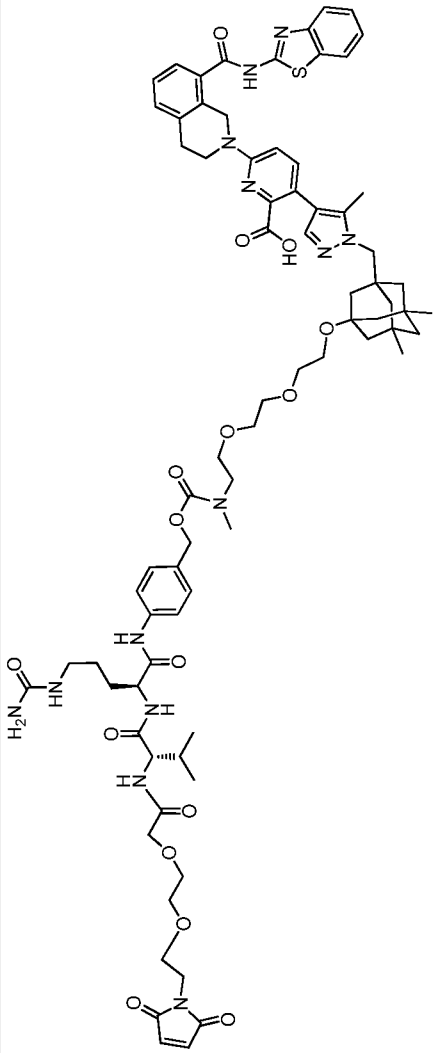
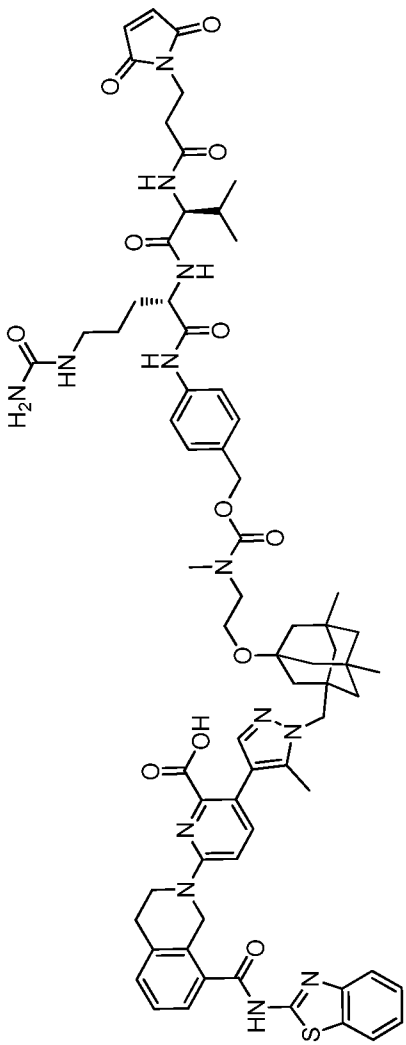
Exemplary synthons useful for making ADCs described herein include, but are not limited to, the following synthons:

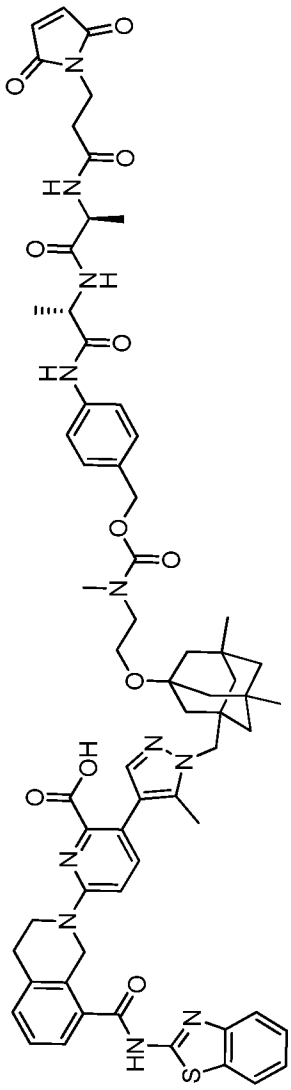
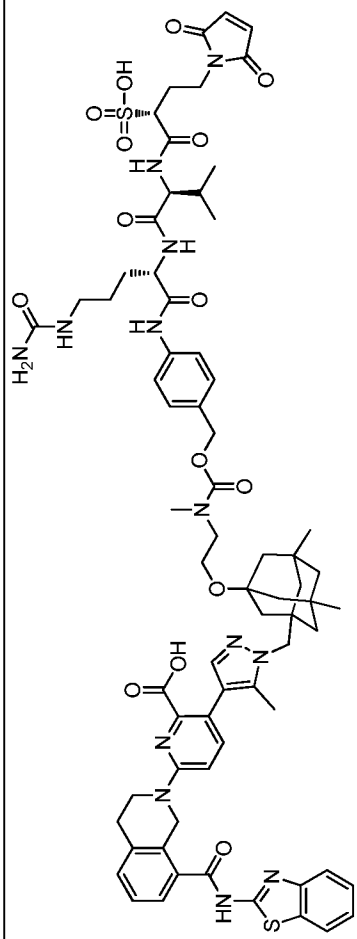
Table 5

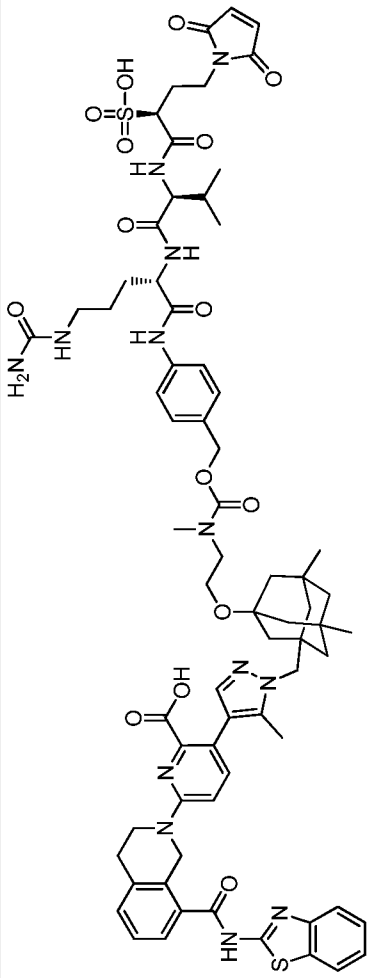
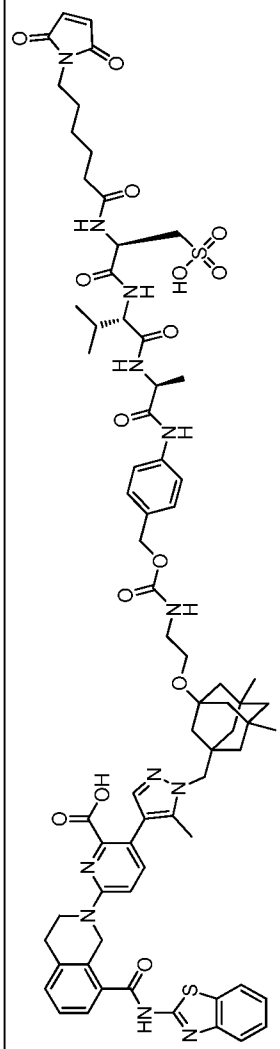
Example No.	Synthon	Synthon structure
2.1	E	
2.2	D	

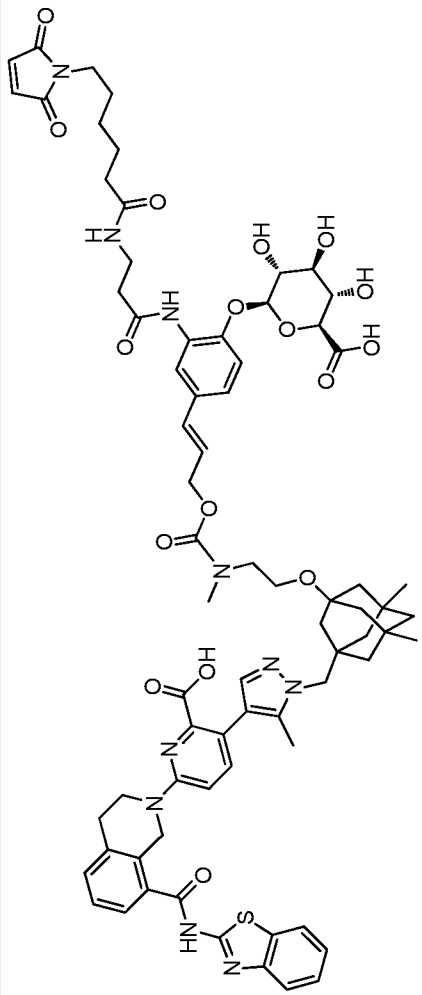
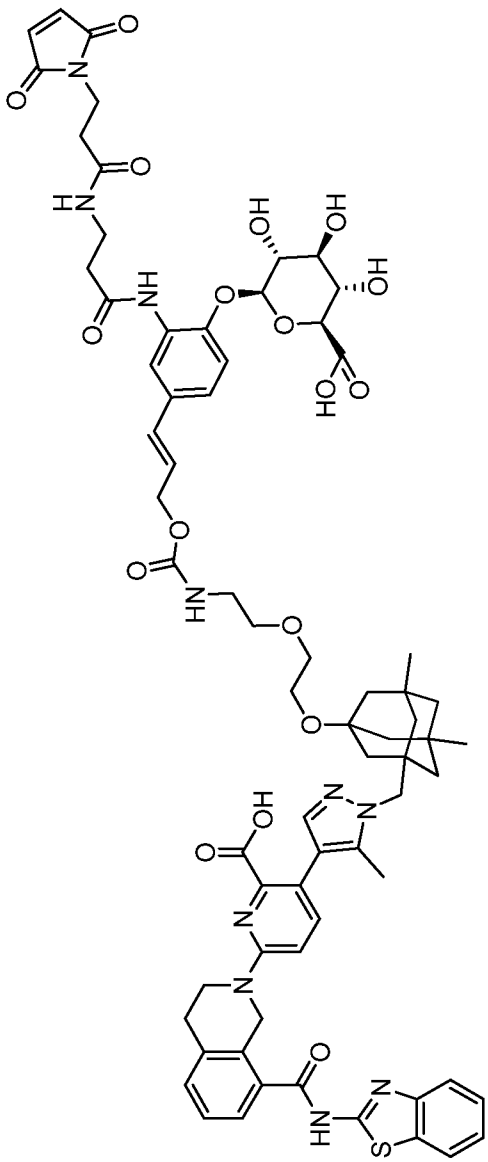
Example No.	Synthon	Synthon structure
2.3	J	
2.4	K	
2.5	L	

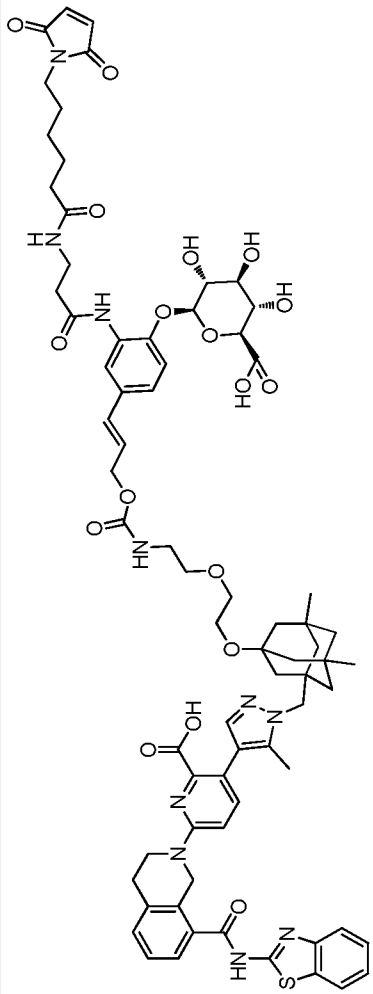
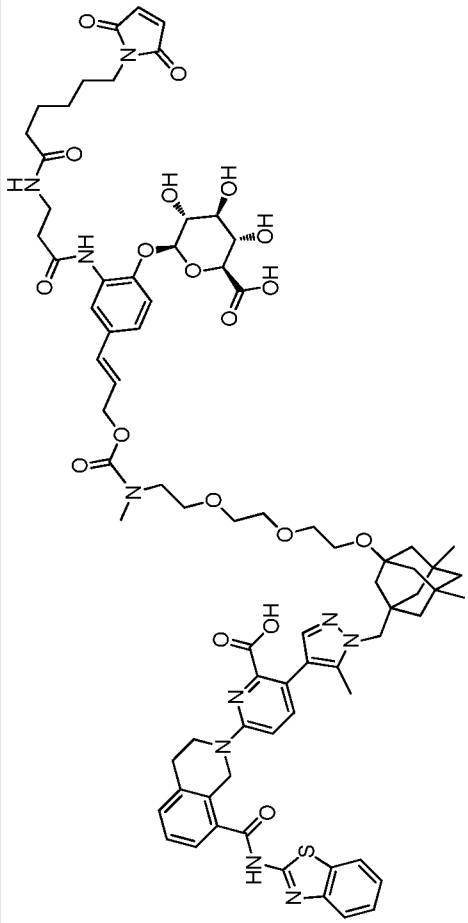
Example No.	Synthon	Synthon structure
2.6	M	
2.7	V	

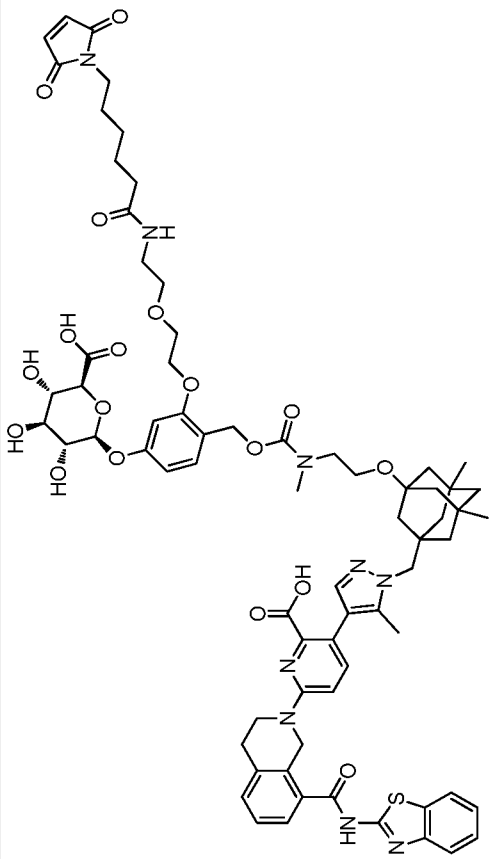
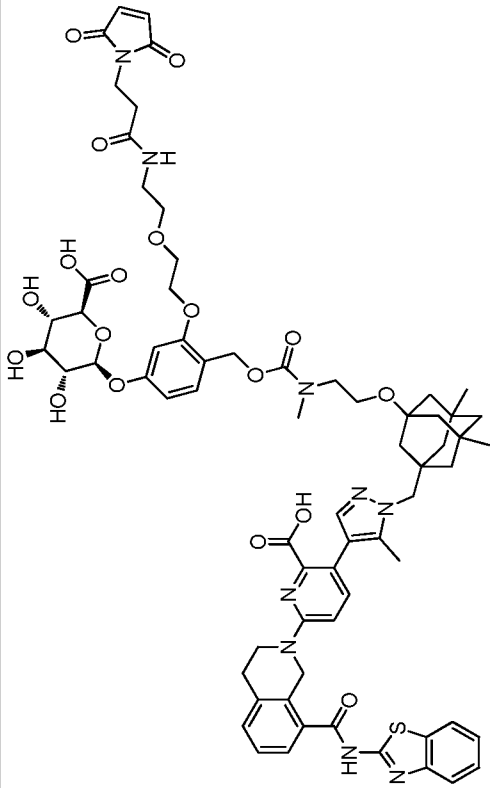
Example No.	Synthon	Synthon structure
2.8	DS	
2.10	BG	

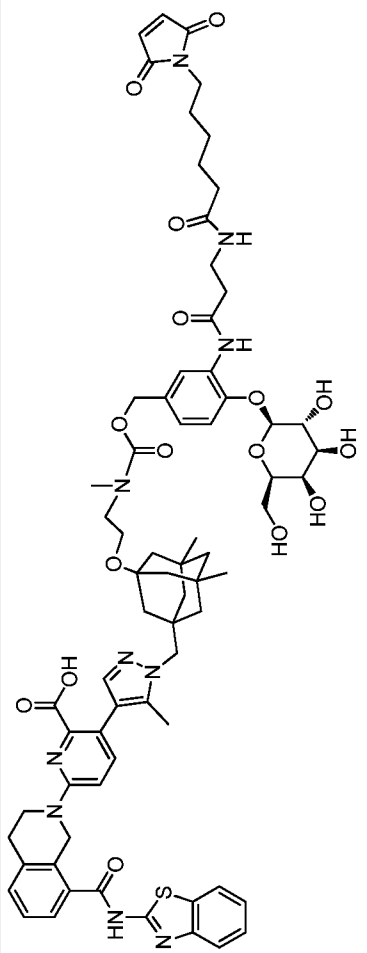
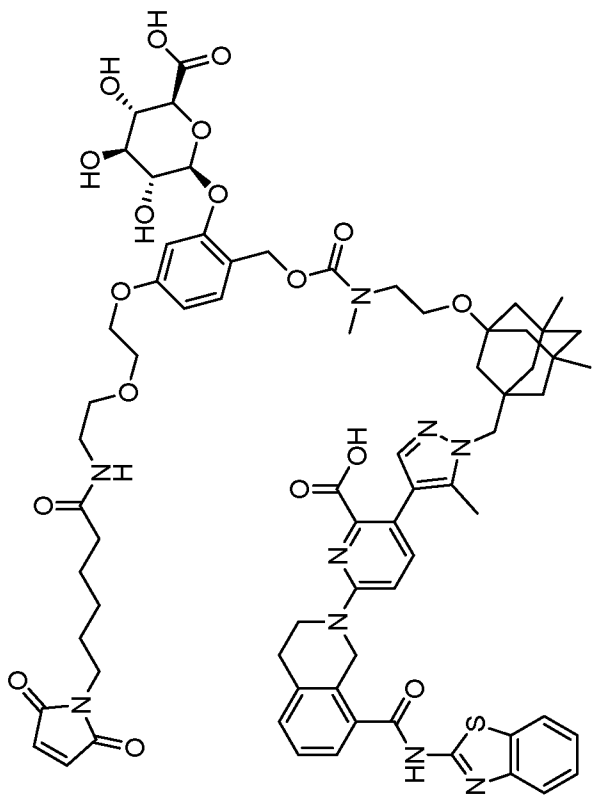
Example No.	Synthon	Synthon structure
2.12	BI	
2.17	BO	

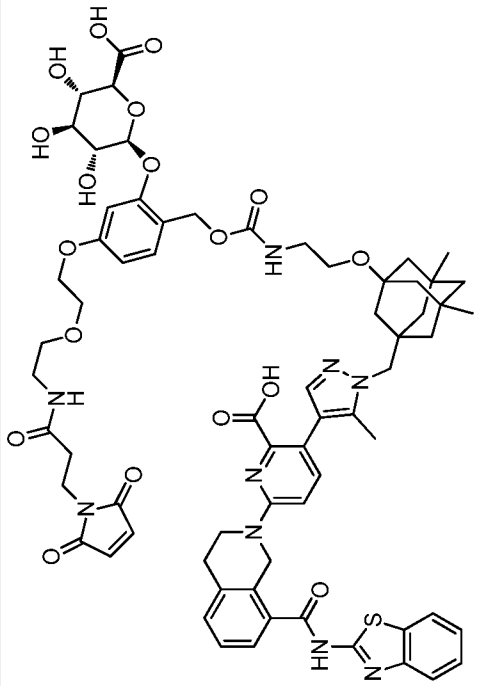
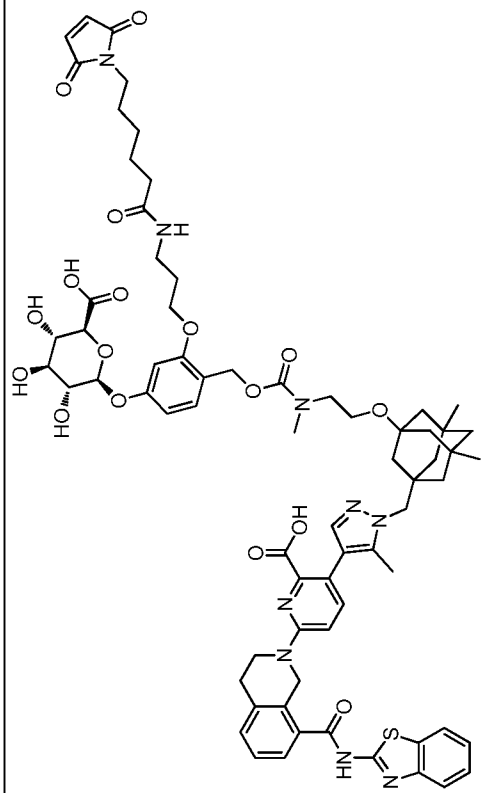
Example No.	Synthon	Synthon structure
2.18	BP	
2.21	IQ	

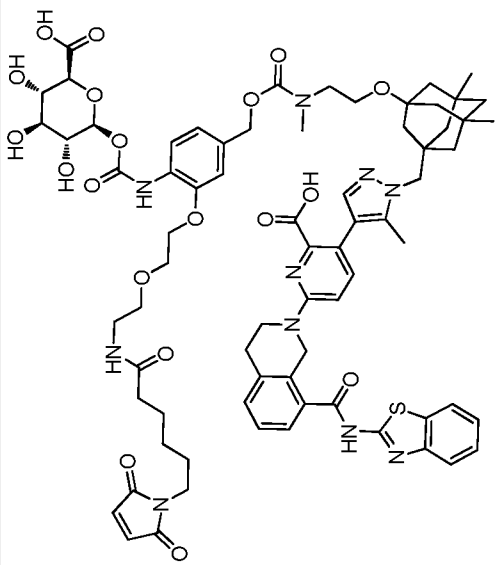
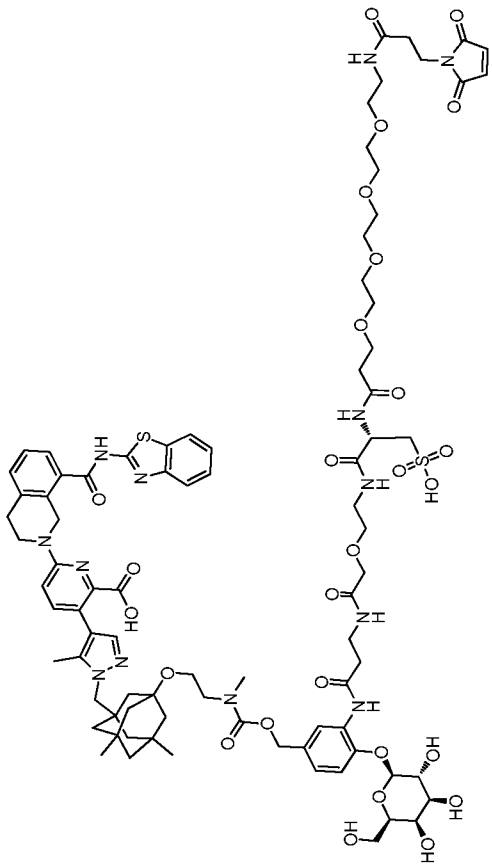
Example No.	Synthon	Synthon structure
2.22	DB	
2.23	DM	

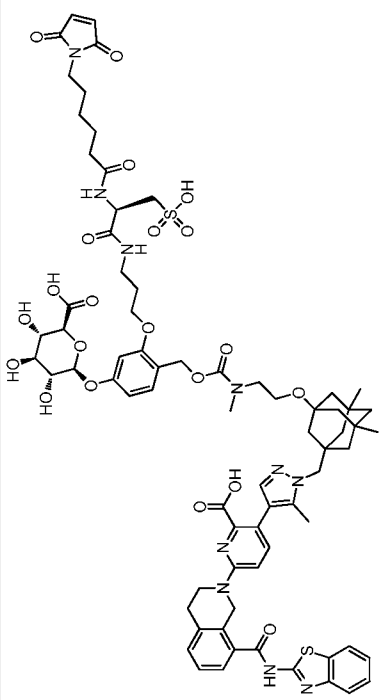
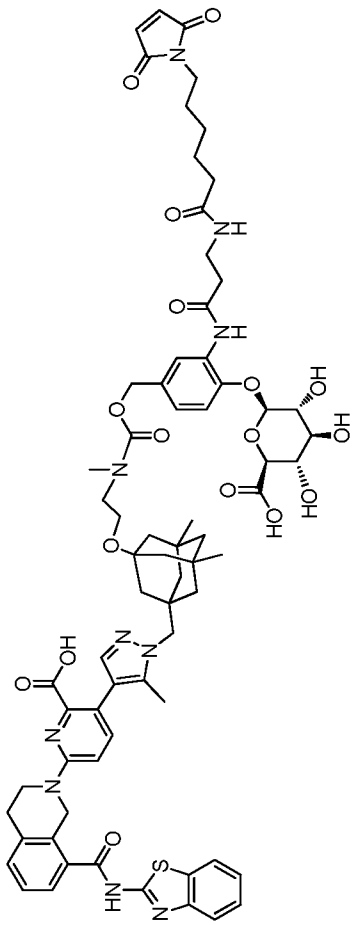
Example No.	Synthon	Synthon structure
2.24	DL	
2.25	DR	

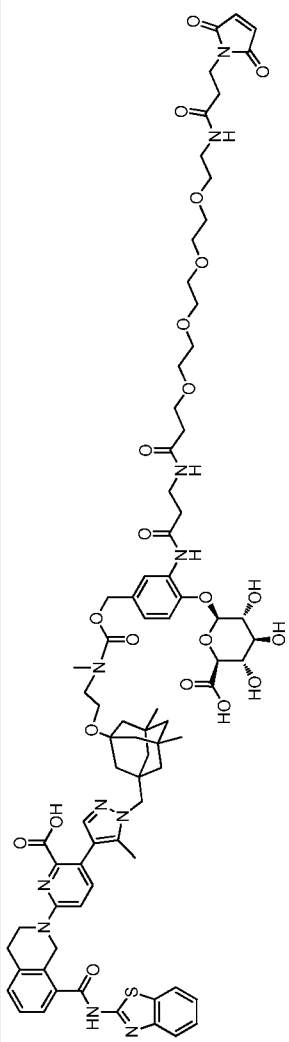
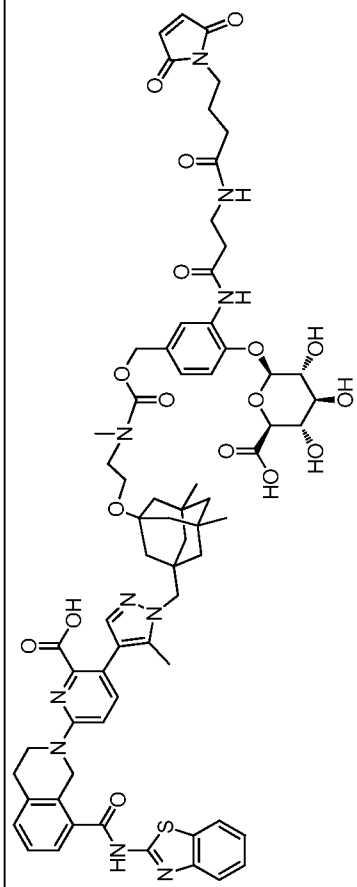
Example No.	Synthon	Synthon structure
2.26	DZ	
2.27	EA	

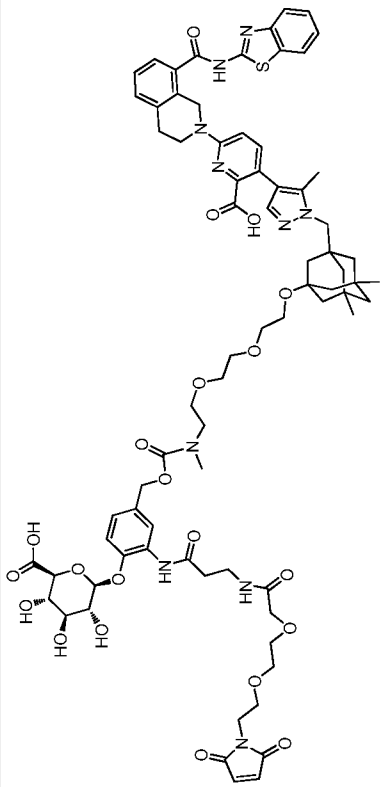
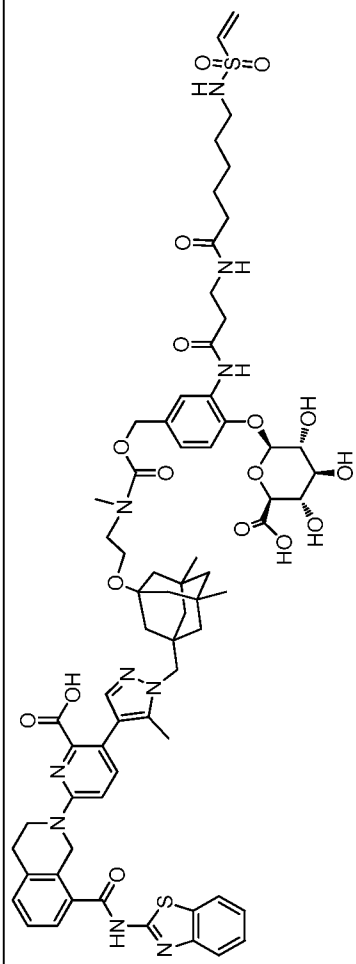
Example No.	Synthon	Synthon structure
2.28	EO	
2.29	FB	

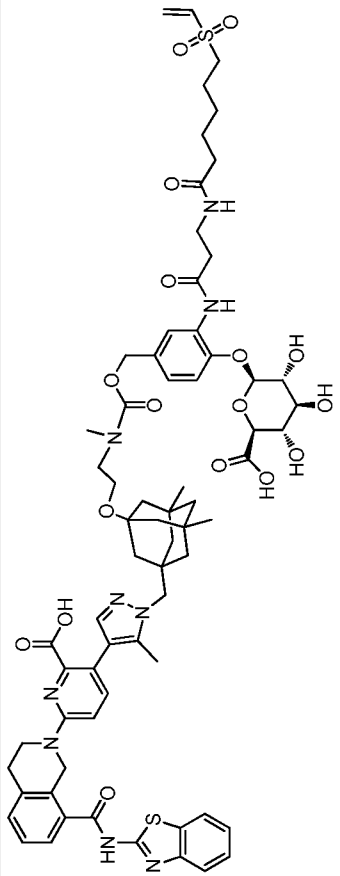
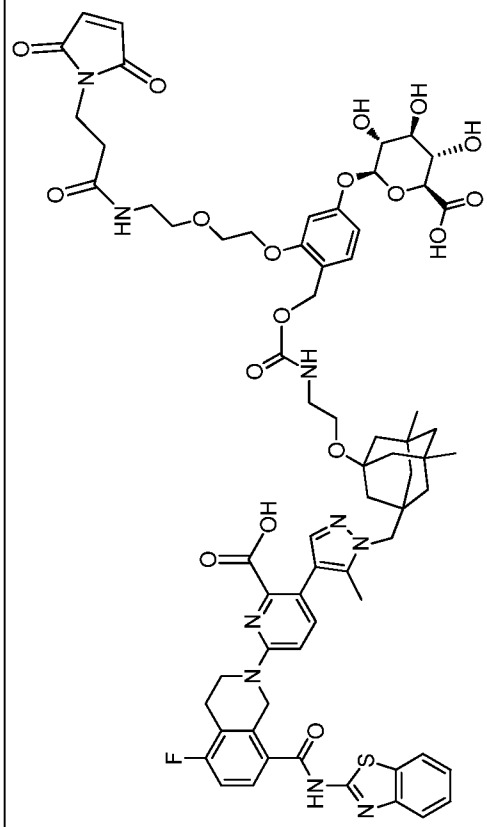
Example No.	Synthon	Synthon structure
2.30	KX	
2.31	FF	

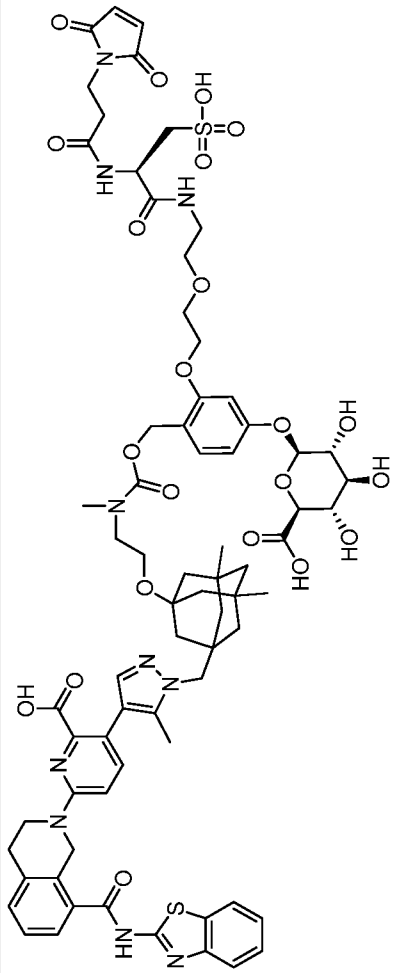
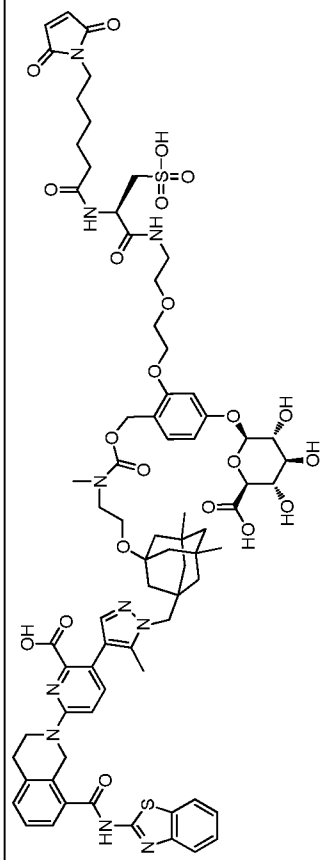
Example No.	Synthon	Synthon structure
2.32	FU	
2.33	GH	

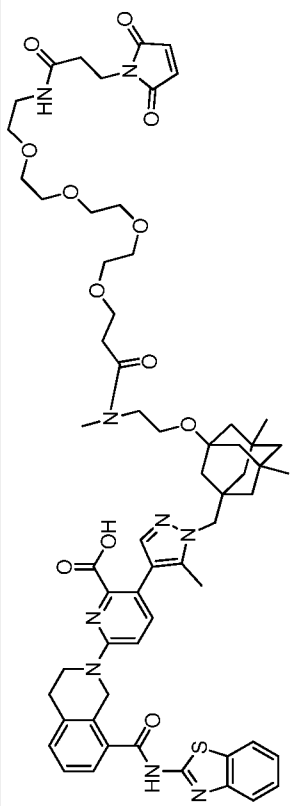
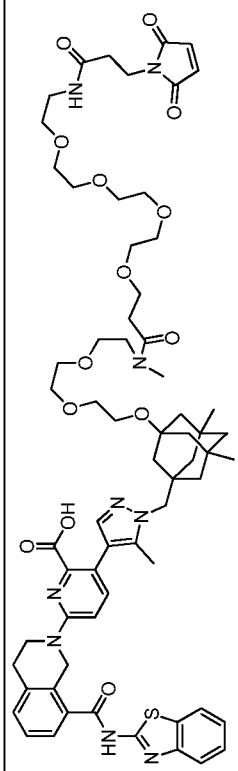
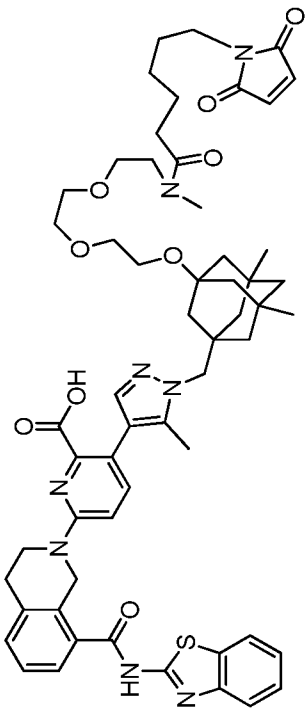
Example No.	Synthon	Synthon structure
2.34	FX	
2.35	H	

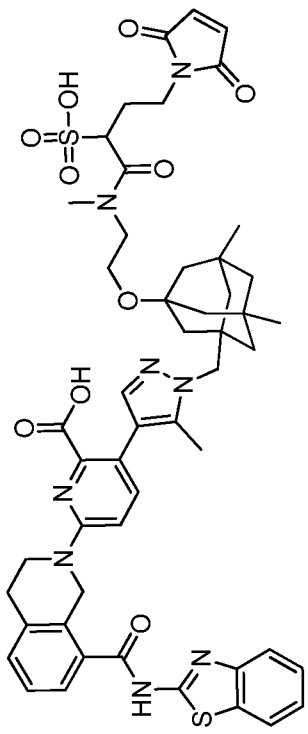
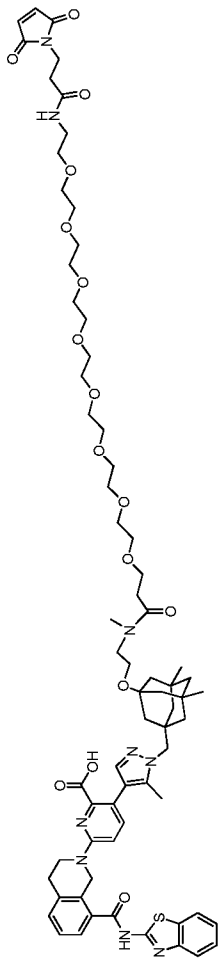
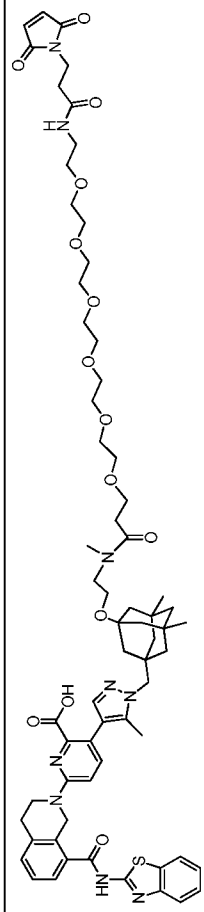
Example No.	Synthon	Synthon structure
2.36	I	
2.37	KQ	

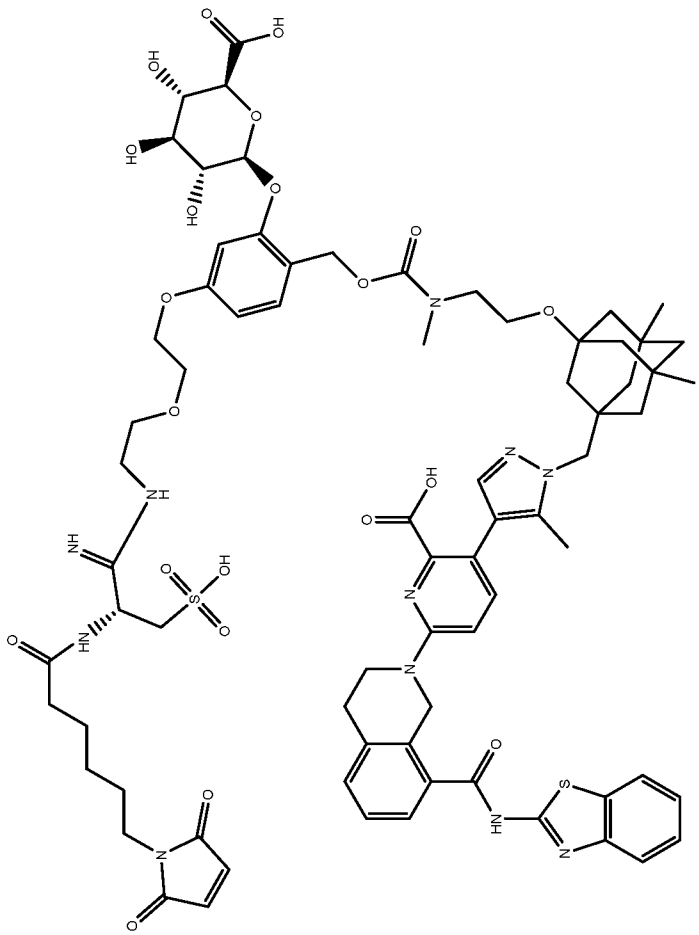
Example No.	Synthon	Synthon structure
2.38	KP	
2.39	HA	

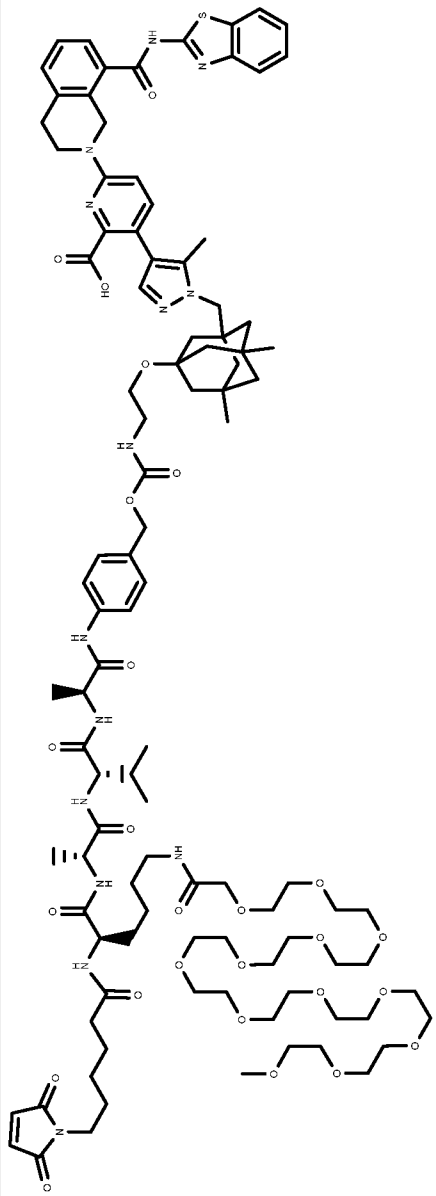
Example No.	Synthon	Synthon structure
2.40	HB	
2.41	LB	

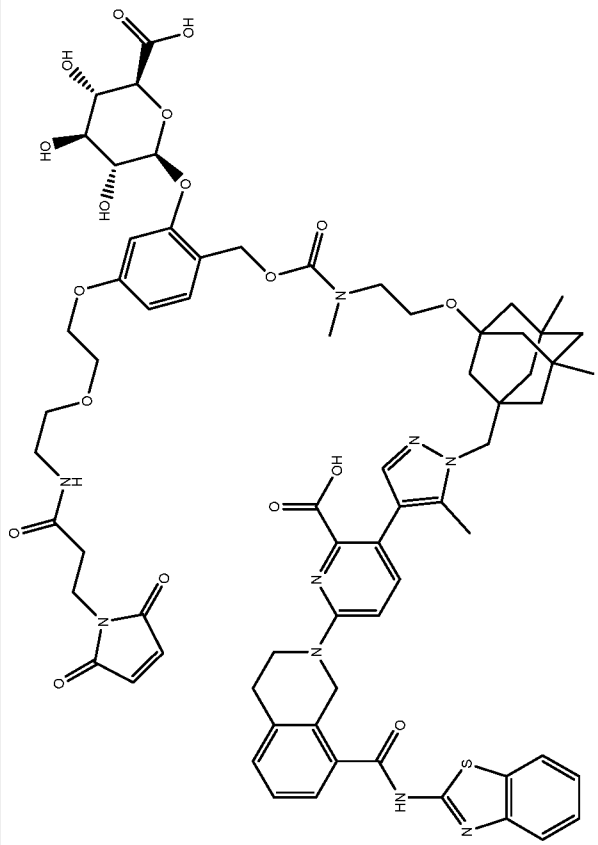
Example No.	Synthon	Synthon structure
2.42	NF	
2.43	NG	

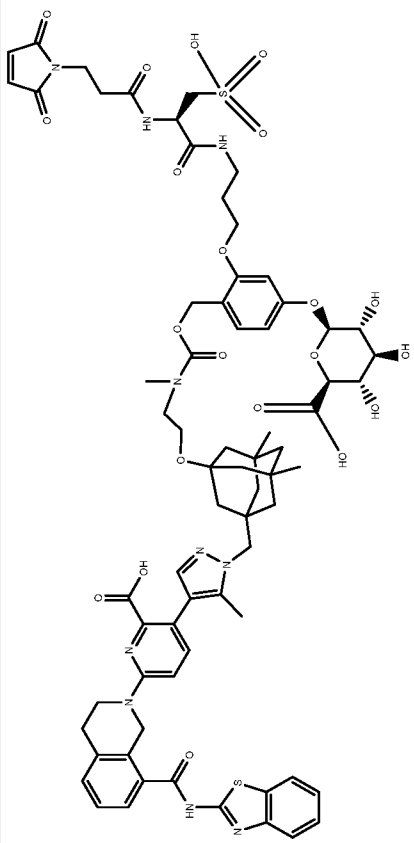
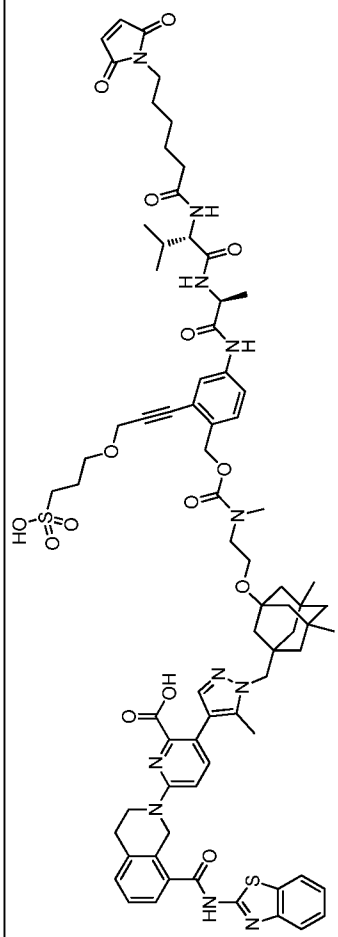
Example No.	Synthon	Synthon structure
2.44	AS	
2.45	AT	
2.46	AU	

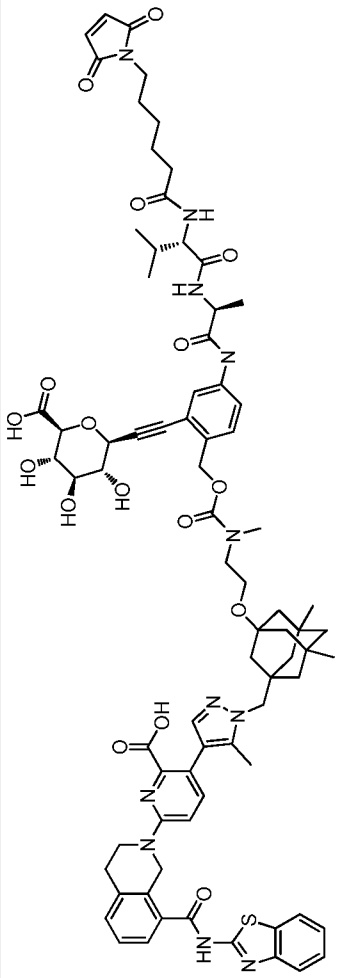
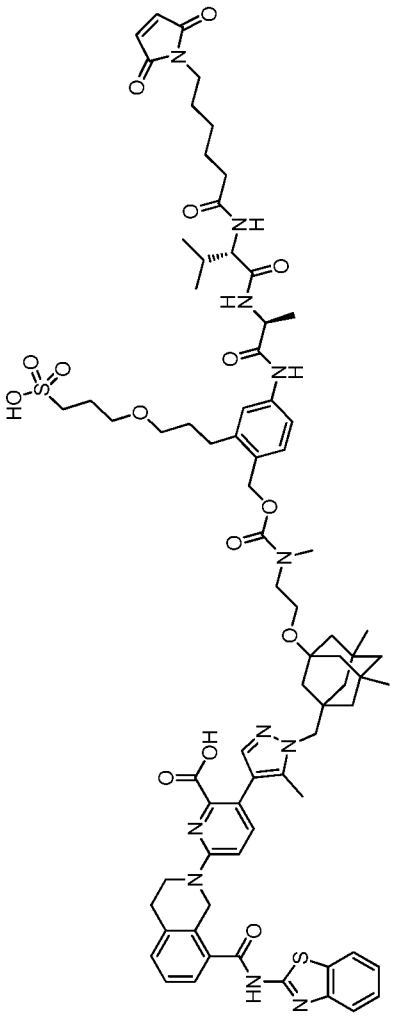
Example No.	Synthon	Synthon structure
2.47	BK	
2.48	BQ	
2.49	BR	

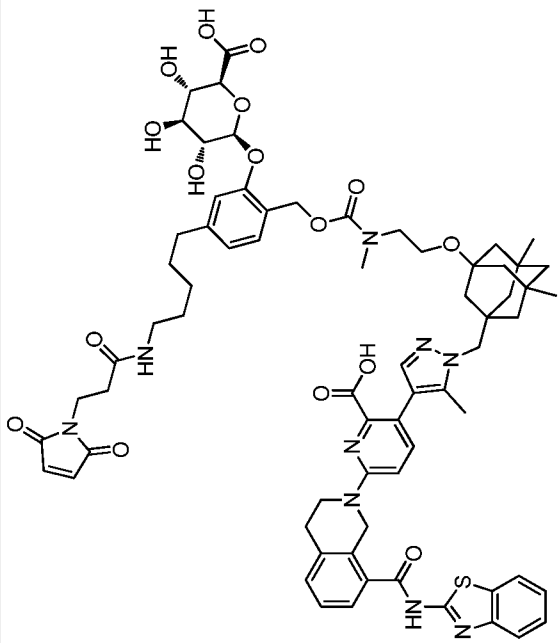
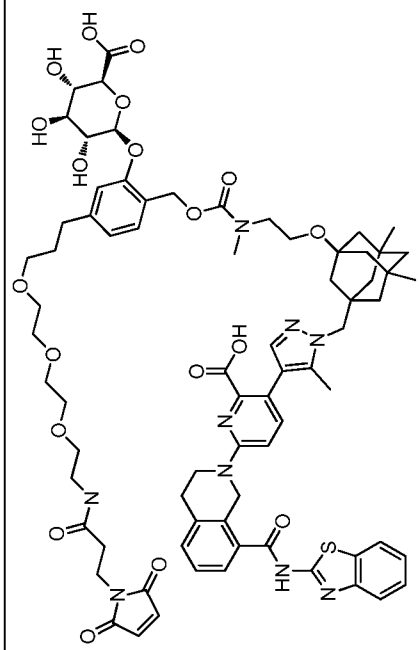
Example No.	Synthon	Synthon structure
2.50	OI	

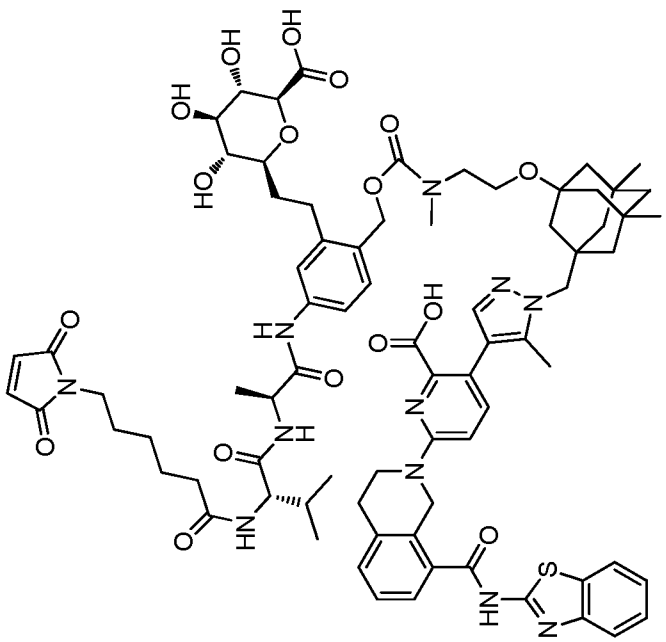
Example No.	Synthon	Synthon structure
2.51	NX	

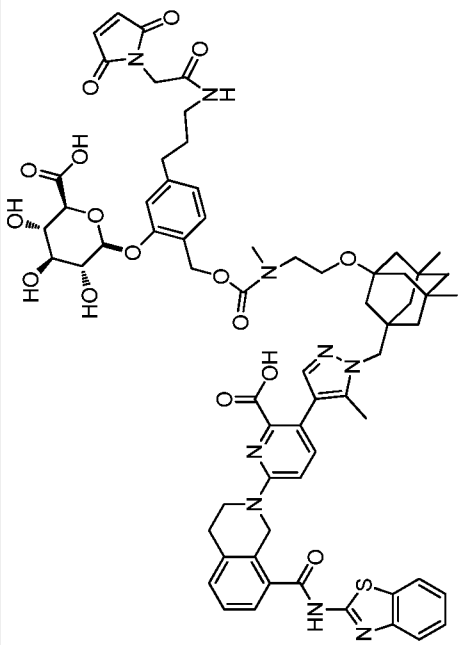
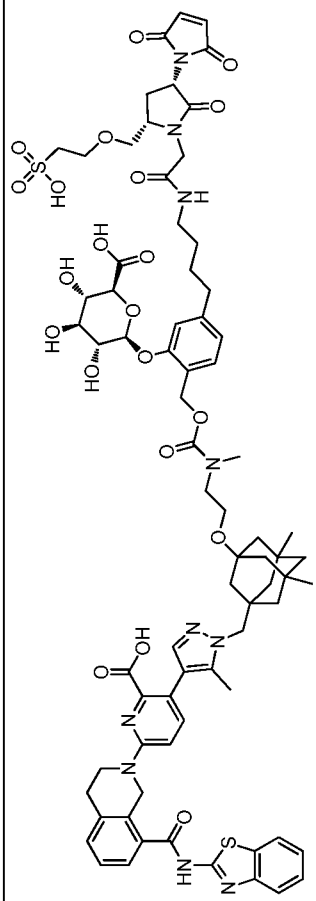
Example No.	Synthon	Synthon structure
2.52	OJ	

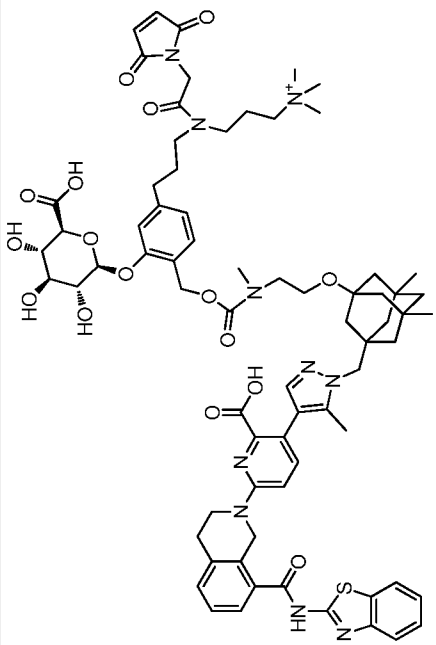
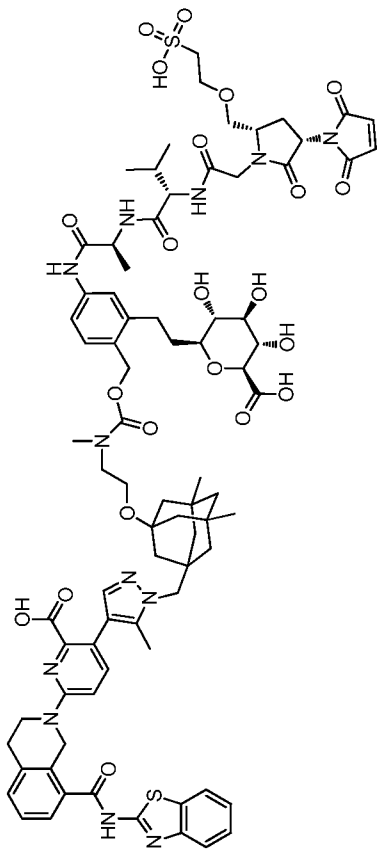
Example No.	Synthon	Synthon structure
2.53	XY	
2.54	LX	

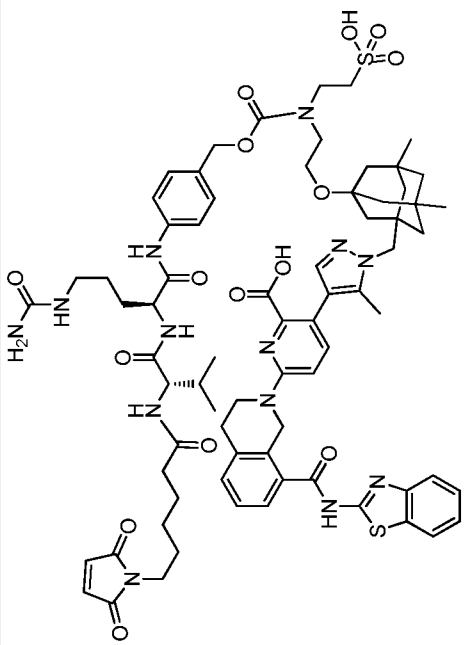
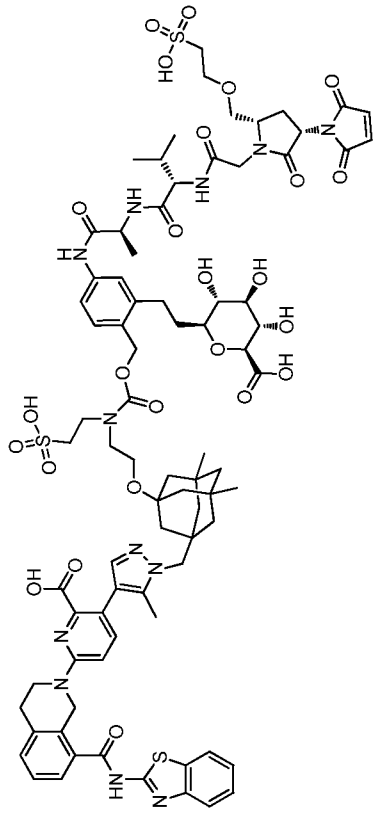
Example No.	Synthon	Synthon structure
2.55	MJ	
2.56	NH	

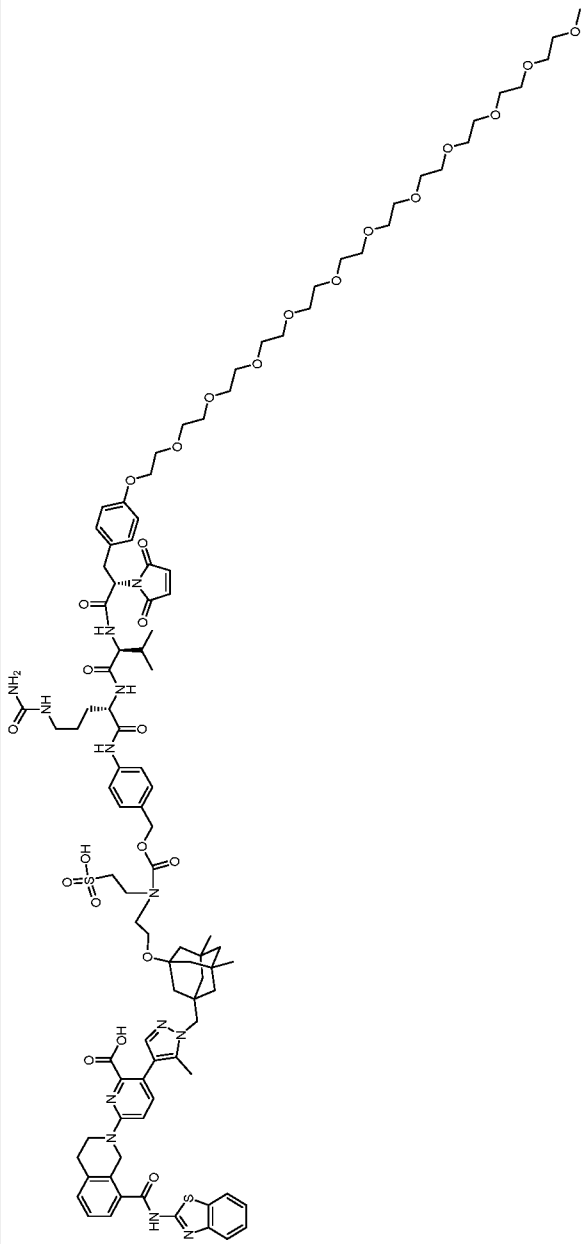
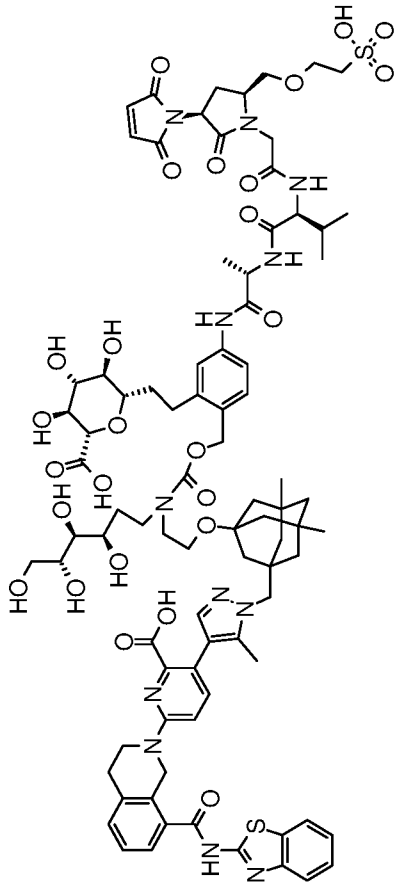
Example No.	Synthon	Synthon structure
2.57	OV	
2.58	QS	

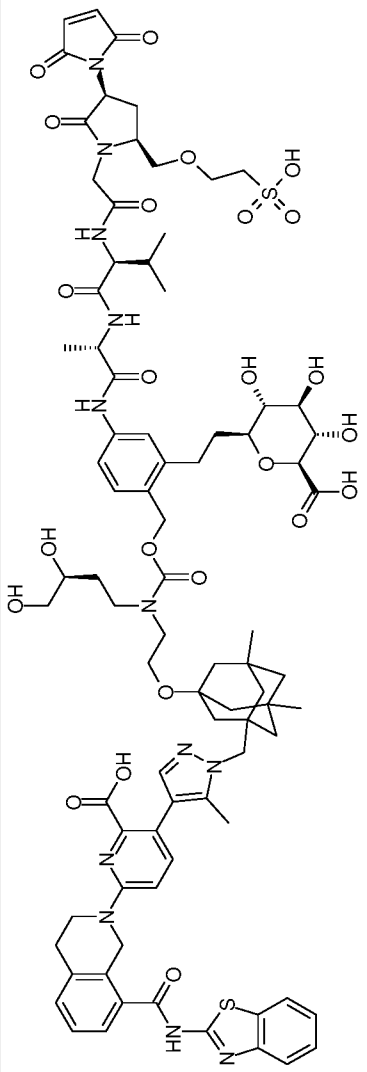
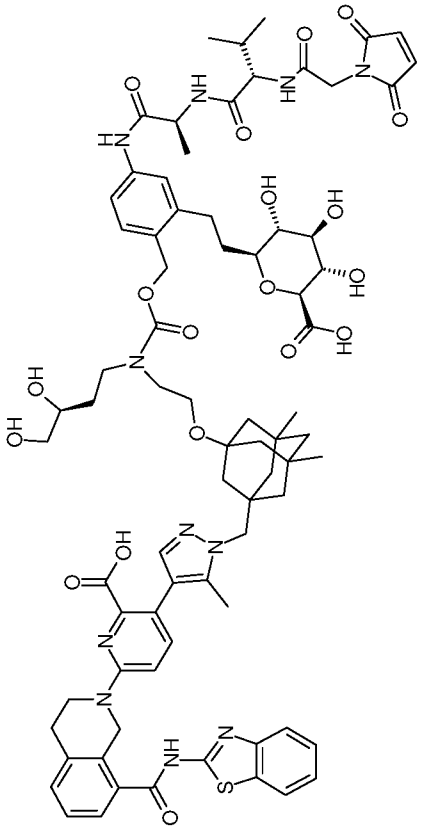
Example No.	Synthon	Synthon structure
2.59	SG	

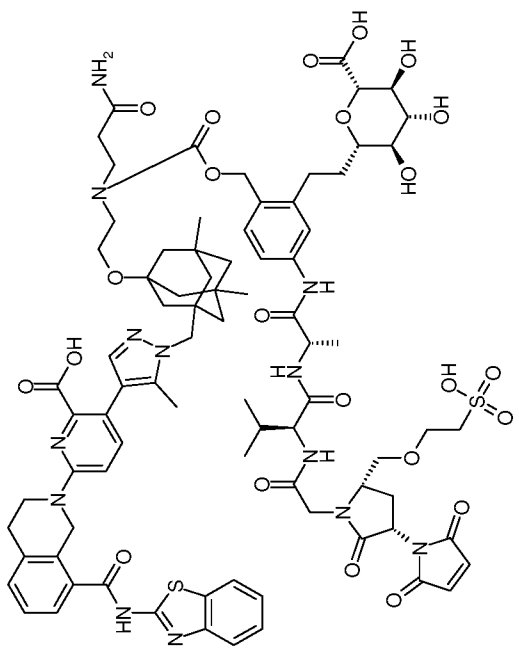
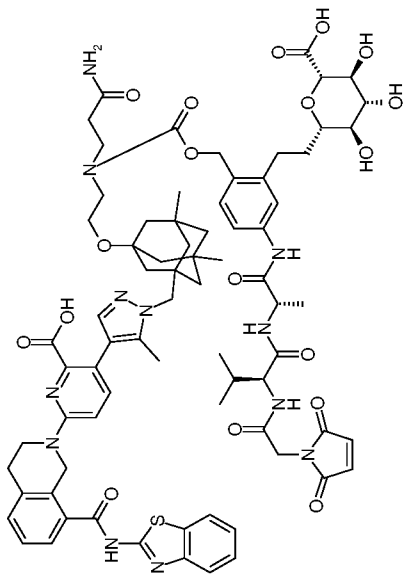
Example No.	Synthon	Synthon structure
2.60	UF	
2.61	VD	

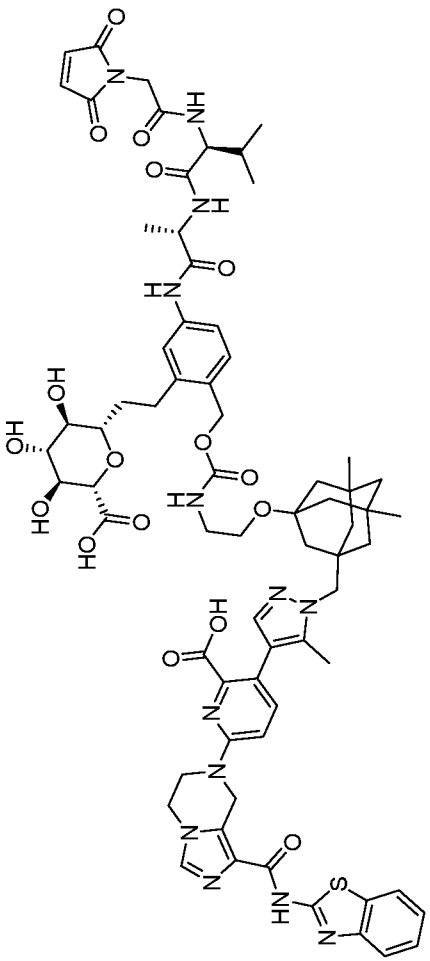
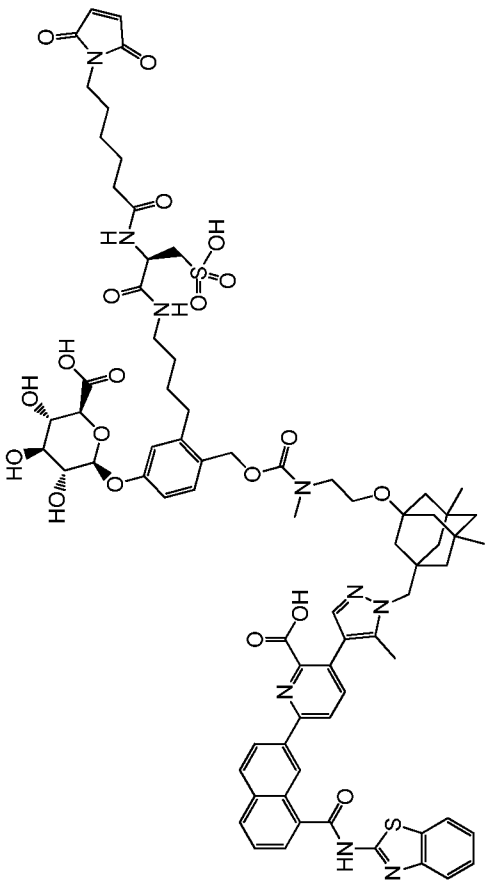
Example No.	Synthon	Synthon structure
2.62	VX	
2.63	WD	

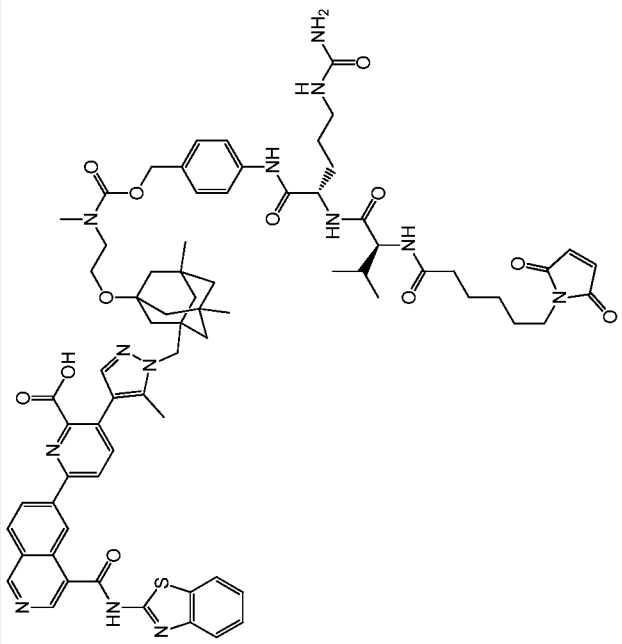
Example No.	Synthon	Synthon structure
2.64 (control)	CZ	
2.65 (control)	TX	

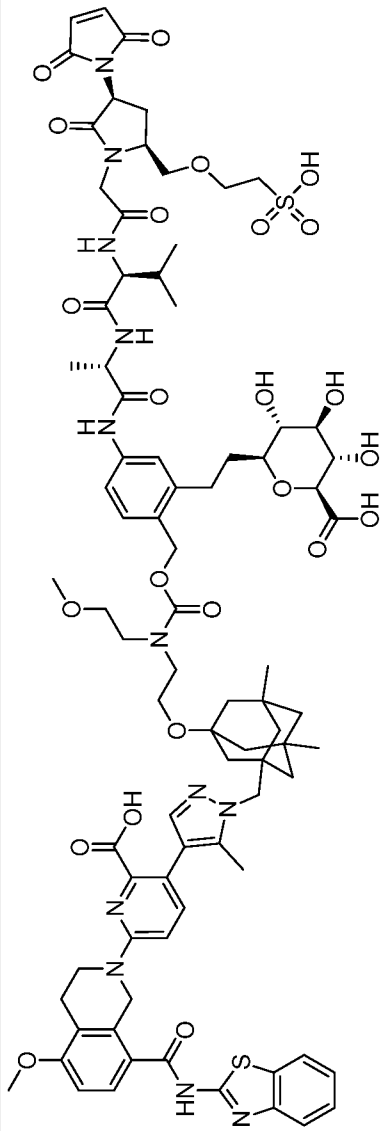
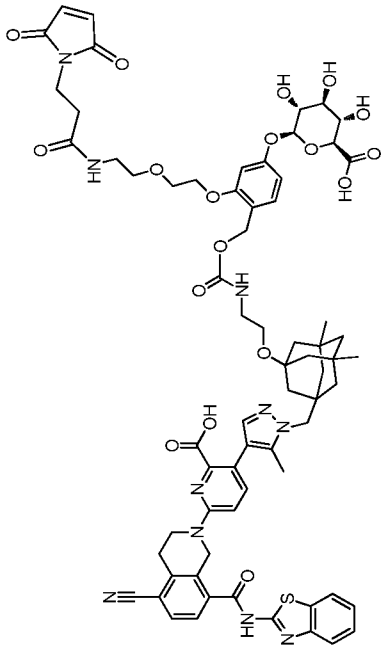
Example No.	Synthon	Synthon structure
2.66 (control)	TV	
2.67 (control)	YY	

Example No.	Synthon	Synthon structure
2.68 (control)	AAA	
2.69 (control)	AAD	

Example No.	Synthon	Synthon structure
2.70 (control)	ZZ	
2.71 (control)	ZT	

Example No.	Synthon	Synthon structure
2.72 (control)	XW	
2.73 (control)	SE	

Example No.	Synthon	Synthon structure
2.74 (control)	SR	

Example No.	Synthon	Synthon structure
2.75 (control)	YG	
2.76 (control)	KZ	

In certain embodiments, the synthon is selected from the group consisting of synthon examples 2.1, 2.2, 2.4, 2.5, 2.6, 2.7, 2.8, 2.10, 2.12, 2.17, 2.18, 2.21, 2.22, 2.23, 2.24, 2.25, 2.26, 2.27, 2.28, 2.29, 2.30, 2.31, 2.32, 2.33, 2.34, 2.35, 2.36, 2.37, 2.38, 2.39, 2.40, 2.41, 2.42, 2.43, 2.44, 2.45, 2.46, 2.47, 2.48, 2.49, 2.50, 2.51, 2.52, 2.53, 2.54, 2.55, 2.56, 2.57, 2.58, 2.59, 2.60, 2.61, 2.62, and 2.63, or a pharmaceutically acceptable salt thereof. The compound names of these synthon are provided below:

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl) carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy) methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({(1s,3s)-3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-

methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[(2S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid;

1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,20-dioxo-7,10,13,16-tetraoxa-3,19-diazadocos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-methyl-10,26-dioxo-3,6,13,16,19,22-hexaoxa-9,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl](methyl)amino}ethoxy)ethoxy]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetratriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,26-dioxo-7,10,13,16,19,22-hexaoxa-3,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide;

(6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid;

(6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{4-[({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)amino]butyl}phenyl beta-D-glucopyranosiduronic acid;

3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium; and

(6S)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{[N-({(3S,5S)-

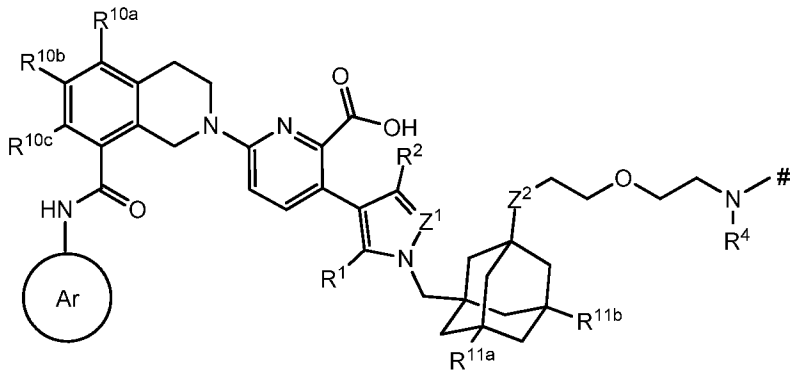
3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid.

In one embodiment, the present invention is directed to a synthon according to structural formula D-L^2-R^x, or a pharmaceutically acceptable salt thereof, wherein:

D is the Bcl-xL inhibitor drug according to structural formula (IIa);

L^2 is the linker selected from the group consisting of IVa.8, IVb.16-IVb.19, IVc.3-IVc.6, IVd.1-IVd.4, Vb.5-Vb.10, Vc.11, Vd.3-Vd.6, VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8 and VIIc.1-VIIc.6; and

R^x is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,



(IIa)

or a pharmaceutically acceptable salt thereof, wherein Ar, R^1, R^2, R^4, R^{10a}, R^{10b}, R^{10c}, R^{11a}, R^{11b}, Z^1, Z^2, and *n* are as previously defined for structural formula (IIa).

In certain embodiments, R^x comprises a maleimide, an acetyl halide, or a vinyl sulfone.

In certain embodiments, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.$1^{3,7}$]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.$1^{3,7}$]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.$1^{3,7}$]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

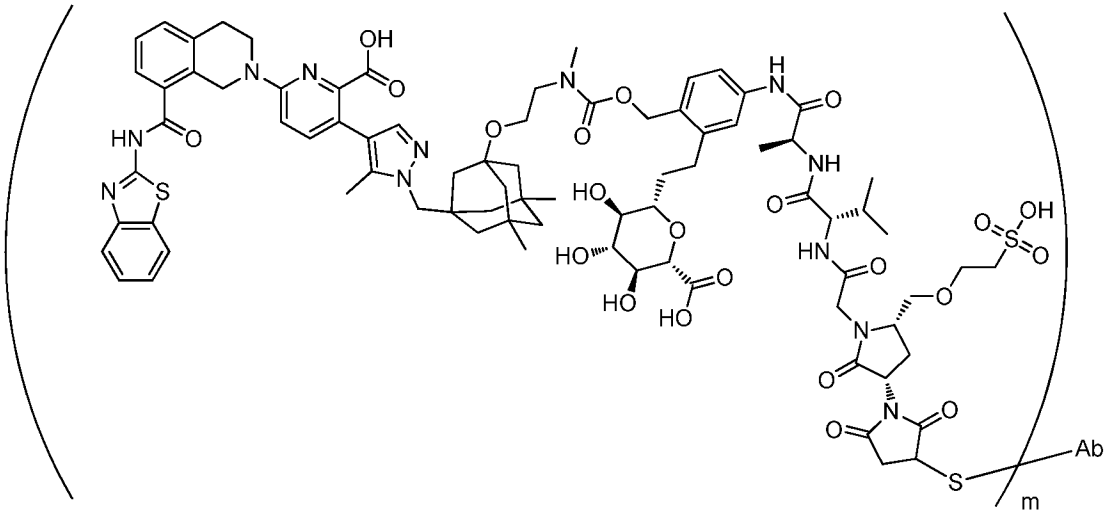
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

and a pharmaceutically acceptable salt thereof.

In certain embodiments, the linker L^2 comprises a segment according to structural formula IVc.5, IVc.6, IVd.3, IVd.4, Vb.9, VIIa.1, VIIa.2, VIIc.1, VIIc.4, VIIc.5, as described above, wherein,  represents the point of attachment of the linker to the Bcl-xL inhibitor,

In certain embodiments, the synthons of the present invention is selected from the group consisting of synthon examples 2.54 (LX), 2.55 (MJ), 2.56 (NH), 2.57 (OV), 2.58 (QS), 2.59 (SG), 2.60 (UF), 2.61 (VD), 2.62 (VX), 2.63 (WD), and a pharmaceutically acceptable salt thereof. In a more specific embodiment, the synthons of the present invention is selected from the group consisting of synthon examples 2.61 (VD) and 2.63 (WD) and a pharmaceutically acceptable salt thereof.

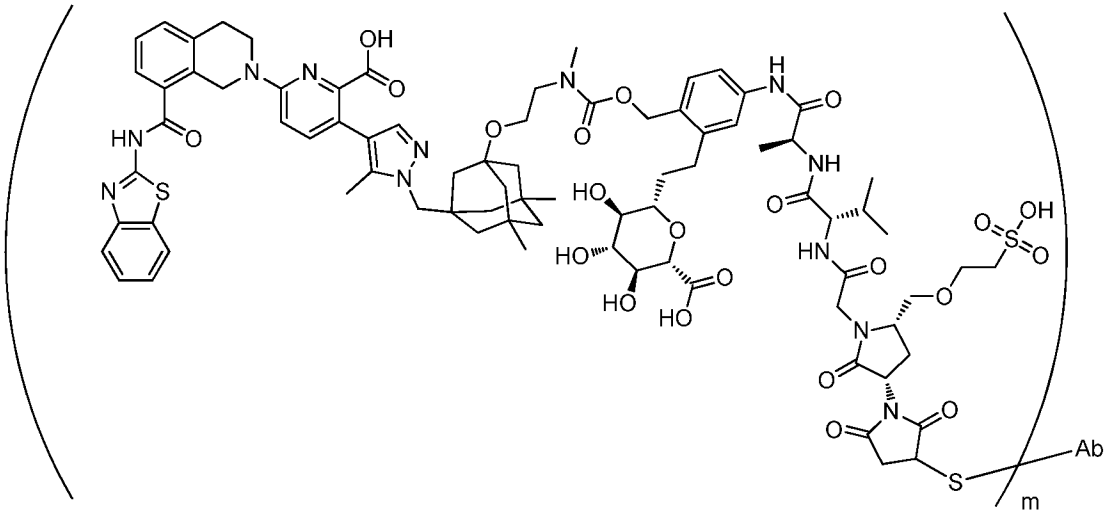
In one embodiment, the ADC , or a pharmaceutically acceptable salt thereof, is:



(i),

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; optionally wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

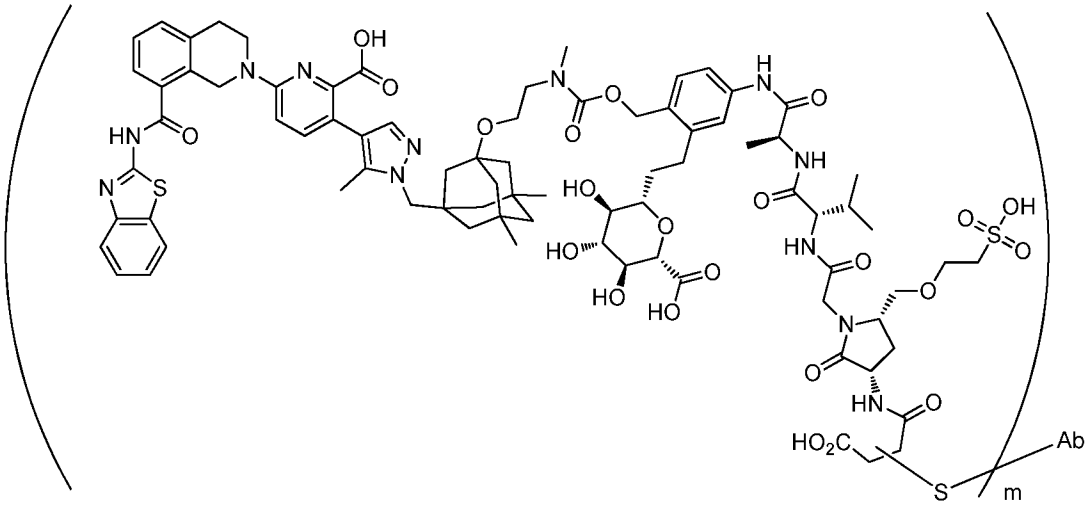
In one embodiment, the ADC , or a pharmaceutically acceptable salt thereof, is:



(i),

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; optionally, wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

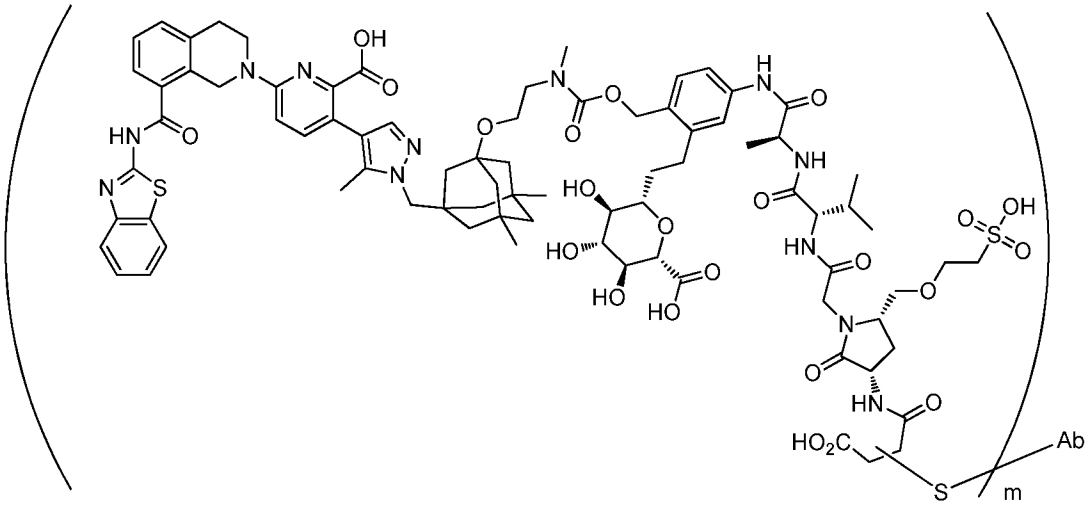
In one embodiment, the ADC , or a pharmaceutically acceptable salt thereof, is:



(ii),

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; optionally wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

In one embodiment, the ADC , or a pharmaceutically acceptable salt thereof, is:



(ii),

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; optionally, wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

Bcl-xL inhibitors, including warheads and synthons, and methods of making the same are described in WO 2016/094505 (AbbVie Inc.), which is incorporated by reference herein.

5. Methods of Synthesis of ADCs

The Bcl-xL inhibitors and synthons described herein may be synthesized using standard, known techniques of organic chemistry. General schemes for synthesizing Bcl-xL inhibitors and synthons that may be used as-is or modified to synthesize the full scope of Bcl-xL inhibitors and synthons described herein are provided below. Specific methods for synthesizing exemplary

Bcl-xL inhibitors and synthons that may be useful for guidance are provided in the Examples section.

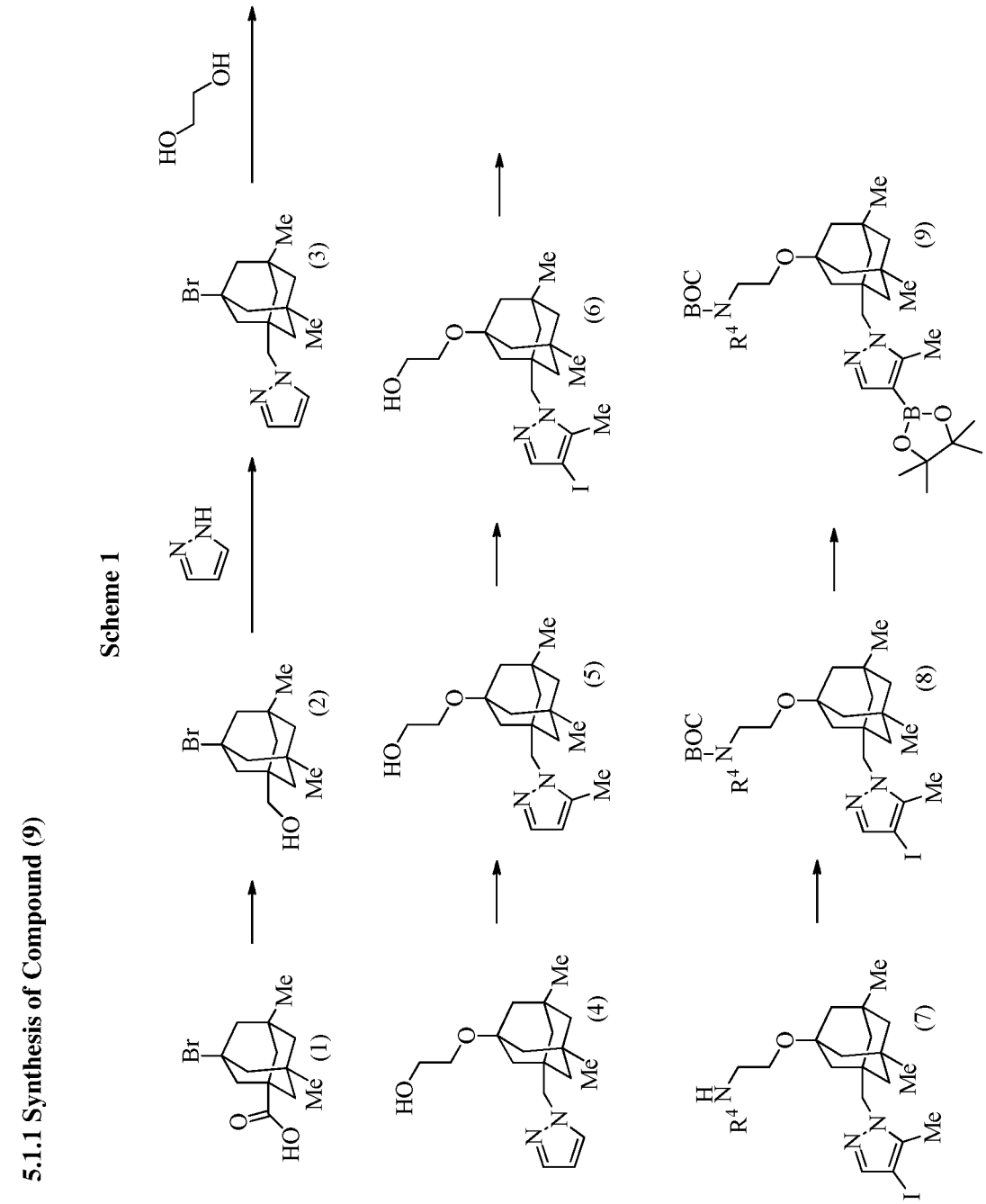
ADCs may likewise be prepared by standard methods, such as methods analogous to those described in Hamblett *et al.*, 2004, "Effects of Drug Loading on the Antitumor Activity of a Monoclonal Antibody Drug Conjugate," *Clin. Cancer Res.* 10:7063-7070; Doronina *et al.*, 2003, "Development of potent and highly efficacious monoclonal antibody auristatin conjugates for cancer therapy," *Nat. Biotechnol.* 21(7):778-784; and Francisco *et al.*, 2003, "cAClO-vcMMAE, an anti-CD30-monomethylauristatin E conjugate with potent and selective antitumor activity," *Blood* 102:1458-1465. For example, ADCs with four drugs per antibody may be prepared by partial reduction of the antibody with an excess of a reducing reagent such as DTT or TCEP at 37 °C for 30 minutes, then the buffer exchanged by elution through SEPHADEX® G-25 resin with 1 mM DTPA in DPBS. The eluent is diluted with further DPBS, and the thiol concentration of the antibody may be measured using 5,5'-dithiobis(2-nitrobenzoic acid) [Ellman's reagent]. An excess, for example 5-fold, of a linker-drug synthon is added at 4 °C for 1 hour, and the conjugation reaction may be quenched by addition of a substantial excess, for example 20-fold, of cysteine. The resulting ADC mixture may be purified on SEPHADEX G-25 equilibrated in PBS to remove unreacted synthons, desalted if desired, and purified by size-exclusion chromatography. The resulting ADC may then be then sterile filtered, for example, through a 0.2 μm filter, and lyophilized if desired for storage. In certain embodiments, all of the interchain cysteine disulfide bonds are replaced by linker-drug conjugates.

Specific methods for synthesizing exemplary ADCs that may be used to synthesize the full range of ADCs described herein are provided in the Examples section.

5.1 General Methods for Synthesizing Bcl-xL Inhibitors

5.1.1 Synthesis of Compound (9)

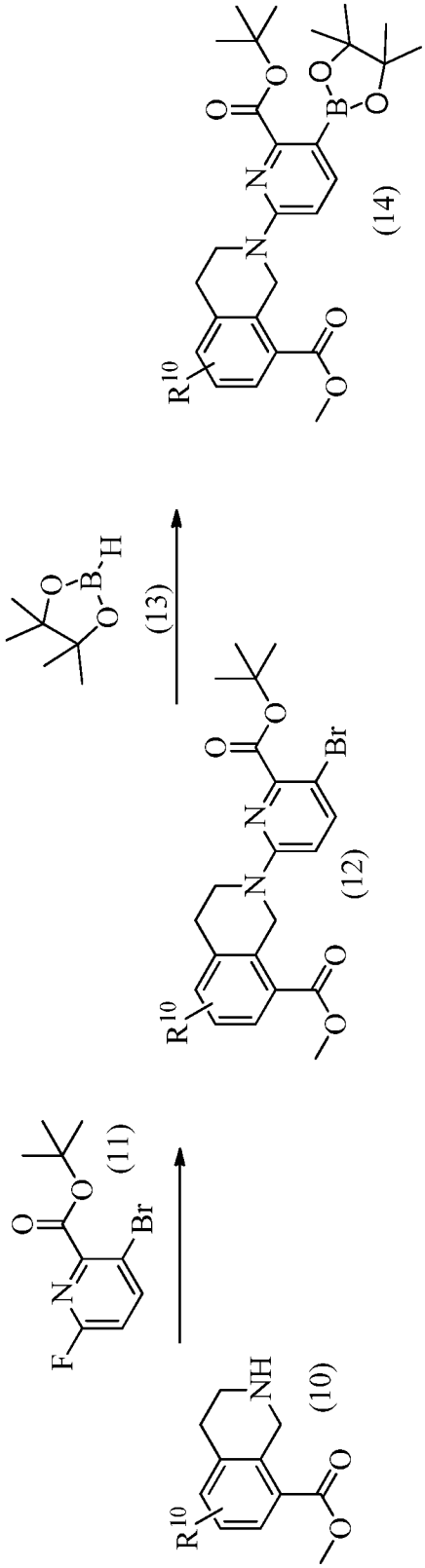
Scheme 1



The synthesis of pyrazole intermediate, formula (9), is described in Scheme 1. 3-Bromo-5,7-dimethyladamantanecarboxylic acid (1) can be treated with BH_3·THF to provide 3-bromo-5,7-dimethyladamantanemethanol (2). The reaction is typically performed at ambient temperature in a solvent, such as, but not limited to, tetrahydrofuran. 1-((3-Bromo-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-1H-pyrazole (3) can be prepared by treating 3-bromo-5,7-dimethyladamantanemethanol (2) with 1H-pyrazole in the presence of cyanomethylenetributylphosphorane. The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to, toluene. 1-((3-Bromo-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-1H-pyrazole (3) can be treated with ethane-1,2-diol in the presence of a base such as, but not limited to, triethylamine, to provide 2-{[3,5-dimethyl-7-(1*H*-pyrazol-1-ylmethyl)tricyclo[3.3.1.1^{3,7}]dec-1-yl]oxy}ethanol (4). The reaction is typically performed at an elevated temperature, and the reaction may be performed under microwave conditions. 2-{[3,5-Dimethyl-7-(1*H*-pyrazol-1-ylmethyl)tricyclo[3.3.1.1^{3,7}]dec-1-yl]oxy}ethanol (4) can be treated with a strong base, such as, but not limited to, n-butyllithium, followed by the addition of iodomethane, to provide 2-({3,5-dimethyl-7-[(5-methyl-1*H*-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethanol (5). The addition and reaction is typically performed in a solvent such as, but not limited to, tetrahydrofuran, at a reduced temperature before warming up to ambient temperature for work up. 2-({3,5-Dimethyl-7-[(5-methyl-1*H*-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethanol (5) can be treated with N-iodosuccinimide to provide 1-({3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole (6). The reaction is typically performed at ambient temperature is a solvent such as, but not limited to, N,N-dimethylformamide. Compounds of formula (7) can be prepared by reacting 1-({3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole (6) with methanesulfonyl chloride, in the presence of a base such as, but not limited to, triethylamine, followed by the addition of amine, H_2NR^4. The reaction with methanesulfonyl chloride is typically performed at low temperature, before increasing the temperature for the reaction with the amine, and the reaction is typically performed in a solvent such as, but not limited to tetrahydrofuran. Compounds of formula (7) can be reacted with di-tert-butyl dicarbonate in the presence of 4-dimethylaminopyridine to provide compounds of formula (8). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to tetrahydrofuran. The borylation of compounds of formula (8) to provide compounds of formula (9) can be performed under conditions described herein and readily available in the literature.

5.1.2 Synthesis of Compound (14)

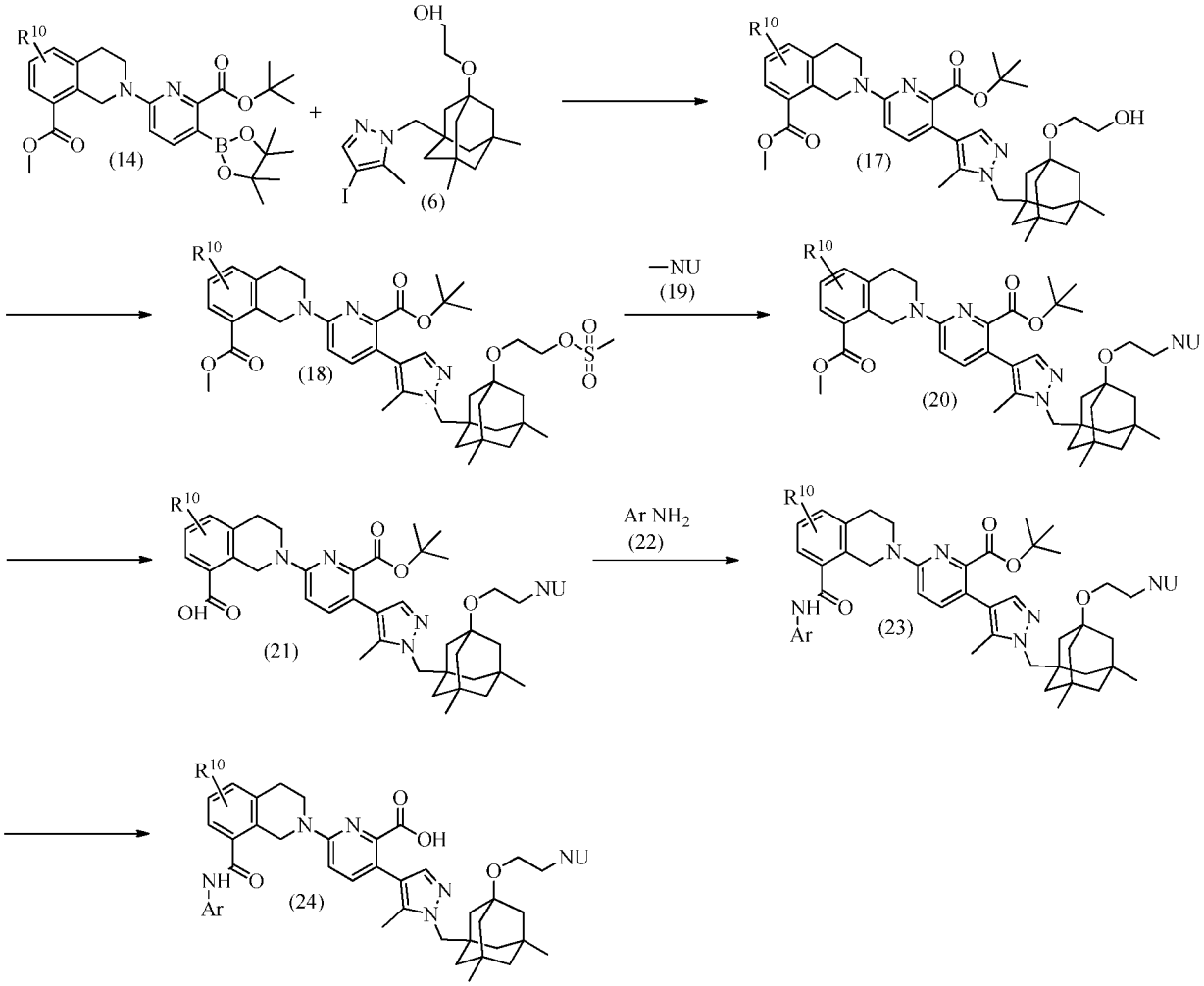
Scheme 2



The synthesis of intermediate, formula (14), is described in Scheme 2. Compounds of formula (12) can be prepared by reacting compounds of formula (10), with *tert*-butyl 3-bromo-6-fluoropicolinate (11) in the presence of a base, such as, but not limited to, N,N-diisopropylethylamine, or trimethylamine. The reaction is typically performed under an inert atmosphere at an elevated temperature in a solvent, such as, but not limited to, dimethyl sulfoxide. Compounds of formula (12) can be reacted with 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (13), under borylation conditions described herein or in the literature to provide compounds of formula (14).

5.1.3 Synthesis of Compound (24)

Scheme 3

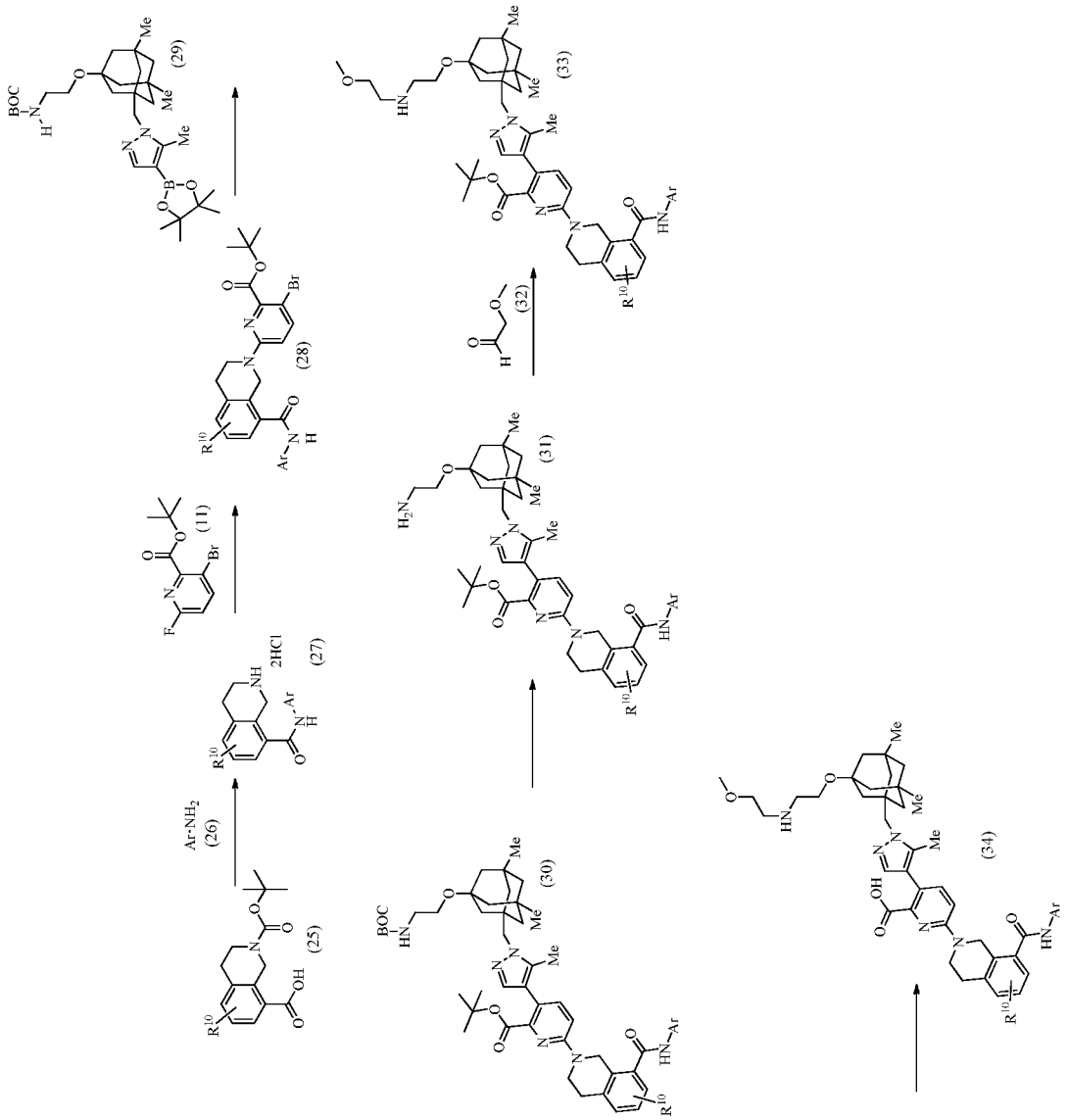


Scheme 3 describes a method to make intermediates that contain –Nu (nucleophile) tethered to an adamantine and a picolinate protected as a t-butyl ester. Methyl 2-(6-(*tert*-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (14) can be reacted with 1-({3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole (6) under

Suzuki Coupling conditions described herein or in the literature to provide methyl 2-(6-(*tert*-butoxycarbonyl)-5-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (17). Methyl 2-(6-(*tert*-butoxycarbonyl)-5-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (17) can be treated with a base such as but not limited to triethylamine, followed by methanesulfonyl chloride to provide methyl 2-(6-(*tert*-butoxycarbonyl)-5-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (18). The addition is typically performed at low temperature before warming up to ambient temperature in a solvent, such as, but not limited to, dichloromethane. Methyl 2-(6-(*tert*-butoxycarbonyl)-5-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (18) can be reacted with a nucleophile (Nu) of formula (19) to provide compounds of formula (20). Examples of nucleophiles include, but are not limited to, sodium azide, methylamine, ammonia and di-tert-butyl iminodicarbonate. Compounds of formula (20) can be reacted with lithium hydroxide to provide compounds of formula (21). The reaction is typically performed at ambient temperature in a solvent such as but not limited to tetrahydrofuran, methanol, water, or mixtures thereof. Compounds of formula (21) can be reacted with compounds of formula (22), wherein Ar is as described herein, under amidation conditions described herein or readily available in the literature to provide compounds of formula (23). Compounds of the formula (23) can be treated with acids, such as trifluoroacetic acid or HCl, in solvents, such as but not limited to dichloromethane or dioxane, to provide compounds of the formula (24).

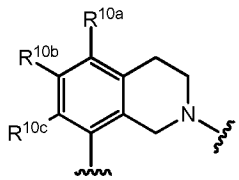
5.1.4 Synthesis of Compound (34)

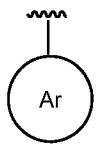
Scheme 4



The synthesis of compound (34) is described in Scheme 4. Compounds of formula (25) can be reacted with compounds of formula (26), wherein Ar is as described herein, under amidation conditions described herein or readily available in the literature to provide compounds of formula (27). Compounds of formula (27) can be reacted with *tert*-butyl 3-bromo-6-fluoropicolinate (11) in the presence of a base such as, but not limited to, cesium carbonate, to provide compounds of formula (28). The reaction is typically performed at elevated temperature in a solvent such as, but not limited to, N,N-dimethylacetamide. Compounds of formula (30) can be prepared by reacting compounds of formula (28) with a boronate ester (or the equivalent boronic acid) of formula (29) under Suzuki Coupling conditions described herein or in the literature. Compounds of formula (31) can be prepared by treating compounds of formula (30) with trifluoroacetic acid. The reaction is typically performed at ambient temperature in a solvent such as but not limited to dichloromethane. Compounds of formula (31) can be reacted with 2-methoxyacetaldehyde (32) followed by a reducing agent such as, but not limited to, sodium borohydride, to provide compounds of formula (33). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, dichloromethane, methanol, or mixtures thereof. Compounds of the formula (33) can be treated with acids, such as trifluoroacetic acid or HCl, in solvents, such as but not limited to dichloromethane or dioxane, to provide compounds of the formula (34).

5.2 General Methods for Synthesizing Synthons

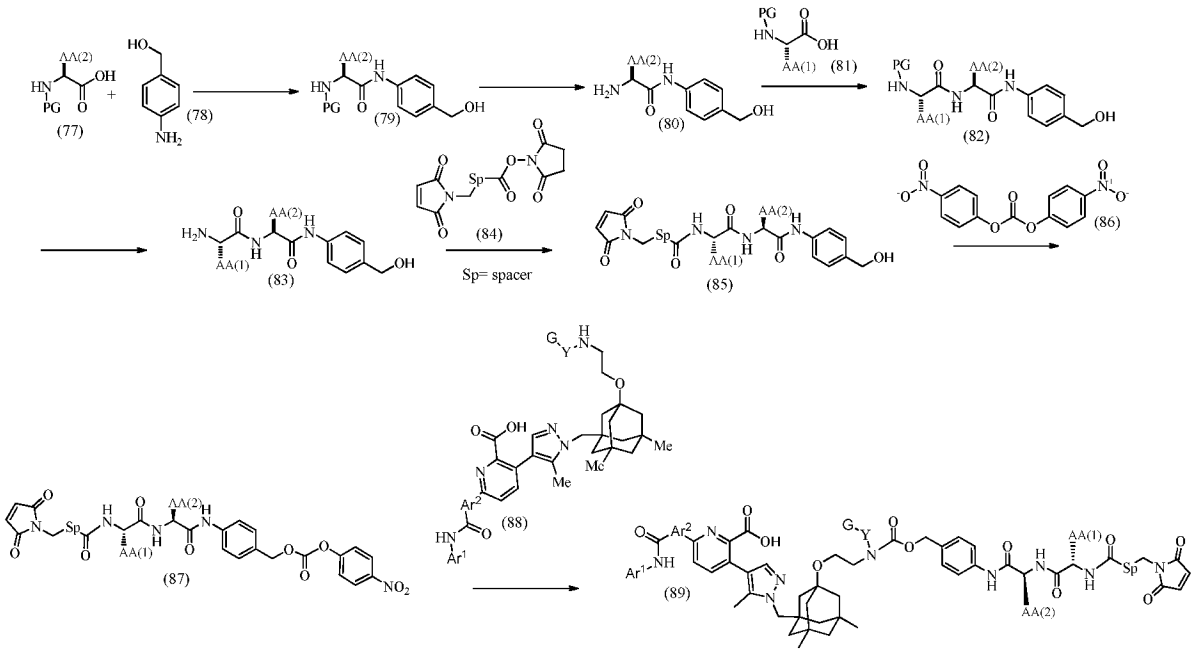
In the following schemes, the variable Ar^2 represents 

in the compound of formula (IIa) and the variable Ar^1 represents 

in the compound of formula (iia).

5.2.1 Synthesis of Compound (89)

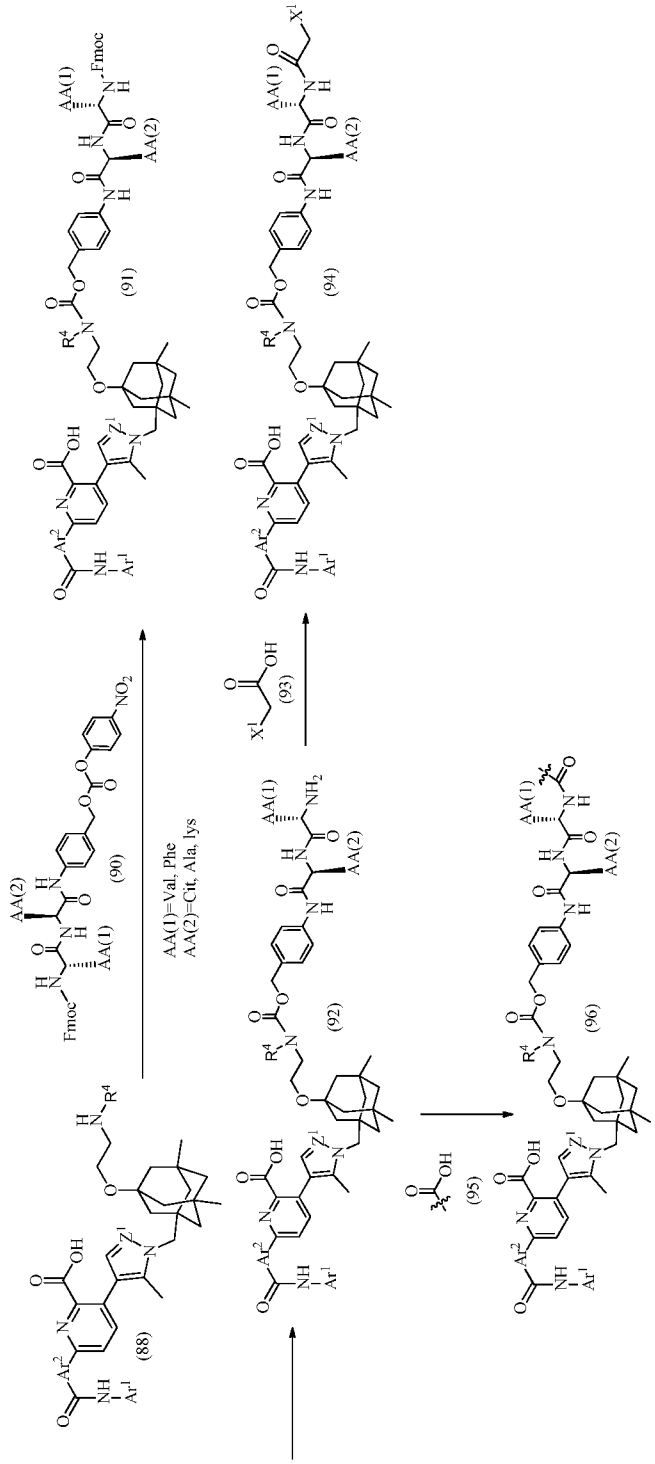
Scheme 5



As shown in scheme 5, compounds of formula (77), wherein PG is an appropriate base labile protecting group and AA(2) is Cit, Ala, or Lys, can be reacted with 4-(aminophenyl)methanol (78), under amidation conditions described herein or readily available in the literature to provide compound (79). Compound (80) can be prepared by reacting compound (79) with a base such as, but not limited to, diethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (81), wherein PG is an appropriate base or acid labile protecting group and AA(1) is Val or Phe, can be reacted with compound (80), under amidation conditions described herein or readily available in the literature to provide compound (82). Compound (83) can be prepared by treating compound (82) with diethylamine or trifluoroacetic acid, as appropriate. The reaction is typically performed at ambient temperature in a solvent such as but not limited to dichloromethane. Compound (84), wherein Sp is a spacer, can be reacted with compound (83) to provide compound (85). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (85) can be reacted with bis(4-nitrophenyl) carbonate (86) in the presence of a base such as, but not limited to N,N-diisopropylethylamine, to provide compounds (87). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compounds (87) can be reacted with compound (88) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide compound (89). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide.

5.2.2 Synthesis of Compounds (94) and (96)

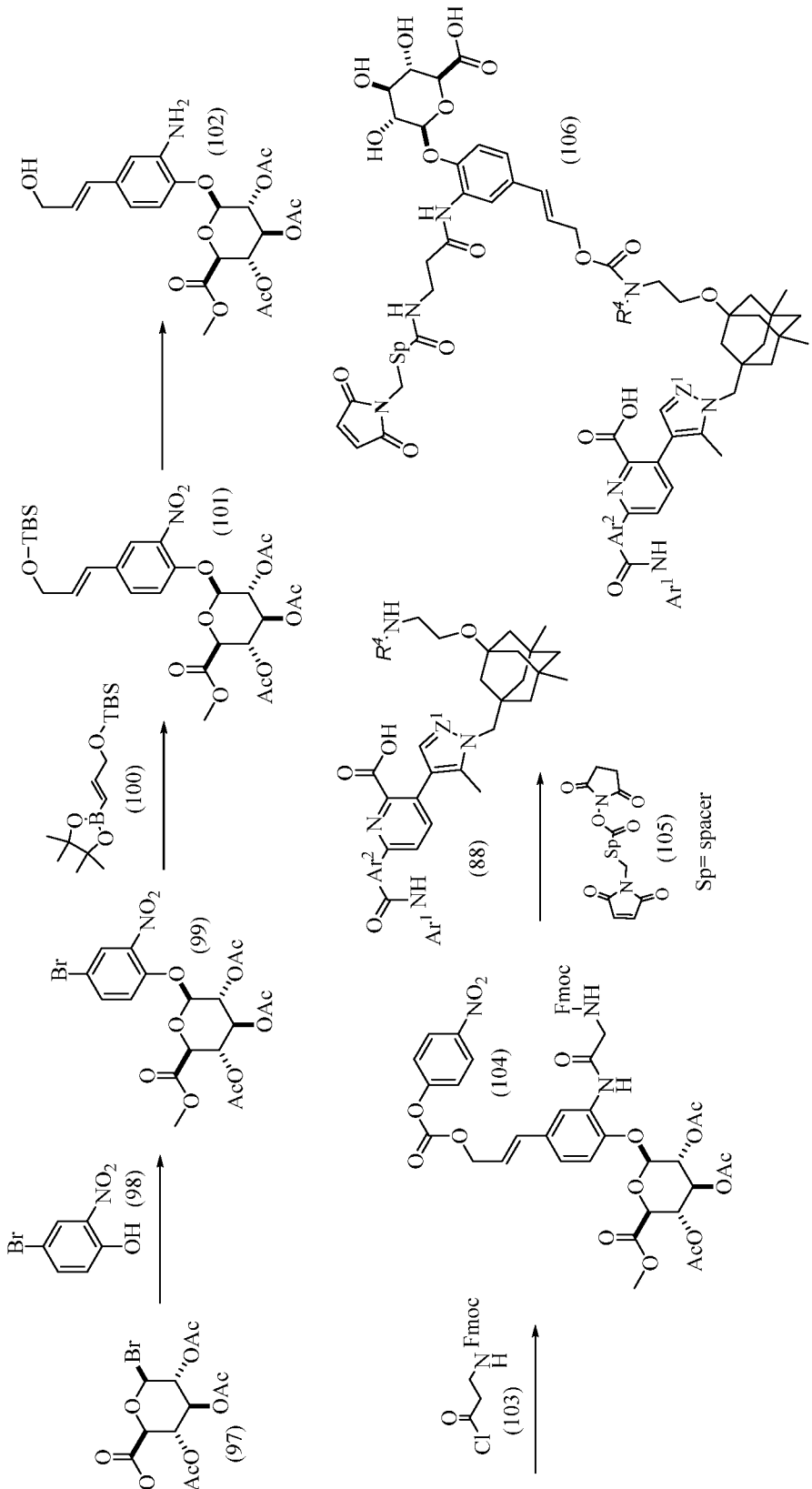
Scheme 6



Scheme 6 describes the installment of alternative mAb-linker attachments to dipeptide synthons. Compound (88) can be reacted with compound (90) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide compound (91). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (92) can be prepared by reacting compound (91) with diethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (93), wherein X^1 is Cl, Br, or I, can be reacted with compound (92), under amidation conditions described herein or readily available in the literature to provide compound (94). Compound (92) can be reacted with compounds of formula (95) under amidation conditions described herein or readily available in the literature to provide compound (96).

5.2.3 Synthesis of Compound (106)

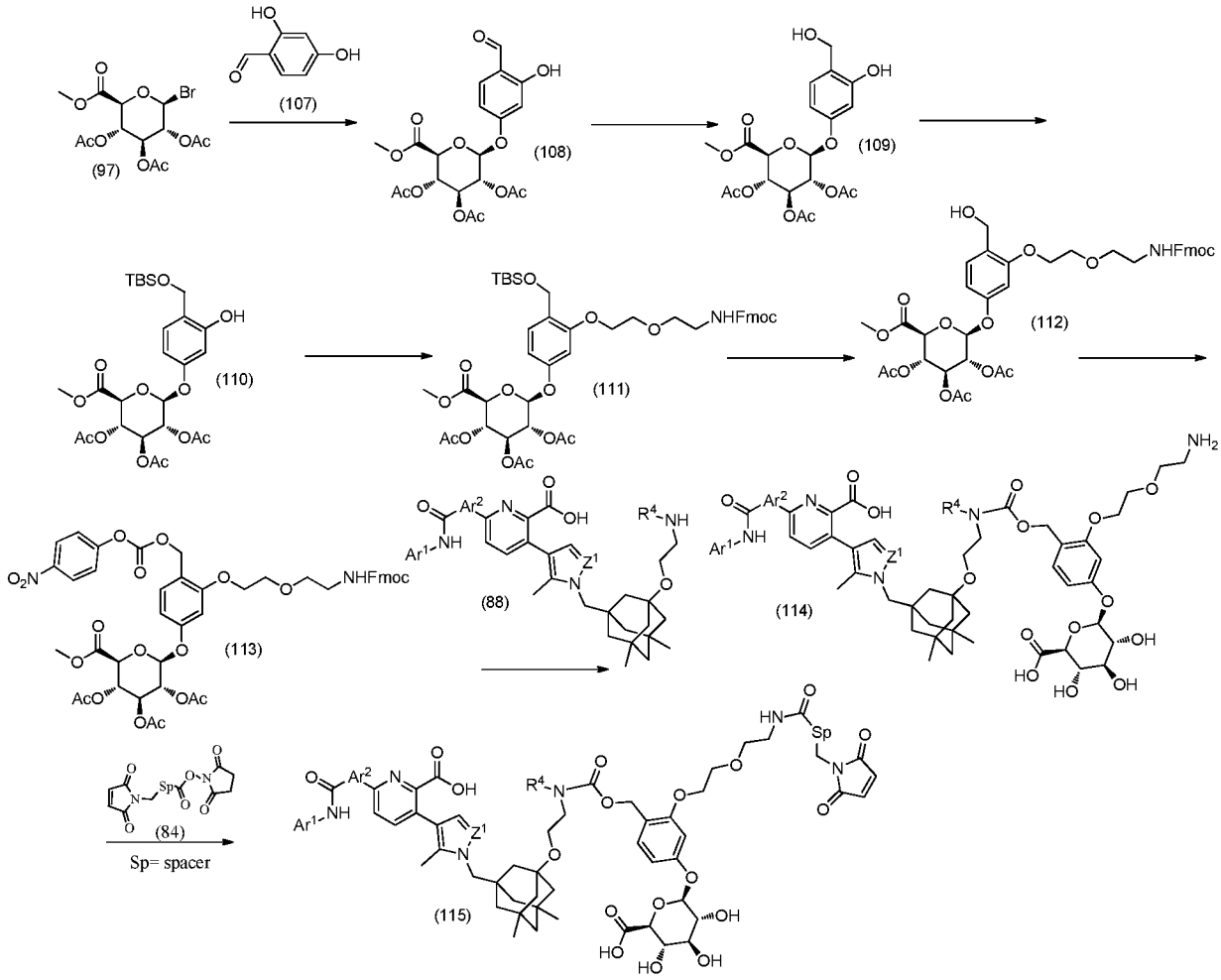
Scheme 7



Scheme 7 describes the synthesis of vinyl glucuronide linker intermediates and synthons. (2R,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (97) can be treated with silver oxide, followed by 4-bromo-2-nitrophenol (98) to provide (2S,3R,4S,5S,6S)-2-(4-bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (99). The reaction is typically performed at ambient temperature in a solvent, such as, but not limited to, acetonitrile. (2S,3R,4S,5S,6S)-2-(4-Bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (99) can be reacted with (E)-*tert*-butyldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane (100) in the presence of a base such as, but not limited to, sodium carbonate, and a catalyst such as but not limited to tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$), to provide (2S,3R,4S,5S,6S)-2-(4-(E)-3-((*tert*-butyldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (101). The reaction is typically performed at an elevated temperature in a solvent, such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (102) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(4-((E)-3-((*tert*-butyldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (101) with zinc in the presence of an acid such as, but not limited to, hydrochloric acid. The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as, but not limited to, tetrahydrofuran, water, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (102) can be reacted with (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate (103), in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (104). The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as, but not limited to, dichloromethane. Compound (88) can be reacted with (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (104) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, followed by work up and reaction with compound of formula (105) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine to provide compound (106). The reactions are typically performed at ambient temperature in a solvent such as, but not limited to N,N-dimethylformamide.

5.2.4 Synthesis of Compound (115)

Scheme 8

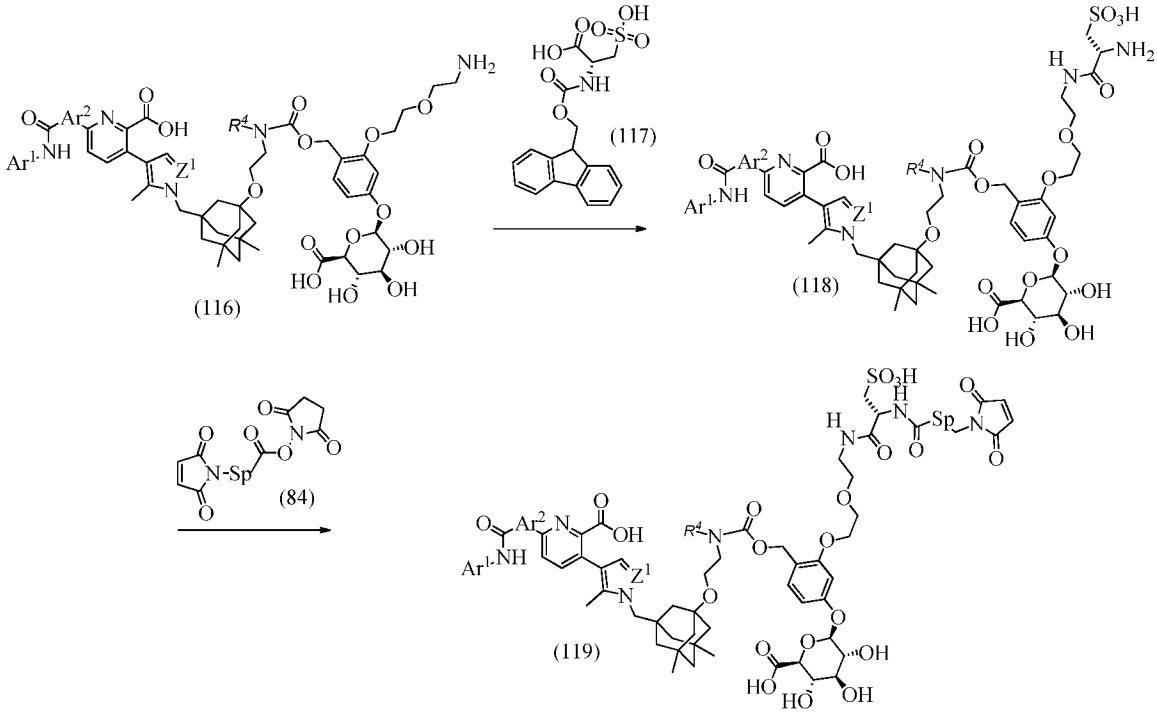


Scheme 8 describes the synthesis of a representative 2-ether glucuronide linker intermediate and synthon. (2S,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (97) can be reacted with 2,4-dihydroxybenzaldehyde (107) in the presence of silver carbonate to provide (2S,3R,4S,5S,6S)-2-(4-formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (108). The reaction is typically performed at an elevated temperature in a solvent, such as, but not limited to, acetonitrile. (2S,3R,4S,5S,6S)-2-(4-Formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (108) can be treated with sodium borohydride to provide (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (109). The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as but not limited to tetrahydrofuran, methanol, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (110) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-

(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (109) with *tert*-butyldimethylsilyl chloride in the presence of imidazole. The reaction is typically performed at low temperature in a solvent, such as, but not limited to, dichloromethane. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (111) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (110) with (9H-fluoren-9-yl)methyl (2-(2-hydroxyethoxy)ethyl)carbamate in in the presence of triphenylphosphine and a azodicarboxylate such as, but not limited to, di-*tert*-butyl diazene-1,2-dicarboxylate. The reaction is typically performed at ambient temperature in a solvent such as but not limited to toluene. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (111) can be treated with acetic acid to provide (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (112). The reaction is typically performed at ambient temperature in a solvent such as but not limited to water, tetrahydrofuran, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (113) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (112) with bis(4-nitrophenyl) carbonate in the presence of a base such as but not limited to N,N-diisopropylethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (113) can be treated with compound (88) in the presence of a base such as but not limited to N,N-diisopropylethylamine, followed by treatment with lithium hydroxide to provide a compound (114). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide, tetrahydrofuran, methanol, or mixtures thereof. Compound (115) can be prepared by reacting compound (114) with compound (84) in the presence of a base such as but not limited to N,N-diisopropylethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide.

5.2.5 Synthesis of Compound (119)

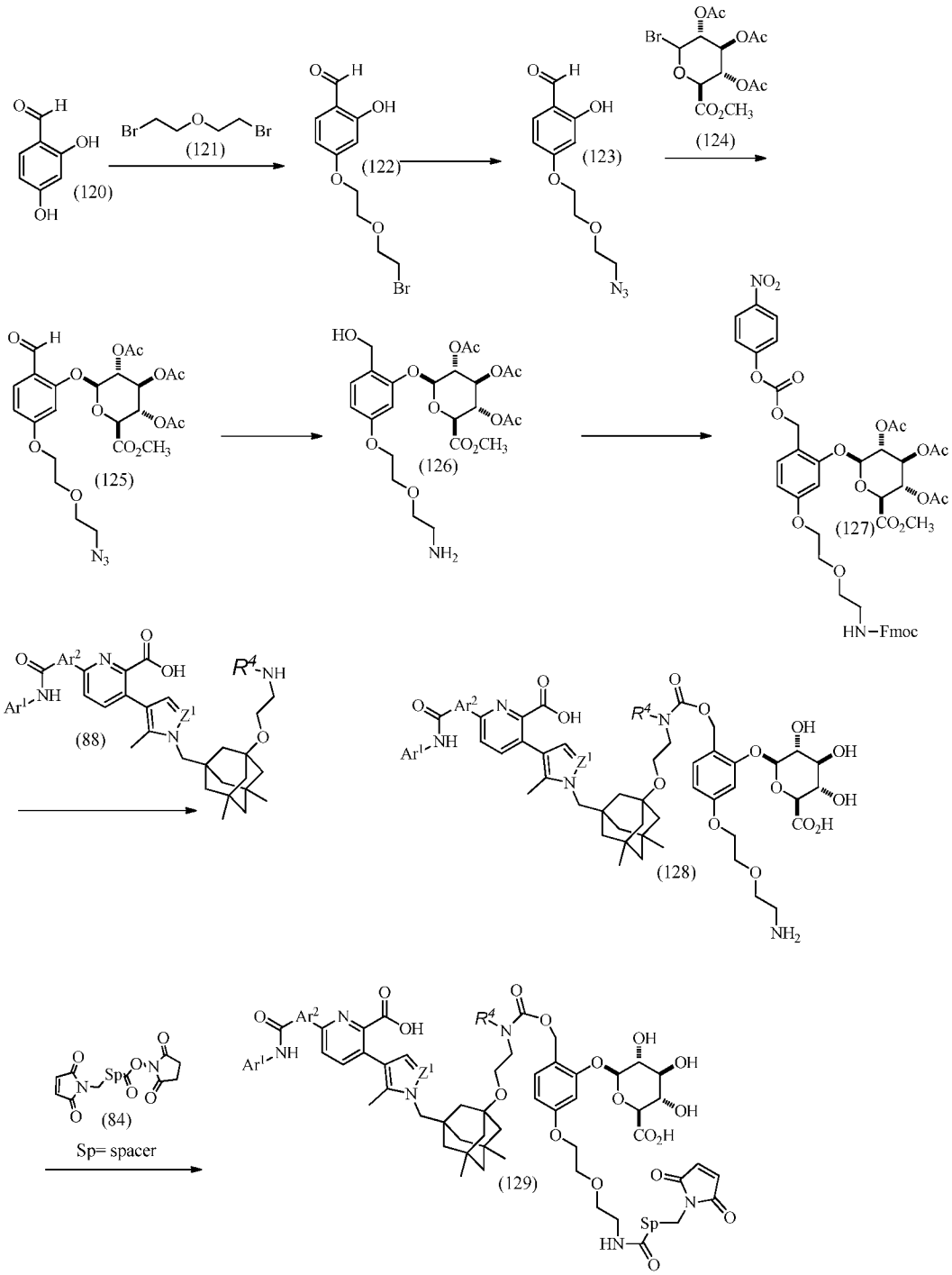
Scheme 9



Scheme 9 describes the introduction of a second solubilizing group to a sugar linker. Compound (116) can be reacted with (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (117), under amidation conditions described herein or readily available in the literature, followed by treatment with a base such as but not limited to diethylamine, to provide compound (118). Compound (118) can be reacted with compound (84), wherein Sp is a spacer, under amidation conditions described herein or readily available in the literature, to provide compound (119).

5.2.6 Synthesis of Compound (129)

Scheme 10

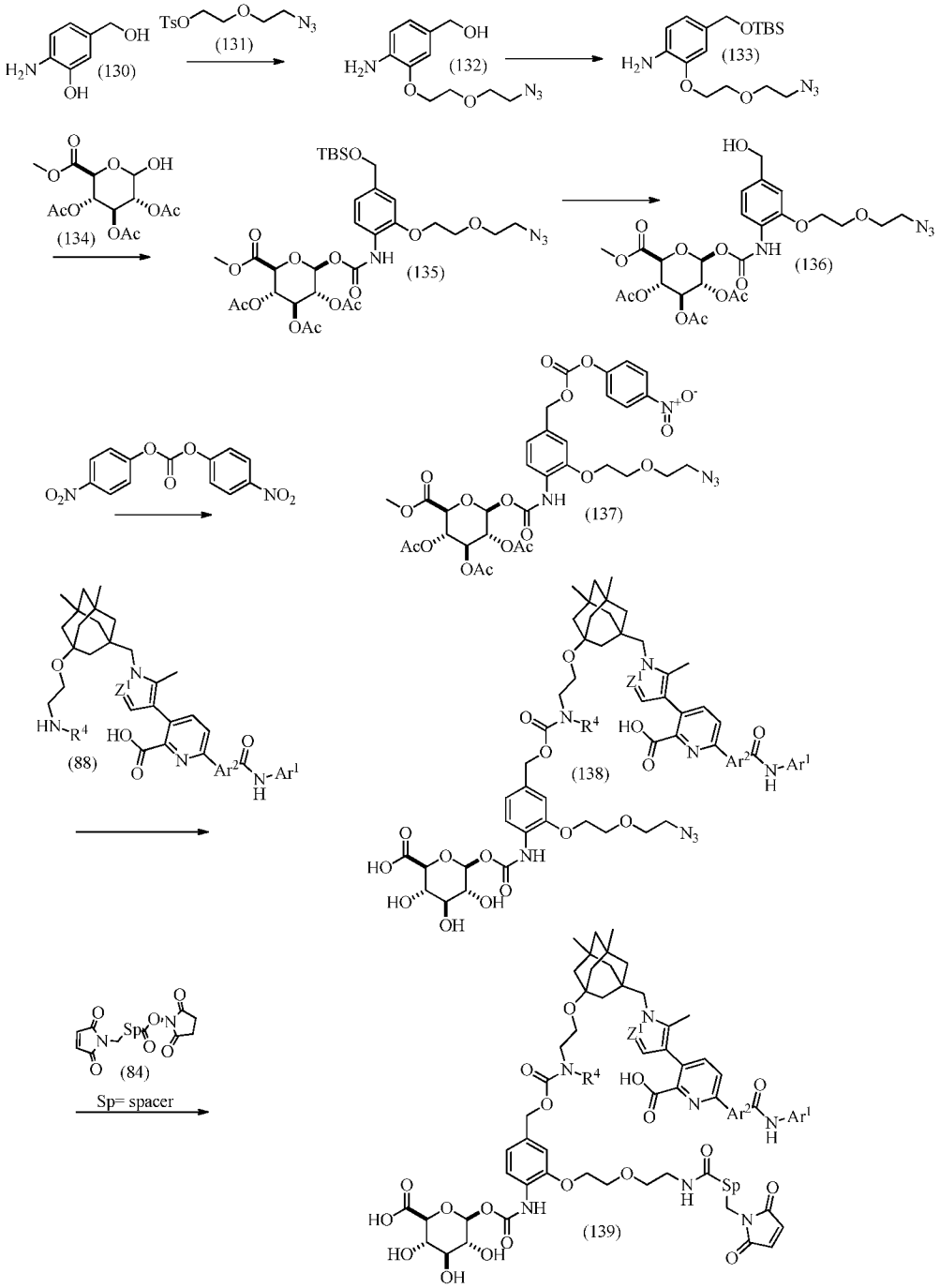


Scheme 10 describes the synthesis of 4-ether glucuronide linker intermediates and synthons. 4-(2-(2-Bromoethoxy)ethoxy)-2-hydroxybenzaldehyde (122) can be prepared by reacting 2,4-dihydroxybenzaldehyde (120) with 1-bromo-2-(2-bromoethoxy)ethane (121) in the presence of a base such as, but not limited to, potassium carbonate. The reaction is typically performed at an elevated temperature in a solvent such as but not limited to acetonitrile. 4-(2-(2-Bromoethoxy)ethoxy)-2-hydroxybenzaldehyde (122) can be treated with sodium azide to provide

4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde (123). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. (2S,3R,4S,5S,6S)-2-(5-(2-(2-Azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (125) can be prepared by reacting 4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde (123) with (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (124) in the presence of silver oxide. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, acetonitrile. Hydrogenation of (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (125) in the presence of Pd/C will provide (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (126). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6S)-2-(5-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (127) can be prepared by treating (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (126) with (9H-fluoren-9-yl)methyl carbonochloridate in the presence of a base, such as, but not limited to, N,N-diisopropylethylamine. The reaction is typically performed at low temperature in a solvent such as, but not limited to, dichloromethane. Compound (88) can be reacted with (2S,3R,4S,5S,6S)-2-(5-(2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (127) in the presence of a base, such as, but not limited to, N,N-diisopropylethylamine, followed by treatment with lithium hydroxide to provide compound (128). The reaction is typically performed at low temperature in a solvent such as, but not limited to, N,N-dimethylformamide. Compound (129) can be prepared by reacting compound (128) with compound (84) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide.

5.2.7 Synthesis of Compound (139)

Scheme 11

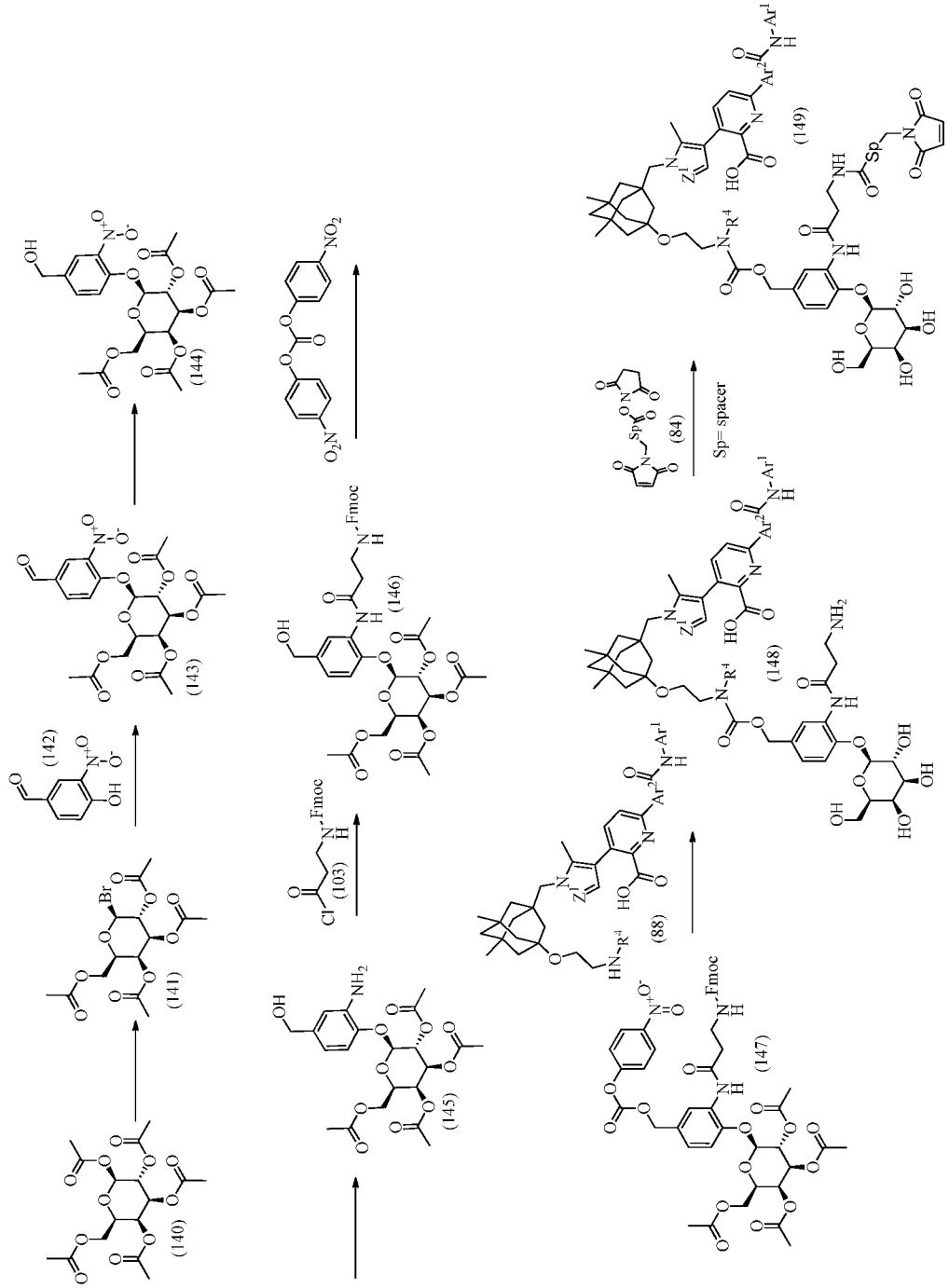


Scheme 11 describes the synthesis of carbamate glucuronide intermediates and synthons. 2-Amino-5-(hydroxymethyl)phenol (130) can be treated with sodium hydride and then reacted with 2-(2-Azidoethoxy)ethyl 4-methylbenzenesulfonate (131) to provide (4-amino-3-(2-(2-azidoethoxy)ethoxy)phenyl)methanol (132). The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to N,N-dimethylformamide. 2-(2-(2-Azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (133) can be prepared by reacting (4-amino-3-(2-(2-azidoethoxy)ethoxy)phenyl)methanol (132) with tert-butyldimethylchlorosilane in the presence of imidazole. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to tetrahydrofuran. 2-(2-(2-

Azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (133) can be treated with phosgene, in the presence of a base such as but not limited to trimethylamine, followed by reaction with (3R,4S,5S,6S)-2-hydroxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (134) in the presence of a base such as but not limited to trimethylamine, to provide (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (135). The reaction is typically performed in a solvent such as, but not limited to, toluene, and the additions are typically performed at low temperature, before warming up to ambient temperature after the phosgene addition and heating at an elevated temperature after the (3R,4S,5S,6S)-2-hydroxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (134) addition. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-(hydroxymethyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (136) can be prepared by reacting 2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (135) with p-toluenesulfonic acid monohydrate. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to methanol. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-(hydroxymethyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (136) can be reacted with bis(4-nitrophenyl)carbonate in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (137). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (137) can be reacted with compound in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, followed by treatment with aqueous lithium hydroxide, to provide compound (138). The first step is typically conducted at ambient temperature in a solvent such as, but not limited to N,N-dimethylformamide, and the second step is typically conducted at low temperature in a solvent such as but not limited to methanol. Compound (138) can be treated with tris(2-carboxyethyl))phosphine hydrochloride, followed by reaction with compound (84) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide compound (139). The reaction with tris(2-carboxyethyl))phosphine hydrochloride is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran, water, or mixtures thereof, and the reaction with N-succinimidyl 6-maleimidohexanoate is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide.

5.2.8 Synthesis of Compound (149)

Scheme 12

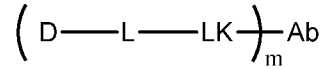


Scheme 12 describes the synthesis of galactoside linker intermediates and synthons. (2S,3R,4S,5S,6R)-6-(Acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (140) can be treated with HBr in acetic acid to provide (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate (141). The reaction is typically performed at ambient temperature under a nitrogen atmosphere. (2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(4-formyl-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (143) can be prepared by treating (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate (141) with silver(I) oxide in the presence of 4-hydroxy-3-nitrobenzaldehyde (142). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, acetonitrile. (2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(4-formyl-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (143) can be treated with sodium borohydride to provide (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (144). The reaction is typically performed at low temperature in a solvent such as but not limited to tetrahydrofuran, methanol, or mixtures thereof. (2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(2-amino-4-(hydroxymethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (145) can be prepared by treating (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (144) with zinc in the presence of hydrochloric acid. The reaction is typically performed at low temperature, under a nitrogen atmosphere, in a solvent such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (146) can be prepared by reacting (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(2-amino-4-(hydroxymethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (145) with (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate (103) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine. The reaction is typically performed at low temperature, in a solvent such as, but not limited to, dichloromethane. (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (146) can be reacted with bis(4-nitrophenyl)carbonate in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (147). The reaction is typically performed at low temperature, in a solvent such as, but not limited to, N,N-dimethylformamide. (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (147) can be reacted with compound (88) in the presence of a base such as, but not

limited to N,N-diisopropylethylamine, followed by treatment with lithium hydroxide, to provide compound (148). The first step is typically performed at low temperature, in a solvent such as, but not limited to, N,N-dimethylformamide, and the second step is typically performed at ambient temperature, in a solvent such as, but not limited to, methanol. Compound (148) can be treated with compound (84), wherein Sp is a spacer, in the presence of a base, such as, but not limited to N,N-diisopropylethylamine, to provide compound (149). The reaction is typically performed at ambient temperature, in a solvent such as, but not limited to, N,N-dimethylformamide.

5.3 General Methods for Synthesizing Anti-EGFR ADCs

The present invention also discloses a process to prepare an anti-EGFR ADC according to structural formula (I):

(I) 

wherein D, L, LK, Ab and m are as defined in the Detailed Description section. The process comprises:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to2.63 (Table 5);

adjusting the pH of the solution to a pH of 7.5 to 8.5; and

allowing the reaction to run for 48 to 80 hours to form the ADC;

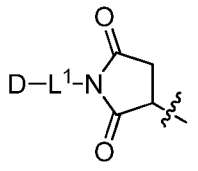
wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

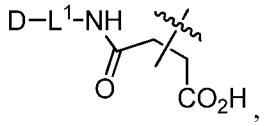
In certain embodiments, Ab is an anti-hEGFR antibody comprises the heavy and light chain CDRs of the anti-hEGFR antibodies disclosed herein, including, AbA, AbB, AbG, and AbK;

The present invention is also directed to an anti-EGFR ADC prepared by the above-described process.

In certain embodiments, the anti-EGFR ADC disclosed in the present application is formed by contacting an antibody that binds an hEGFR cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the drug-linker synthon covalently links to the antibody through a maleimide moiety as shown in formula (IId) or (IIe),



(IId)



(IIe),

wherein D is the Bcl-xL inhibitor drug according to structural formula (IIa) as described above and L^1 is the portion of the linker not formed from the maleimide upon attachment of the synthon to the antibody; and wherein the drug-linker synthon is selected from the group consisting of synthon examples 2.1 to 2.63 (Table 5), or a pharmaceutically acceptable salt thereof.

In certain embodiments, the contacting step is carried out under conditions such that the anti-EGFR ADC has a DAR of 2, 3 or 4.

6. Purification of Anti-EGFR ADCs

Purification of the ADCs may be achieved in such a way that ADCs having certain DARs are collected. For example, HIC resin may be used to separate high drug loaded ADCs from ADCs having optimal drug to antibody ratios (DARs), *e.g.* a DAR of 4 or less. In one embodiment, a hydrophobic resin is added to an ADC mixture such that undesired ADCs, *i.e.,* higher drug loaded ADCs, bind the resin and can be selectively removed from the mixture. In certain embodiments, separation of the ADCs may be achieved by contacting an ADC mixture (*e.g.,* a mixture comprising a drug loaded species of ADC of 4 or less and a drug loaded species of ADC of 6 or more) with a hydrophobic resin, wherein the amount of resin is sufficient to allow binding of the drug loaded species which is being removed from the ADC mixture. The resin and ADC mixture are mixed together, such that the ADC species being removed (*e.g.,* a drug loaded species of 6 or more) binds to the resin and can be separated from the other ADC species in the ADC mixture. The amount of resin used in the method is based on a weight ratio between the species to be removed and the resin, where the amount of resin used does not allow for significant binding of the drug loaded species that is desired. Thus, methods may be used to reduce the average DAR to less than 4. Further, the purification methods described herein may be used to isolate ADCs having any desired range of drug loaded species, *e.g.,* a drug loaded species of 4 or less, a drug loaded species of 3 or less, a drug loaded species of 2 or less, a drug loaded species of 1 or less.

Certain species of molecule(s) binds to a surface based on hydrophobic interactions between the species and a hydrophobic resin. In one embodiment, method of the invention refers to a purification process that relies upon the intermixing of a hydrophobic resin and a mixture of ADCs, wherein the amount of resin added to the mixture determines which species (*e.g.*, ADCs with a DAR of 6 or more) will bind. Following production and purification of an antibody from an expression system (e.g., a mammalian expression system), the antibody is reduced and coupled to a drug through a conjugation reaction. The resulting ADC mixture often contains ADCs

having a range of DARs, *e.g.,* 1 to 8. In one embodiment, the ADC mixture comprises a drug loaded species of 4 or less and a drug loaded species of 6 or more. According to the methods of the invention, the ADC mixture may be purified using a process, such as, but not limited to, a batch process, such that ADCs having a drug loaded species of 4 or less are selected and separated from ADCs having a higher drug load (e.g., ADCs having a drug loaded species of 6 or more). Notably, the purification methods described herein may be used to isolate ADCs having any desired range of DAR, *e.g.,* a DAR of 4 or less, a DAR of 3 or less, or a DAR of 2 or less.

Thus, in one embodiment, an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more may be contacted with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, the method of the invention comprises contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

The ADC separation method described herein method may be performed using a batch purification method. The batch purification process generally includes adding the ADC mixture to the hydrophobic resin in a vessel, mixing, and subsequently separating the resin from the supernatant. For example, in the context of batch purification, a hydrophobic resin may be prepared in or equilibrated to the desired equilibration buffer. A slurry of the hydrophobic resin may thus be obtained. The ADC mixture may then be contacted with the slurry to adsorb the specific species of ADC(s) to be separated by the hydrophobic resin. The solution comprising the desired ADCs that do not bind to the hydrophobic resin material may then be separated from the slurry, e.g., by filtration or by allowing the slurry to settle and removing the supernatant. The resulting slurry can be subjected to one or more washing steps. In order to elute bound ADCs, the salt concentration can be decreased. In one embodiment, the process used in the invention includes no more than 50 g of hydrophobic resin.

Thus, a batch method may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a batch method is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

Alternatively, in a separate embodiment, purification may be performed using a circulation process, whereby the resin is packed in a container and the ADC mixture is passed over the hydrophobic resin bed until the specific species of ADC(s) to be separated have been removed. The supernatant (containing the desired ADC species) is then pumped from the container and the resin bed may be subjected to washing steps.

A circulation process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a circulation process is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less

than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

Alternatively, a flow through process may be used to purify an ADC mixture to arrive at a composition comprising a majority of ADCs having a certain desired DAR. In a flow through process, resin is packed in a container, *e.g.,* a column, and the ADC mixture is passed over the packed resin such that the desired ADC species does not substantially bind to the resin and flows through the resin, and the undesired ADC species is bound to the resin. A flow through process may be performed in a single pass mode (where the ADC species of interest are obtained as a result of a single pass through the resin of the container) or in a multi-pass mode (where the ADC species of interest are obtained as a result of multiple passes through the resin of the container). The flow through process is performed such that the weight of resin selected binds to the undesired ADC population, and the desired ADCs (*e.g.*, DAR 2-4) flow over the resin and are collected in the flow through after one or multiple passes.

A flow through process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected after one or multiple passes, such that the composition comprising the desired ADCs (e.g. DAR 2-4) is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a flow through process is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin by passing the ADC mixture over the resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the amount of hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

Following a flow through process, the resin may be washed with a one or more washes following in order to further recover ADCs having the desired DAR range (found in the wash filtrate). For example, a plurality of washes having decreasing conductivity may be used to

further recover ADCs having the DAR of interest. The elution material obtained from the washing of the resin may be subsequently combined with the filtrate resulting from the flow through process for improved recovery of ADCs having the DAR of interest.

The aforementioned batch, circulation, and flow through process purification methods are based on the use of a hydrophobic resin to separate high vs. low drug loaded species of ADC. Hydrophobic resin comprises hydrophobic groups which interact with the hydrophobic properties of the ADCs. Hydrophobic groups on the ADC interact with hydrophobic groups within the hydrophobic resin. The more hydrophobic a protein is the stronger it will interact with the hydrophobic resin.

Hydrophobic resin normally comprises a base matrix (e.g., cross-linked agarose or synthetic copolymer material) to which hydrophobic ligands (e.g., alkyl or aryl groups) are coupled. Many hydrophobic resins are available commercially. Examples include, but are not limited to, Phenyl Sepharose™ 6 Fast Flow with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™. t-Butyl Supports (Bio-Rad, California); WP HI-Propyl (C_3)™ (J. T. Baker, New Jersey); and Toyopearl™ ether, hexyl, phenyl or butyl (TosoHaas, PA). In one embodiment, the hydrophobic resin is a butyl hydrophobic resin. In another embodiment, the hydrophobic resin is a phenyl hydrophobic resin. In another embodiment, the hydrophobic resin is a hexyl hydrophobic resin, an octyl hydrophobic resin, or a decyl hydrophobic resin. In one embodiment, the hydrophobic resin is a methacrylic polymer having n-butyl ligands (*e.g.* TOYOPEARL Butyl-600M).

Further methods for purifying ADC mixtures to obtain a composition having a desired DAR are described in U.S. Application No. 14/210,602 (U.S. Patent Appln. Publication No. US 2014/0286968), incorporated by reference in its entirety.

In certain embodiments of the invention, ADCs described herein having a DAR2 are purified from ADCs having higher or lower DARs. Such purified DAR2 ADCs are referred to herein as "E2". Purification methods for achieving a composition having E2 anti-EGFR ADCs. In one embodiment, of the invention provides a composition comprising an ADC mixture, wherein at least 75% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 80% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 85% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition

comprising an ADC mixture, wherein at least 90% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2.

7. Uses of Anti-EGFR ADCs

The Bcl-xL inhibitors included in the ADCs, as well as the synthons delivered by the ADCs, inhibit Bcl-xL activity and induce apoptosis in cells expressing Bcl-xL. Accordingly, the Bcl-xL inhibitors and/or ADCs may be used in methods to inhibit Bcl-xL activity and/or induce apoptosis in cells.

For Bcl-xL inhibitors, the method generally involves contacting a cell whose survival depends, at least in part, upon Bcl-xL expression with an amount of a Bcl-xL inhibitor sufficient to inhibit Bcl-xL activity and/or induce apoptosis. For ADCs, the method generally involves contacting a cell whose survival depends, at least in part upon Bcl-xL expression, and that expresses a cell-surface antigen, *i.e.,* EGFR, for the antibody of the ADC with an ADC under conditions in which the ADC binds the antigen.

ABV122internalization of the ADC into the cell, where the Bcl-xL inhibitory synthon is delivered. The method may be carried out *in vitro* in a cellular assay to inhibit Bcl-xL activity and/or inhibit apoptosis, or *in vivo* as a therapeutic approach towards treating diseases in which inhibition of apoptosis and/or induction of apoptosis would be desirable.

Dysregulated apoptosis has been implicated in a variety of diseases, including, for example, autoimmune disorders (*e.g.*, systemic lupus erythematosus, rheumatoid arthritis, graft-versus-host disease, myasthenia gravis, or Sjogren's syndrome), chronic inflammatory conditions (*e.g.*, psoriasis, asthma or Crohn's disease), hyperproliferative disorders (*e.g.*, breast cancer, lung cancer), viral infections (*e.g.*, herpes, papilloma, or HIV), and other conditions, such as osteoarthritis and atherosclerosis. The Bcl-xL inhibitor or ADCs described herein may be used to treat or ameliorate any of these diseases. Such treatments generally involve administering to a subject suffering from the disease an amount of a Bcl-xL inhibitor or ADC described herein sufficient to provide therapeutic benefit. For ADCs, identity of the antibody of the ADC administered will depend upon the disease being treated – to the antibody should bind a cell-surface antigen expressed in the cell type where inhibition of Bcl-xL activity would be beneficial. The therapeutic benefit achieved will also depend upon the specific disease being treated. In certain instances, the Bcl-xL inhibitor or ADC may treat or ameliorate the disease itself, or symptoms of the disease, when administered as monotherapy. In other instances, the Bcl-xL inhibitor or ADC may be part of an overall treatment regimen including other agents that, together with the inhibitor or ADC, treat or ameliorate the disease being treated, or symptoms of the disease. Agents useful to treat or ameliorate specific diseases that may be administered

adjunctive to, or with, the Bcl-xL inhibitors and/or ADCs described herein will be apparent to those of skill in the art.

Although absolute cure is always desirable in any therapeutic regimen, achieving a cure is not required to provide therapeutic benefit. Therapeutic benefit may include halting or slowing the progression of the disease, regressing the disease without curing, and/or ameliorating or slowing the progression of symptoms of the disease. Prolonged survival as compared to statistical averages and/or improved quality of life may also be considered therapeutic benefit.

One particular class of diseases that involve dysregulated apoptosis and that are significant health burden world-wide are cancers. In a specific embodiment, the Bcl-xL inhibitors and/or ADCs described herein may be used to treat cancers. The cancer may be, for example, solid tumors or hematological tumors. Cancers that may be treated with the ADCs described herein include, but are not limited to include, but are not limited to bladder cancer, brain cancer, breast cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, 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, chronic lymphocytic leukemia, myeloma, prostate cancer, or spleen cancer. ADCs may be especially beneficial in the treatment of cancers because the antibody can be used to target the Bcl-xL inhibitory synthon specifically to tumor cells, thereby potentially avoiding or ameliorating undesirable side-effects and/or toxicities that may be associated with systemic administration of unconjugated inhibitors. One embodiment pertains to a method of treating a disease involving dysregulated intrinsic apoptosis, comprising administering to a subject having a disease involving dysregulated apoptosis an amount of an ADC described herein effective to provide therapeutic benefit, wherein the antibody of the ADC binds a cell surface receptor on a cell whose intrinsic apoptosis is dysregulated. One embodiment pertains to a method of treating cancer, comprising administering to a subject having cancer an ADC described herein that is capable of binding a cell surface receptor or a tumor associated antigen expressed on the surface of the cancer cells, in an amount effective to provide therapeutic benefit.

In certain embodiments, the cancer may be characterized as having EGFR overexpression.

In other embodiments, the cancer is characterized as having an activating EGFR mutation, *e.g.* a mutation(s) that activates the EGFR signaling pathway and/or mutation(s) that lead to overexpression of the EGFR protein. In specific exemplary embodiments, the activating EGFR mutation may be a mutation in the EGFR gene. In particular embodiments, the activating EGFR mutation is an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and/or combinations thereof.

In the context of tumorigenic cancers, therapeutic benefit, in addition to including the effects discussed above, may also specifically include halting or slowing progression of tumor growth, regressing tumor growth, eradicating one or more tumors and/or increasing patient survival as compared to statistical averages for the type and stage of the cancer being treated. In one embodiment, the cancer being treated is a tumorigenic cancer.

The ADCs of the invention are capable of neutralizing human EGFR activity both *in vivo* and *in vitro*. Accordingly, such ADCs of the invention can be used to inhibit hEGFR activity, *e.g.,* in a cell culture containing hEGFR, in human subjects or in other mammalian subjects having EGFR with which an antibody of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting hEGFR activity comprising contacting hEGFR with an antibody or antibody portion of the invention such that hEGFR activity is inhibited. For example, in a cell culture containing, or suspected of containing hEGFR, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hEGFR activity in the culture.

In another embodiment, the invention features a method for reducing hEGFR activity in a subject, advantageously from a subject suffering from a disease or disorder in which EGFR activity is detrimental. The invention provides methods for reducing EGFR activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject an ADC of the invention such that EGFR activity in the subject is reduced. Preferably, the EGFR is human EGFR, and the subject is a human subject. Alternatively, the subject can be a mammal expressing an EGFR to which ADCs of the invention are capable of binding. Still further the subject can be a mammal into which EGFR has been introduced (*e.g.*, by administration of EGFR or by expression of an EGFR transgene). ADCs of the invention can be administered to a human subject for therapeutic purposes. Moreover, ADCs of the invention can be administered to a non-human mammal expressing an EGFR with which the antibody 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 antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the term "a disorder in which EGFR activity is detrimental" is intended to include diseases and other disorders in which the presence of EGFR in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which EGFR activity is detrimental is a disorder in which reduction of EGFR activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of EGFR in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of EGFR in a tumor, serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for

example, using an anti-EGFR antibody as described above. Non-limiting examples of disorders that can be treated with the ADCs of the invention, for example, an ADC comprising AbA, include those disorders discussed below. For example, suitable disorders include, but are not limited to, a variety of cancers including, but not limited to, breast cancer, lung cancer, a glioma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, and kidney cancer. Other examples of cancer that may be treated using the compositions and methods disclosed herein include squamous cell carcinoma (*e.g.,* squamous lung cancer or squamous head and neck cancer), triple negative breast cancer, non-small cell lung cancer, colorectal cancer, and mesothelioma. In one embodiment, the ADCs disclosed herein are used to treat a solid tumor, *e.g.,* inhibit growth of or decrease size of a solid tumor, overexpressing EGFR or which is EGFR positive. In one embodiment, the invention is directed to the treatment of EGFR amplified squamous lung cancer. In one embodiment, the ADCs disclosed herein are used to treat EGFR amplified squamous head and neck cancer. In another embodiment, the ADCs disclosed herein are used to treat triple negative breast cancer (TNBC). Diseases and disorders described herein may be treated by anti-EGFR ADCs of the invention, as well as pharmaceutical compositions comprising such anti-EGFR ADCs.

In certain embodiments, the ADCs disclosed herein are administered to a subject in need thereof in order to treat advanced solid tumor types likely to exhibit elevated levels of Epidermal Growth Factor Receptor (EGFR). Examples of such tumors include, but are not limited to, head and neck squamous cell carcinoma, non-small cell lung cancer, triple negative breast cancer, colorectal carcinoma, and glioblastoma multiforme.

In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an anti-EGFR ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. In certain embodiments, the solid tumor is a non-small cell lung carcinoma or a glioblastoma. In further embodiments, the solid tumor is an EGFRvIII positive tumor or an EGFR-expressing solid tumors. In further embodiments, the solid tumor is an EGFR amplified solid tumor or an EGFR overexpressing solid tumors. In certain embodiments the anti-EGFR ADCs described herein are administered to a subject having glioblastoma multiforme, alone or in combination with an additional agent, *e.g.,* radiation and/or temozolomide.

In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor which was identified as an EGFR expressing or EGFR overexpressing tumor (or an EGFRvIII expressing tumor), said method comprising administering an anti-EGFR ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. Methods for identifying EGFR expressing tumors (*e.g.*, EGFR overexpressing tumors) are known in the art, and include FDA-approved tests

and validation assays. For example, the EGFR pharmDx™ assay (Dako North America, Inc.) is a qualitative immunohistochemical (IHC) kit system used to identify EGFR expression in normal and neoplastic tissues routinely-fixed for histological evaluation. EGFR pharmDx specifically detects the EGFR (HER1) protein in EGFR-expressing cells. In addition, PCR-based assays may also be used for identifying EGFR overexpressing tumors. For example, these assays may use primers that are specific for the variant EGFR gene (e.g., SEQ ID NO: 33) and/or cDNA and result in the amplification of the EGFR gene/cDNA, or a portion thereof. The amplified PCR products may be subsequently analyzed, for example, by gel electrophoresis using standard methods known in the art to determine the size of the PCR products. Such tests may be used to identify tumors that may be treated with the methods and compositions described herein.

Any of the methods for gene therapy available in the art can be used according to the invention. For general reviews of the methods of gene therapy, see Goldspiel *et al.*, 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, *Science* 260:926- 932 (1993); and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy is provided in US20050042664 A1 which is incorporated herein by reference.

In another aspect, this application features a method of treating (*e.g.*, curing, suppressing, ameliorating, delaying or preventing the onset of, or preventing recurrence or relapse of) or preventing a EGFR-associated disorder, in a subject. The method includes: administering to the subject an EGFR binding agent (particularly an antagonist), *e.g.*, an anti-EGFR antibody or fragment thereof as described herein, in an amount sufficient to treat or prevent the EGFR-associated disorder. The EGFR antagonist, *e.g.*, the anti-EGFR antibody or fragment thereof, can be administered to the subject, alone or in combination with other therapeutic modalities as described herein.

ADCs of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the ADCs of the invention can be used alone or in combination with an additional agent, *e.g.*, a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the ADC of the invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, *e.g.*, an agent which affects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, *e.g.*, two or three additional agents if the combination is such that the formed composition can perform its intended function.

The combination therapy can include anti-EGFR antagonists ADCs of the invention formulated with, and/or co-administered with, one or more additional therapeutic agents, *e.g.*, one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents (*e.g.*, systemic anti-inflammatory agents), anti-fibrotic agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, mitotic inhibitors, antitumor antibiotics, immunomodulating agents, vectors for gene therapy, alkylating agents, antiangiogenic agents, antimetabolites, boron-containing agents, chemoprotective agents, hormones, antihormone agents, corticosteroids, photoactive therapeutic agents, oligonucleotides, radionuclide agents, topoisomerase inhibitors, kinase inhibitors, or radiosensitizers, as described in more herein.

In a particular embodiment, the anti-EGFR ADCs described herein, are used in combination with an anti-cancer agent or an antineoplastic agent. The terms “anti-cancer agent” and “antineoplastic agent” refer to drugs used to treat malignancies, such as cancerous growths. Drug therapy may be used alone, or in combination with other treatments such as surgery or radiation therapy. Several classes of drugs may be used in cancer treatment, depending on the nature of the organ involved. For example, breast cancers are commonly stimulated by estrogens, and may be treated with drugs which inactive the sex hormones. Similarly, prostate cancer may be treated with drugs that inactivate androgens, the male sex hormone. Anti-cancer agents that may be used in conjunction with the anti-EGFR ADCs of the invention include, among others, the following agents:

Anti-Cancer Agent	Comments	Examples
Antibodies (a) antibodies other than anti-EGFR antibodies; and (b) anti-EGFR antibodies which bind different epitopes	Antibodies which bind IGF-1R (insulin-like growth factor type 1 receptor), which is expressed on the cell surface of most human cancers	A12 (fully humanized mAb) 19D12 (fully humanized mAb) Cp751-871 (fully humanized mAb) H7C10 (humanized mAb) alphaIR3 (mouse) ScFV/FC (mouse/human chimera) EM/164 (mouse)
	Antibodies which bind EGFR (epidermal growth factor receptor); Mutations affecting EGFR expression or activity could result in cancer	Matuzumab (EMD72000) Erbitux® / Cetuximab (Imclone) Vectibix® / Panitumumab (Amgen) mAb 806 Nimotuxumab (TheraCIM)
	Antibodies which bind cMET (Mesenchymal epithelial transition factor); a member of the MET family of receptor tyrosine kinases)	AVEO (AV299) (AVEO) AMG102 (Amgen) 5D5 (OA-5d5) (Genentech) H244G11 (Pierre Fabre)
	Anti-ErbB3 antibodies which bind different epitopes	Ab #14 (MM 121-14) Herceptin® (Trastuzumab; Genentech) 1B4C3; 2D1D12 (U3 Pharma AG)
Small Molecules Targeting IGF1R	Insulin-like growth factor type 1 receptor which is expressed on the cell surface of many human cancers	NVP-AEW541-A BMS-536,924 (1H-benzoimidazol-2-yl)-1H-pyridin-2-one) BMS-554,417 Cycloligan TAE226 PQ401
Small Molecules Targeting EGFR	EGFR (epidermal growth factor receptor); Overexpression or mutations affecting EGFR expression or activity could result in cancer	Iressa® / Gefitinib (AstraZeneca) CI-1033 (PD 183805) (Pfizer) Lapatinib (GW-572016) (GlaxoSmithKline) Tykerb® / Lapatinib Ditosylate (Smith Kline Beecham) Tarceva ® / Erlotinib HCL (OSI-774) (OSI Pharma) PKI-166 (Novartis) PD-158780 EKB-569 Tyrphostin AG 1478 (4-(3-Chloroanillino)-6,7-dimethoxyquinazoline)
Small Molecules Targeting cMET	cMET (Mesenchymal epithelial transition factor);	PHA665752 ARQ 197

	a member of the MET family of receptor tyrosine kinases)	
Antimetabolites		Flourouracil (5-FU) Capecitabine / XELODA® (HLR Roche) 5-Trifluoromethyl-2'-deoxyuridine Methotrexate sodium (Trexall) (Barr) Raltitrexed/ Tomudex® (AstraZeneca) Pemetrexed / Alimta® (Lilly) Tegafur Cytosine Arabinoside (Cytarabine, Ara-C) / Thioguanine® (GlaxoSmithKline) 5-azacytidine 6-mercaptopurine (Mercaptopurine, 6-MP) Azathioprine / Azasan® (AAIPHARMA LLC) 6-thioguanine (6-TG) / Purinethol® (TEVA) Pentostatin / Nipent® (Hospira Inc.) Fludarabine phosphate / Fludara® (Bayer Health Care) Cladribine (2-CdA, 2-chlorodeoxyadenosine) / Leustatin® (Ortho Biotech)
Alkylating agents	An alkylating antineoplastic agent is an alkylating agent that attaches an alkyl group to DNA. Since cancer cells generally proliferate unrestrictively more than do healthy cells they are more sensitive to DNA damage, and alkylating agents are used clinically to treat a variety of tumors.	Ribonucleotide Reductase Inhibitor (RNR) Cyclophosphamide / Cytoxan (BMS) Neosar (TEVA) Ifosfamide / Mitoxana® (ASTA Medica) Thiotepa (Bedford, Abraxis, Teva) BCNU→ 1,3-bis(2-chloroethyl)-1-nitosourea CCNU→ 1, -(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (methyl CCNU) Hexamethylmelamine (Altretamine, HMM) / Hexalen® (MGI Pharma Inc.) Busulfan / Myleran (GlaxoSmithKline) Procarbazine HCL/ Matulane (Sigma Tau Pharmaceuticals, Inc.) Dacarbazine (DTIC) Chlorambucil / Leukara® (SmithKline Beecham) Melphalan / Alkeran® (GlaxoSmithKline) Cisplatin (Cisplatinum, CDDP) / Platinol (Bristol Myers) Carboplatin / Paraplatin (BMS) Oxaliplatin /Eloxitan® (Sanofi-Aventis US)
Topoisomerase inhibitors	Topoisomerase inhibitors are chemotherapy agents designed to interfere with	Doxorubicin HCL / Doxil® (Alza) Daunorubicin citrate / Daunoxome® (Gilead) Mitoxantrone HCL / Novantrone

	the action of topoisomerase enzymes (topoisomerase I and II), which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle.	(EMD Serono) Actinomycin D Etoposide / Vepesid® (BMS)/ Etopophos® (Hospira, Bedford, Teva Parenteral, Etc.) Topotecan HCL / Hycamtin® (GlaxoSmithKline) Teniposide (VM-26) / Vumon® (BMS) Irinotecan HCL(CPT-ll) / Camptosar® (Pharmacia & Upjohn)
Microtubule targeting agents	Microtubules are one of the components of the cytoskeleton. They have diameter of ~24 nm and length varying from several micrometers to possibly millimeters in axons of nerve cells. Microtubules serve as structural components within cells and are involved in many cellular processes including mitosis, cytokinesis, and vesicular transport.	Vincristine / Oncovin® (Lilly) Vinblastine sulfate / Velban®(discontinued) (Lilly) Vinorelbine tartrate / Navelbine® (PierreFabre) Vindesine sulphate / Eldisine® (Lilly) Pac1itaxel / Taxol® (BMS) Docetaxel / Taxotere® (Sanofi Aventis US) Nanoparticle paclitaxel (ABI-007) / Abraxane® (Abraxis BioScience, Inc.) Ixabepilone / IXEMPRA™ (BMS)
Kinase inhibitors	Kinases are enzymes that catalyze the transfer of phosphate groups from high-energy, phosphate-donating molecules to specific substrates, and are utilized to transmit signals and regulate complex processes in cells.	Imatinib mesylate / Gleevec (Novartis) Sunitinib malate / Sutent® (Pfizer) Sorafenib tosylate / Nexavar® (Bayer) Nilotinib hydrochloride monohydrate / Tasigna® (Novartis), Osimertinib, Cobimetinib, Trametinib, Dabrafenib, Dinaciclib
Protein synthesis inhibitors	Induces cell apoptosis	L-asparaginase / Elspar® (Merck & Co.)
Immunotherapeutic agents	Induces cancer patients to exhibit immune responsiveness Antibody / small molecule immune checkpoint modulators	Alpha interferon Angiogenesis Inhibitor / Avastin® (Genentech) IL-2→ Interleukin 2 (Aldesleukin) / Proleukin ® (Chiron) IL-12→ Interleukin 12 Anti-CTLA-4 and PR-1 therapies Yervoy® (ipilimumab; Bristol-Myers Squibb) Opdivo® (nivolumab; Bristol-Myers Squibb) Keytrada® (pembrolizumab; Merck)
Hormones	Hormone therapies	Toremifene citrate / Fareston® (GTX, Inc.)

	associated with menopause and aging seek to increase the amount of certain hormones in your body to compensate for age- or disease-related hormonal declines. Hormone therapy as a cancer treatment either reduces the level of specific hormones or alters the cancer's ability to use these hormones to grow and spread.	Fulvestrant / Faslodex® (AstraZeneca) Raloxifene HCL / Evista® (Lilly) Anastrazole / Arimidex® (AstraZeneca) Letrozole / Femara® (Novartis) Fadrozole (CGS 16949A) Exemestane / Aromasin® (Pharmacia & Upjohn) Leuprolide acetate / Eligard® (QTL USA) Lupron® (TAP Pharm) Goserelin acetate / Zoladex® (AstraZeneca) Triptorelin pamoate / Trelstar® (Watson Labs) Buserelin / Suprefact® (Sanofi Aventis) Nafarelin / Synarel® (Pfizer) Cetrorelix / Cetrotide® (EMD Serono) Bicalutamide / Casodex® (AstraZeneca) Nilutamide / Nilandron® (Aventis Pharm.) Megestrol acetate / Megace® (BMS) Somatostatin Analogs (Octreotide acetate / Sandostatin® (Novartis)
Glucocorticoids	Anti-inflammatory drugs used to reduce swelling that causes cancer pain.	Prednisolone Dexamethasone / Decadron® (Wyeth)
Aromatose inhibitors	Includes imidazoles	Ketoconazole
mTOR inhibitors	The mTOR signaling pathway was originally discovered during studies of the immunosuppressive agent rapamycin. This highly conserved pathway regulates cell proliferation and metabolism in response to environmental factors, linking cell growth factor receptor signaling via phosphoinositide-3-kinase(PI-3K) to cell growth, proliferation, and angiogenesis.	Sirolimus (Rapamycin) / Rapamune® (Wyeth) Temsirolimus (CCI-779) / Torisel® (Wyeth) Deforolimus (AP23573) / (Ariad Pharm.) Everolimus (RAD00I) / Certican® (Novartis)

In addition to the above anti-cancer agents, the anti-EGFRADCs described herein may be administered in combination with the agents described in section II. Further, the aforementioned anti-cancer agents may also be used in the ADCs of the invention.

In particular embodiments, the ADCs of the invention can be administered alone or with another anti-cancer agent which acts in conjunction with or synergistically with the antibody to treat the disease associated with EGFR activity. Such anti-cancer agents include, for example, agents well known in the art (*e.g.*, cytotoxins, chemotherapeutic agents, small molecules and

radiation). Examples of anti-cancer agents include, but are not limited to, Panorex (Glaxo-Welcome), Rituxan (IDEC/Genentech/Hoffman la Roche), Mylotarg (Wyeth), Campath (Millennium), Zevalin (IDEC and Schering AG), Bexxar (Corixa/GSK), Erbitux (Imclone/BMS), Avastin (Genentech) and Herceptin (Genentech/Hoffman la Roche). Other anti-cancer agents include, but are not limited to, those disclosed in U.S. Patent No. 7,598,028 and International Publication No. WO2008/100624, the contents of which are hereby incorporated by reference. One or more anti-cancer agents may be administered either simultaneously or before or after administration of an antibody or antigen binding portion thereof of the invention.

In particular embodiments of the invention, the ADCs described herein can be used in a combination therapy with an inhibitor of NAMPT (see examples of inhibitors in US 2013/0303509; AbbVie, Inc., incorporated by reference herein) to treat a subject in need thereof. NAMPT (also known as pre-B-cell-colony-enhancing factor (PBEF) and visfatin) is an enzyme that catalyzes the phosphoribosylation of nicotinamide and is the rate-limiting enzyme in one of two pathways that salvage NAD. In one embodiment of the invention, anti-EGFR antibodies and ADCs described herein are administered in combination with a NAMPT inhibitor for the treatment of cancer in a subject.

In particular embodiments of the invention, the ADCs described herein can be used in a combination therapy with SN-38, which is the active metabolite of the topoisomerase inhibitor irinotecan.

In other embodiments of the invention, the ADCs described herein can be used in a combination therapy with a PARP (poly ADP ribose polymerase) inhibitor, *e.g.*, veliparib, to treat cancer, including breast, ovarian and non-small cell lung cancers.

Further examples of additional therapeutic agents that can be co-administered and/or formulated with anti-EGFR ADCs described herein, include, but are not limited to, one or more of: inhaled steroids; beta-agonists, *e.g.*, short-acting or long- acting beta-agonists; antagonists of leukotrienes or leukotriene receptors; combination drugs such as ADVAIR; IgE inhibitors, *e.g.*, anti-IgE antibodies (*e.g.*, XOLAIR, omalizumab); phosphodiesterase inhibitors (*e.g.*, PDE4 inhibitors); xanthines; anticholinergic drugs; mast cell-stabilizing agents such as cromolyn; IL-4 inhibitors; IL-5 inhibitors; eotaxin/CCR3 inhibitors; antagonists of histamine or its receptors including H1, H2, H3, and H4, and antagonists of prostaglandin D or its receptors (DP1 and CRTH2). Such combinations can be used to treat, for example, asthma and other respiratory disorders. Other examples of additional therapeutic agents that can be co-administered and/or formulated with anti-EGFR ADCs described herein, include, but are not limited to, one or more of, temozolomide, ibrutinib, duvelisib, and idelalisib. Additional examples of therapeutic agents that can be co-administered and/or formulated with one or more anti-EGFR antibodies or fragments thereof include one or more of: TNF antagonists (*e.g.*, a soluble fragment of a TNF

receptor, *e.g.*, p55 or p75 human TNF receptor or derivatives thereof, *e.g.*, 75 kD TNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBREL)); TNF enzyme antagonists, *e.g.*, TNF converting enzyme (TACE) inhibitors; muscarinic receptor antagonists; TGF-beta antagonists; interferon gamma; perfenidone; chemotherapeutic agents, *e.g.*, methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, *e.g.*, CCI-779; COX2 and cPLA2 inhibitors; NSAIDs; immunomodulators; p38 inhibitors, TPL-2, MK-2 and NFkB inhibitors, among others.

Other preferred combinations are cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-31, interferons, EMAP-II, GM-CSF, FGF, EGF, PDGF, and edothelin-1, as well as the receptors of these cytokines and growth factors. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA, CTLA-4, PD-1, or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, adalimumab, (HUMIRA; D2E7; PCT Publication No. WO 97/29131 and U.S. Patent No. 6,090,382, incorporated by reference herein), CA2 (Remicade®), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel®) or p55TNFR1gG (Lenercept), and also TNF converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 4.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount 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.

The amount of ADC administered will depend upon a variety of factors, including but not limited to, the particular disease being treated, the mode of administration, the desired therapeutic benefit, the stage or severity of the disease, the age, weight and other characteristics of the patient, etc. Determination of effective dosages is within the capabilities of those skilled in the art.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions 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 mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an ADC, is 0.1-20 mg/kg, more preferably 1-10 mg/kg. In one embodiment, the dose of the ADCs described herein is 1 to 6 mg/kg, including the individual doses recited therein, *e.g.*, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, and 6 mg/kg. In another embodiment, the dose of the ADCs described herein is 1 to 200 μg/kg, including the individual doses recited therein, *e.g.*, 1 μg/kg, 2 μg/kg, 3 μg/kg, 4 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, 30 μg/kg, 40 μg/kg, 50 μg/kg, 60 μg/kg, 80 μg/kg, 100 μg/kg, 120 μg/kg, 140 μg/kg, 160 μg/kg, 180 μg/kg and 200 μg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

In one embodiment, an anti-EGFR ADC described herein, *e.g.*, an ADC comprising AbA, is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 0.1 to 30 mg/kg. In another embodiment, the anti-EGFR ADC, *e.g.*, an ADC comprising AbA, is administered to a subject in need thereof, *e.g.*, a subject having cancer, as an ADC at a dose of 1 to 15 mg/kg. In another embodiment, the anti-EGFR ADC, *e.g.*, an ADC comprising AbA, is

administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 1 to 10 mg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 2 to 3 mg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 1 to 4 mg/kg.

In one embodiment, an anti-EGFR ADC described herein, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 1 to 200 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 150 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 100 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 90 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 80 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 70 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 5 to 60 μg/kg. In another embodiment, the anti-EGFR ADC, *e.g.,* an ADC comprising AbA, is administered to a subject in need thereof, *e.g.,* a subject having cancer, as an ADC at a dose of 10 to 80 μg/kg.

In one embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of .1 to 6 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of .5 to 4 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of 1.8 to 2.4 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of 1 to 4 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of about 1 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of 3 to 6 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.,* a subject having cancer, at a dose of 3 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need

thereof, *e.g.*, a subject having cancer, at a dose of 2 to 3 mg/kg. In another embodiment, an anti-EGFR ADC described herein, is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 6 mg/kg.

In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 1 to 200 μg/kg. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 5 to 100 μg/kg. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 5 to 90 μg/kg. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 5 to 80 μg/kg. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 5 to 70 μg/kg. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, *e.g.*, a subject having cancer, at a dose of 5 to 60 μg/kg.

In another aspect, this application provides a method for detecting the presence of EGFR in a sample *in vitro* (*e.g.*, a biological sample, such as serum, plasma, tissue, and biopsy). The subject method can be used to diagnose a disorder, *e.g.*, a cancer. The method includes: (i) contacting the sample or a control sample with the anti-EGFR ADC as described herein; and (ii) detecting formation of a complex between the anti-EGFR ADC and the sample or the control sample, wherein a statistically significant change in the formation of the complex in the sample relative to the control sample is indicative of the presence of EGFR in the sample.

Given their ability to bind to human EGFR, the ADCs of the invention can be used to detect human EGFR (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. In one aspect, the invention provides a method for detecting human EGFR in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to human EGFR or unbound antibody (or antibody portion), to thereby detect human EGFR in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a

luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

Alternative to labeling the antibody, human EGFR can be assayed in biological fluids by a competition immunoassay utilizing rhEGFR standards labeled with a detectable substance and an unlabeled anti-human EGFR ADC. In this assay, the biological sample, the labeled rhEGFR standards and the anti-human EGFR antibody are combined and the amount of labeled rhEGFR standard bound to the unlabeled antibody is determined. The amount of human EGFR in the biological sample is inversely proportional to the amount of labeled rhEGFR standard bound to the anti-EGFR antibody. Similarly, human EGFR can also be assayed in biological fluids by a competition immunoassay utilizing rhEGFR standards labeled with a detectable substance and an unlabeled anti-human EGFR ADC.

8. Pharmaceutical Compositions

The Bcl-xL inhibitors and/or ADCs described herein may be in the form of compositions comprising the inhibitor or ADC and one or more carriers, excipients and/or diluents. The compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (*e.g.*, dry powder, liquid formulation, *etc.*) and the excipients, diluents and/or carriers used will depend upon the intended uses of the inhibitors and/or ADCs and, for therapeutic uses, the mode of administration.

For therapeutic uses, the Bcl-xL inhibitor and/or ADC compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intrathecally, topically or locally. The most suitable route for administration in any given case will depend on the particular Bcl-xL inhibitor or ADC, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the Bcl-xL inhibitors will be administered orally or parenterally, and ADC pharmaceutical composition will be administered intravenously or subcutaneously.

Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of Bcl-xL inhibitor or an ADC described herein per dose. The quantity of inhibitor or ADC included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. For Bcl-xL inhibitors, such unit dosages may be in the form of tablets, capsules, lozenges, *etc.* containing an amount of Bcl-xL inhibitor suitable for a single administration. For ADCs, such unit dosages may be in the form of a lyophilized dry powder containing an amount of ADC suitable for a single administration, or in

the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of ADC suitable for a single administration.

The pharmaceutical compositions may also be supplied in bulk form containing quantities of ADC suitable for multiple administrations

Pharmaceutical compositions of ADCs may be prepared for storage as lyophilized formulations or aqueous solutions by mixing an ADC having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), *i.e.*, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. *See*, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at concentrations ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, *etc.*), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, *etc.*), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, *etc.*), gluconate buffers (*e.g.*, gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, *etc.*), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, *etc.*), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, *etc.*) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, *etc.*). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be

added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*e.g.*, peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccacharides such as raffinose; and polysaccharides such as dextran.

Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188 *etc.*), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, *etc.*). Non-ionic surfactants may be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, for example about 0.07 mg/ml to about 0.2 mg/ml.

Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1. Synthesis of Exemplary Bcl-xL Inhibitors

This Example provides synthetic methods for exemplary Bcl-xL inhibitory compounds W1.01-W1.08. Bcl-xL inhibitors (W1.01-W1.08) and synthons (Examples 2.1-2.63) were named using ACD/Name 2012 release (Build 56084, 05 April 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 October 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA), or ChemDraw® Professional Ver. 15.0.0.106. Bcl-xL inhibitor and synthon intermediates were named with ACD/Name 2012 release (Build 56084, 05 April 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 October 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA) or ChemDraw® Professional Ver. 15.0.0.106 (PerkinElmer Informatics, Inc.).

1.1. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W1.01)

1.1.1. 3-bromo-5,7-dimethyladamantanecarboxylic acid

In a 50 mL round-bottomed flask at 0°C was added bromine (16 mL). Iron powder (7 g) was then added, and the reaction was stirred at 0°C for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g) was then added. The mixture was warmed up to room temperature and stirred for 3 days. A mixture of ice and concentrated HCl was poured into the reaction mixture. The resulting suspension was treated twice with Na_2SO_3 (50 g in 200 mL water) to destroy bromine and was extracted three times with dichloromethane. The combined organics were washed with 1N aqueous HCl, dried over Na_2SO_4, filtered, and concentrated to give the crude title compound.

1.1.2. 3-bromo-5,7-dimethyladamantanemethanol

To a solution of Example 1.1.1 (15.4 g) in tetrahydrofuran (200 mL) was added BH_3 (1M in tetrahydrofuran, 150 mL). The mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched by adding methanol dropwise. The mixture was then concentrated under vacuum, and the residue was balanced between ethyl acetate (500 mL) and 2N aqueous HCl (100 mL). The aqueous layer was further extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over Na_2SO_4, and filtered. Evaporation of the solvent gave the title compound.

1.1.3. 1-((3-bromo-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-1H-pyrazole

To a solution of Example 1.1.2 (8.0 g) in toluene (60 mL) was added 1H-pyrazole (1.55 g) and cyanomethylenetributylphosphorane (2.0 g). The mixture was stirred at 90°C overnight. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (10:1 heptane:ethyl acetate) to give the title compound. MS (ESI) m/e 324.2 $(M+H)^+$.

1.1.4. 2-{[3,5-dimethyl-7-(1*H*-pyrazol-1-ylmethyl)tricyclo[3.3.1.1^{3,7}]dec-1-yl]oxy}ethanol

To a solution of Example 1.1.3 (4.0 g) in ethane-1,2-diol (12 mL) was added triethylamine (3 mL). The mixture was stirred at 150°C under microwave conditions (Biotage Initiator) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, and filtered. Evaporation of the solvent gave the crude product, which was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, followed by 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 305.2 $(M+H)^+$.

1.1.5. 2-({3,5-dimethyl-7-[(5-methyl-1*H*-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethanol

To a cooled (-78°C) solution of Example 1.1.4 (6.05 g) in tetrahydrofuran (100 mL) was added n-BuLi (40 mL, 2.5M in hexane). The mixture was stirred at -78°C for 1.5 hours. Iodomethane (10 mL) was added through a syringe, and the mixture was stirred at -78°C for 3 hours. The reaction mixture was then quenched with aqueous NH_4Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was purified by silica gel column chromatography, eluting with 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 319.5 $(M+H)^+$.

1.1.6. 1-({3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole

To a solution of Example 1.1.5 (3.5 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with **aqueous** $NaHSO_3$, water, and brine. After drying over Na_2SO_4, the solution was filtered and concentrated and the residue was purified by silica gel chromatography (20% ethyl acetate in dichloromethane) to give the title compound. MS (ESI) m/e 445.3 $(M+H)^+$.

1.1.7. 2-({3-[(4-iodo-5-methyl-1*H*-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl methanesulfonate

To a cooled solution of Example 1.1.6 (6.16 g) in dichloromethane (100 mL) was added triethylamine (4.21 g) followed by methanesulfonyl chloride (1.6 g). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/e 523.4 $(M+H)^+$.

1.1.8. 1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole

A solution of Example 1.1.7 (2.5 g) in 2M methylamine in methanol (15 mL) was stirred at 100°C for 20 minutes under microwave conditions (Biotage Initiator). The reaction mixture was concentrated under vacuum. The residue was then diluted with ethyl acetate (400 mL) and washed with aqueous $NaHCO_3$, water and brine. After drying over Na_2SO_4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/e 458.4 $(M+H)^+$.

1.1.9. *tert*-butyl [2-({3-[(4-iodo-5-methyl-1*H*-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]methylcarbamate

To a solution of Example 1.1.8 (2.2 g) in tetrahydrofuran (30 mL) was added di-*tert*-butyl dicarbonate (1.26 g) and a catalytic amount of 4-dimethylaminopyridine. The mixture was stirred at room temperature for 1.5 hours and diluted with ethyl acetate (300 mL). The solution was washed with saturated aqueous $NaHCO_3$, water (60 mL), and brine (60 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 558.5 $(M+H)^+$.

1.1.10. 6-fluoro-3-bromopicolinic acid

A slurry of 6-amino-3-bromopicolinic acid (25 g) in 400 mL 1:1 dichloromethane/chloroform was added to nitrosonium tetrafluoroborate (18.2 g) in dichloromethane (100 mL) at 5°C over 1 hour, and the resulting mixture was stirred for another 30 minutes, then warmed to 35°C and stirred overnight. The reaction was cooled to room temperature, and then adjusted to pH 4 with aqueous NaH_2PO_4 solution. The resulting solution was extracted three times with dichloromethane, and the combined extracts were washed with brine, dried over sodium sulfate, filtered and concentrated to provide the title compound.

1.1.11. Tert-butyl 3-bromo-6-fluoropicolinate

Para-toluenesulfonyl chloride (27.6 g) was added to a solution of Example 1.1.10 (14.5 g) and pyridine (26.7 mL) in dichloromethane (100 mL) and tert-butanol (80 mL) at 0°C. The reaction was stirred for 15 minutes, warmed to room temperature, and stirred overnight. The solution was concentrated and partitioned between ethyl acetate and aqueous Na_2CO_3 solution. The layers were separated, and the aqueous layer extracted with ethyl acetate. The organic layers were combined, rinsed with aqueous Na_2CO_3 solution and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound.

1.1.12. methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 1.1.11 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL). The mixture was stirred at 50°C for 24 hours. The mixture was then diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 448.4 $(M+H)^+$.

1.1.13. methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a solution of Example 1.1.12 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL). The mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent, and silica gel chromatography (eluted with 20% ethyl acetate in heptane) gave the title compound. MS (ESI) m/e 495.4 $(M+H)^+$.

1.1.14. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a solution of Example 1.1.13 (4.94 g) in tetrahydrofuran (60 mL) and water (20 mL) was added Example 1.1.9 (5.57 g), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (412 mg), tris(dibenzylideneacetone)dipalladium(0) (457 mg), and K_3PO_4 (11 g). The mixture was stirred at reflux for 24 hours. The reaction mixture was cooled, diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na_2SO_4. Filtration and

evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 799.1 $(M+H)^+$.

1.1.15. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

To a solution of Example 1.1.14 (10 g) in tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g). The mixture was stirred at room temperature for 24 hours. The reaction mixture was neutralized with 2% aqueous HCl and concentrated under vacuum. The residue was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the title compound. MS (ESI) m/e 785.1 $(M+H)^+$.

1.1.16. *tert*-butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-3-{1-[(3-{2-[(*tert*-butoxycarbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1*H*-pyrazol-4-yl}pyridine-2-carboxylate

To a solution of Example 1.1.15 (10 g) in N,N-dimethylformamide (20 mL) was added benzo[d]thiazol-2-amine (3.24 g), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (5.69 g) and N,N-diisopropylethylamine (5.57 g). The mixture was stirred at 60^{o}C for 3 hours. The reaction mixture was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 915.5 $(M+H)^+$.

1.1.17. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

To a solution of Example 1.1.16 (5 g) in dichloromethane (20 mL) was added trifluoroacetic acid (10 mL). The mixture was stirred overnight. The solvent was evaporated under vacuum, and the residue was dissolved in dimethyl sulfoxide/methanol (1:1, 10 mL), and chromatographed via reverse-phase using an Analogix system and a C18 cartridge (300 g), eluting with 10-85% acetonitrile and 0.1% trifluoroacetic acid in water, to give the title compound as a TFA salt. ^{1}H NMR (300 MHz, dimethyl sulfoxide d_6) δ ppm 12.85 (s, 1H), 8.13-8.30 (m, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.32-7.54 (m, 3H), 7.28 (d, 1H), 6.96 (d, 1H), 4.96 (dd, 1H), 3.80-3.92

(m, 4H), 3.48-3.59 (m, 1H), 2.91-3.11 (m, 2H), 2.51-2.59 (m, 4H), 2.03-2.16 (m, 2H), 1.21-1.49 (m, 6H), 0.97-1.20 (m, 4H), 0.87 (s, 6H). MS (ESI) m/e 760.4 $(M+H)^+$.

1.2.Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Compound W1.02)

1.2.1. 2-(2-(2-(((3-((1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

To a solution of Example 1.1.3 (2.65 g) in 2,2'-(ethane-1,2-diylbis(oxy))diethanol (15 g) was added triethylamine (3 mL). The mixture was stirred at 180°C under microwave conditions (Biotage Initiator) for 120 minutes. The mixture was diluted with water and acetonitrile (1:1, 40 mL). The crude material was added to a reverse phase column (C18, SF65-800g) and was eluted with 10-100% acetonitrile in water with 0.1% trifluoroacetic acid to afford the title compound. MS (ESI) m/e 393.0 $(M+H)^+$.

1.2.2. 2-(2-(2-((3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

To a cooled (0°C) solution of Example 1.2.1 (2.69 g) in tetrahydrofuran (20 mL) was added n-BuLi (10 mL, 2.5M in hexane). The mixture was stirred at 0°C for 1.5 hours. Iodomethane (1 mL) was added through a syringe, and the mixture was stirred at 0°C for 1.5 hours. The reaction mixture was quenched with trifluoroacetic acid (1 mL). After evaporation of the solvents, the residue was used directly in the next step. MS (ESI) m/e 407.5 $(M+H)^+$.

1.2.3. 2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

To a cooled (0°C) solution of Example 1.2.2 (2.78 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (1.65 g). The mixture was stirred at room temperature for 2 hours. The crude product was added to a reverse phase column (C-18, SF65-800g) and was eluted with 10-100% acetonitrile in water with 0.1% trifluoroacetic acid to afford the title compound. MS (ESI) m/e 533.0 $(M+H)^+$.

1.2.4. 2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethoxy)-N-methylethanamine

To a cooled (0°C) solution of Example 1.2.3 (2.45 g) in tetrahydrofuran (10 mL) was added triethylamine (1 mL) followed by methanesulfonyl chloride (0.588 g). The mixture was stirred at room temperature for 2 hours. Methanamine (10 mL, 2M in methanol) was added to the reaction

mixture and transferred to a 20 mL microwave tube. The mixture was heated under microwave conditions (Biotage Initiator) at 100 °C for 60 minutes. After cooling to room temperature, the solvent was removed under vacuum. The residue was added to a reverse phase column (C18, SF40-300g) and eluted with 40-100% acetonitrile in water with 0.1% trifluoroacetic acid to afford the title compound. MS (ESI) m/e 546.0 $(M+H)^+$.

1.2.5. tert-butyl (2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethoxy)ethyl)(methyl)carbamate

To a solution of Example 1.2.4 (1.41 g) in tetrahydrofuran (20 mL) was added di-tert-butyl dicarbonate (1 g) and 4-dimethylaminopyridine (0.6 g). The mixture was stirred at room temperature for 3 hours, and the solvent was removed by vacuum. The residue was purified by silica gel chromatography, eluting with 10-100% ethyl acetate in hexane, to give the title compound. MS (ESI) m/e 645.8 $(M+H)^+$.

1.2.6. tert-butyl (2-(2-(2-((3,5-dimethyl-7-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethyl)(methyl)carbamate

To a solution of Example 1.2.5 (1.25 g), dicyclohexylphosphino-2',6'-dimethoxybiphenyl (0.09 g), pinacolborane (1.5 mL) and triethylamine (1.5 mL) in dioxane (20 mL) was added bis(benzonitrile)palladium(II) chloride (0.042 g). After degassing, the mixture was stirred at 90 °C overnight. Evaporation of the solvent and silica gel column purification (eluting with 20-100% ethyl acetate in hexane) gave the title compound. MS (ESI) m/e 646.1 $(M+H)^+$.

1.2.7. tert-butyl 8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate

To a solution of 2-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid (6.8 g) and benzo[*d*]thiazol-2-amine (5.52 g) in dichloromethane (80 mL) was added 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (9.4 g) and 4-dimethylaminopyridine (6 g). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (400 mL), washed with 5% aqueous HCl, water, and brine, and dried over Na_2SO_4. The mixture was filtered, and the filtrate was concentrated under reduced pressure to provide the title compound.

1.2.8. *N*-(benzo[*d*]thiazol-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxamide dihydrochloride

To a solution of Example 1.2.7 (8.5 g) in dichloromethane (80 mL) was added 2N HCl in diethyl ether (80 mL). The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure to provide the title compound.

1.2.9. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-bromopicolinate

Example 1.1.11 (0.736 g), Example 1.2.8 (1.62 g), and Cs_2CO_3 (4.1 g) were stirred in 12 mL of anhydrous N,N-dimethylacetamide at 120 °C for 12 hours. The cooled reaction mixture was then diluted with ethyl acetate and 10% citric acid. The organic phase was washed three times with citric acid, once with water and brine, and dried over Na_2SO_4. Filtration and concentration afforded crude material, which was chromatographed on silica gel using 0-40% ethyl acetate in hexanes to provide the title compound.

1.2.10. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1s,7s)-3,5-dimethyl-7-((2,2,5-trimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)oxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

To a solution of Example 1.2.6 (0.135 g) in tetrahydrofuran (1 mL) and water (1 mL) was added Example 1.2.9 (0.12 g), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (0.023 g), tris(dibenzylideneacetone)dipalladium(0) (0.015 g), and K_3PO_4 (0.2 g). The mixture was stirred at 140 °C for 5 minutes under microwave conditions (Biotage Initiator). The reaction mixture was diluted with toluene (5 mL) and filtered. Evaporation of solvent and silica gel purification (20-100% ethyl acetate in heptane) gave the title compound. MS (ESI) m/e 1004.8 $(M+H)^+$.

1.2.11. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

Example 1.2.10 (1.42 g) in dichloromethane (10 mL) was treated with trifluoroacetic acid (6 mL), and the reaction was stirred at room temperature for 24 hours. The volatiles were removed under reduced pressure. The residue was purified by reverse phase chromatography using a Gilson system (C18, SF40-300g) eluting with 30-100% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound as a TFA salt. ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (br.s, 1H), 8.33 (br.s, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.41-7.54 (m, 3H), 7.32-7.40 (m, 2H), 7.28 (s, 1H), 6.95 (d, 1H), 4.95 (s, 2H), 3.85-3.93 (m, 2H), 3.81 (s, 2H), 3.60-3.66 (m, 2H), 3.52-3.58 (m, 4H), 3.45 (s, 3H), 2.97-3.12 (m, 4H), 2.56 (t, 2H), 2.10 (s, 3H), 1.34-1.41 (m, 2H), 1.18-1.31 (m, 4H), 0.95-1.18 (m, 6H), 0.85 (s, 6H). MS (ESI) m/e 848.2 $(M+H)^+$.

1.3.Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Compound W1.03)

1.3.1. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a solution of Example 1.1.13 (2.25 g) in tetrahydrofuran (30 mL) and water (10 mL) was added Example 1.1.6 (2.0 g), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadmante (329 mg), tris(dibenzylideneacetone)dipalladium(0) (206 mg) and potassium phosphate tribasic (4.78 g). The mixture was refluxed overnight, cooled, and diluted with ethyl acetate (500 mL). The resulting mixture was washed with water and brine, and the organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes and then with 5% methanol in dichloromethane to provide the title compound.

1.3.2. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a cold solution of Example 1.3.1 (3.32 g) in dichloromethane (100 mL) in an ice-bath was sequentially added triethylamine (3 mL) and methanesulfonyl chloride (1.1 g). The reaction mixture was stirred at room temperature for 1.5 hours, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to provide the title compound.

1.3.3. methyl 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

To a solution of Example 1.3.2 (16.5 g) in N,N-dimethylformamide (120 mL) was added sodium azide (4.22 g). The mixture was heated at 80°C for 3 hours, cooled, diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes to provide the title compound.

1.3.4. 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

To a solution of Example 1.3.3 (10 g) in a mixture of tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g). The mixture was stirred at room temperature overnight and neutralized with 2% aqueous HCl. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate (800 mL), and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to provide the title compound.

1.3.5. tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

The title compound was prepared by following the procedure described in 1.1.16, replacing Example 1.1.15 with Example 1.3.4.

1.3.6. tert-butyl 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

To a solution of Example 1.3.5 (2.0 g) in tetrahydrofuran (30 mL) was added Pd/C (10%, 200 mg). The mixture was stirred under hydrogen atmosphere overnight. The reaction was filtered, and the filtrate was concentrated to provide the title compound.

1.3.7. 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

Example 1.3.6 (300 mg) in dichloromethane (3 mL) was treated with trifluoroacetic acid (3 mL) overnight. The reaction mixture was concentrated ,and the residue was purified by reverse phase chromatography using a Gilson system (300g C18 column), eluting with 10-70% acetonitrile in 0.1% trifluoroacetic acid water solution, to provide the title compound as a trifluoroacetic acid salt. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H) 8.03 (d, 1H) 7.79 (d, 1H) 7.59-7.73 (m, 4H) 7.41-7.53 (m, 3H) 7.32-7.40 (m, 2H) 7.29 (s, 1H) 6.96 (d, 1H) 4.96 (s, 2H) 3.89 (t, 2H) 3.83 (s, 2H) 3.50 (t, 2H) 3.02 (t, 2H) 2.84-2.94 (m, 2H) 2.11 (s, 3H) 1.41 (s, 2H) 1.21-1.36 (m, 4H) 1.08-1.19 (m, 4H) 0.96-1.09 (m, 2H) 0.87 (s, 6H). MS (ESI) m/e 744.3 $(M-H)^-$.

1.4.Synthesis of 3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Compound W1.04)

1.4.1. 2-(2-((3-((1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethanol

The title compound was prepared as described in Example 1.1.4 by substituting ethane-1,2-diol with 2,2'-oxydiethanol. MS (ESI) m/e 349.2 $(M+H)^+$.

1.4.2. 2-(2-((3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethanol

The title compound was prepared as described in Example 1.1.5 by substituting Example 1.1.4 with Example 1.4.1. MS (ESI) m/e 363.3 $(M+H)^+$.

1.4.3. 2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethanol

The title compound was prepared as described in Example 1.1.6 by substituting Example 1.1.5 with Example 1.4.2. MS (ESI) m/e 489.2 $(M+H)^+$.

1.4.4. 2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethyl methanesulfonate

The title compound was prepared as described in Example 1.1.7 by substituting Example 1.1.6 with Example 1.4.3. MS (ESI) m/e 567.2 $(M+H)^+$.

1.4.5. 2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethanamine

The title compound was prepared as described in Example 1.1.8 by substituting Example 1.1.7 with Example 1.4.4, and 2N methylamine in methanol with 7N ammonia in methanol. MS (ESI) m/e 488.2 $(M+H)^+$.

1.4.6. tert-butyl (2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethoxy)ethyl)carbamate

The title compound was prepared as described in Example 1.1.9 by substituting Example 1.1.8 with Example 1.4.5. MS (ESI) m/e 588.2 $(M+H)^+$.

1.4.7. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(2-((tert-butoxycarbonyl)amino)ethoxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

The title compound was prepared as described in Example 1.1.14 by substituting Example 1.1.9 with Example 1.4.6. MS (ESI) m/e 828.5 $(M+H)^+$.

1.4.8. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(2-((tert-butoxycarbonyl)amino)ethoxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

The title compound was prepared as described in Example 1.1.15 by substituting Example 1.1.14 with Example 1.4.7. MS (ESI) m/e 814.5 $(M+H)^+$.

1.4.9. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(2-((tert-butoxycarbonyl)amino)ethoxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

The title compound was prepared as described in Example 1.1.16 by substituting Example 1.1.15 with Example 1.4.8. MS (ESI) m/e 946.2 $(M+H)^+$.

1.4.10. 3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

The title compound was prepared as described in Example 1.1.17 by substituting Example 1.1.16 with Example 1.4.9. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 7.99-8.08 (m, 1H), 7.60-7.82 (m, 4H), 7.20-7.52 (m, 5H), 6.93-6.99 (m, 1H), 4.96 (s, 2H), 3.45-3.60 (m, 6H), 2.09-2.14 (m, 4H), 0.95-1.47 (m, 19H), 0.81-0.91 (m, 6H). MS (ESI) m/e 790.2 $(M+H)^+$.

1.5.Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Compound W1.05)

1.5.1. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,3r)-3-(2-((2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

A solution of Example 1.3.6 (0.050 g) and 2-methoxyacetaldehyde (6.93 mg) were stirred together in dichloromethane(0.5 mL) at room temperature for 1 hour. To the reaction was added a suspension of sodium borohydride (2 mg) in methanol (0.2 mL). After stirring for 30 minutes,

the reaction was diluted with dichloromethane (2 mL) and quenched with saturated aqueous sodium bicarbonate (1 mL). The organic layer was separated, dried over magnesium sulfate, filtered, and concentrated to give the title compound. MS (ELSD) m/e 860.5 $(M+H)^+$.

1.5.2. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

A solution of Example 1.5.1 in dichloromethane (1 mL) was treated with trifluoroacetic acid (0.5 mL). After stirring overnight, the reaction was concentrated, dissolved in N,N-dimethylformamide (1.5 mL) and water (0.5 mL) and was purified by Prep HPLC using a Gilson system eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound as a TFA salt. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 2H), 8.39 (s, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.53-7.42 (m, 3H), 7.40-7.33 (m, 2H), 7.29 (s, 1H), 6.96 (d, 1H), 4.96 (s, 2H), 3.89 (t, 2H), 3.83 (s, 2H), 3.61-3.53 (m, 10H), 3.29 (s, 3H), 3.17-3.09 (m, 2H), 3.09-2.97 (m, 4H), 2.10 (s, 3H), 1.41 (s, 2H), 1.35-1.23 (m, 4H), 1.20-1.10 (m, 4H), 1.10-0.98 (m, 2H). MS (ESI) m/e 804.3 $(M+H)^+$.

1.6.Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Compound W1.06)

1.6.1. 3-Cyanomethyl-4-fluorobenzoic acid methyl ester

To a solution of trimethylsilanecarbonitrile (1.49 mL) in tetrahydrofuran (2.5 mL) was added 1M tetrabutylammonium fluoride (11.13 mL) dropwise over 20 minutes. The solution was then stirred at room temperature for 30 minutes. Methyl 4-fluoro-3-(bromomethyl)benzoate (2.50 g) was dissolved in acetonitrile (12 mL) and was added to the first solution dropwise over 10 minutes. The solution was then heated to 80 °C for 60 minutes and cooled. The solution was concentrated under reduced pressure and was purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound.

1.6.2. 3-(2-Aminoethyl)-4-fluorobenzoic acid methyl ester

Example 1.6.1 (1.84 g) was dissolved in tetrahydrofuran (50 mL), and 1 M borane (in tetrahydrofuran, 11.9 mL) was added. The solution was stirred at room temperature for 16 hours and was slowly quenched with methanol. 4 M Aqueous hydrochloric acid (35 mL) was added,

and the solution was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure, and the pH was adjusted to between 11 and 12 using solid potassium carbonate. The solution was then extracted with dichloromethane (3x 100 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 10- 20% methanol in dichloromethane. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 198 $(M+H)^+$.

1.6.3. 4-Fluoro-3-[2-(2,2,2-trifluoroacetylamino)ethyl]benzoic acid methyl ester

Example 1.6.2 (1.207 g) was dissolved in dichloromethane (40 mL), and N,N-diisopropylethylamine (1.3 mL) was added. Trifluoroacetic anhydride (1.0 mL) was then added dropwise. The solution was stirred for 15 minutes. Water (40 mL) was added, and the solution was diluted with ethyl acetate (100 mL). 1 M Aqueous hydrochloric acid was added (50 mL), and the organic layer was separated, washed with 1 M aqueous hydrochloric acid, and then washed with brine. The solution was dried on anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to provide the title compound.

1.6.4. 5-Fluoro-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

Example 1.6.3 (1.795 g) and paraformaldehyde (0.919 g) were placed in a flask and concentrated sulfuric acid (15 mL) was added. The solution was stirred at room temperature for one hour. Cold water (60 mL) was added, and the solution was extracted with ethyl acetate (2x 100 mL). The extracts were combined, washed with saturated aqueous sodium bicarbonate (100 mL) and water (100 mL), and dried over anhydrous sodium sulfate. The solution was filtered, concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 10-20% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 323 $(M+NH_4)^+$.

1.6.5. 5-Fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

Example 1.6.4 (685 mg) was dissolved in methanol (6 mL) and tetrahydrofuran (6 mL). Water (3 mL) was added followed by potassium carbonate (372 mg). The reaction was stirred at room temperature for three hours, and then diluted with ethyl acetate (100 mL). The solution was washed with saturated aqueous sodium bicarbonate and dried on anhydrous sodium sulfate. The solvent was filtered and evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 210 $(M+H)^+$.

1.6.6. 2-(5-Bromo-6-tert-butoxycarbonylpyridin-2-yl)-5-fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.6.5 for methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride in 1.1.12. MS (ESI) m/e 465, 467 $(M+H)^+$.

1.6.7. 2-[6-tert-Butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-5-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.6.6 for Example 1.1.12 in Example 1.1.13. MS (ESI) m/e 513 $(M+H)^+$.

1.6.8. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethanamine

A solution of Example 1.1.7 (4.5 g) in 7N ammonium in methanol (15 mL) was stirred at 100°C for 20 minutes under microwave conditions (Biotage Initiator). The reaction mixture was concentrated under vacuum, and the residue was diluted with ethyl acetate (400 mL) and washed with aqueous $NaHCO_3$, water (60 mL) and brine (60 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was used in the next reaction without further purification. MS (ESI) m/e 444.2 $(M+H)^+$.

1.6.9. tert-butyl (2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)carbamate

To a solution of Example 1.6.8 (4.4 g) in tetrahydrofuran (100 mL) was added di-t-butyl dicarbonate (2.6 g) and N,N-dimethyl-4-aminopyridine (100 mg). The mixture was stirred for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (300 mL) and washed with aqueous $NaHCO_3$, water (60 mL) and brine (60 mL). After drying (anhydrous Na_2SO_4), the solution was filtered and concentrated and the residue was purified by silica gel column chromatography (20% ethyl acetate in dichloromethane) to give the title compound. MS (ESI) m/e 544.2 $(M+H)^+$.

1.6.10. 2-(6-tert-Butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-5-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.6.7 for Example 1.1.13 and Example 1.6.9 for Example 1.1.9 in Example 1.1.14. MS (ESI) m/e 802 $(M+H)^+$.

1.6.11. 2-(6-tert-Butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-5-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid

The title compound was prepared by substituting Example 1.6.10 for Example 1.1.14 in Example 1.1.15. MS (ESI) m/e 788 $(M+H)^+$.

1.6.12. 6-[8-(Benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydro-1H-isoquinolin-2-yl]-3-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridine-2-carboxylic acid tert-butyl ester

The title compound was prepared by substituting Example 1.6.11 for Example 1.1.15 in Example 1.1.16. MS (ESI) m/e 920 $(M+H)^+$.

1.6.13. 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

The title compound was prepared by substituting Example 1.6.12 for Example 1.1.16 in Example 1.1.17. ^{1}H NMR (400MHz, dimethyl sulfoxide-d_6) δ ppm 12.88 (bs, 1H), 8.03 (d, 1H), 7.79 (d, 1H), 7.73 (m, 1H), 7.63 (m, 2H), 7.52 (d, 1H), 7.48 (t, 1H), 7.36 (t, 1H), 7.28 (dd, 2H), 7.04 (d, 1H), 5.02 (s, 2H), 3.95 (t, 2H), 3.83 (s, 2H), 3.49 (t, 2H), 2.90 (m, 4H), 2.11 (s, 3H), 1.41 (s, 2H), 1.35-1.23 (m, 4H), 1.19-0.99 (m, 6H), 0.87 (bs, 6H). MS (ESI) m/e 764 $(M+H)^+$.

1.7 Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (W1.07)

1.7.1 (3-bromo-5-fluoro-phenyl)-acetonitrile

The title compound was prepared by substituting 1-bromo-3-(bromomethyl)-5-fluorobenzene for methyl 4-fluoro-3-(bromomethyl)benzoate in Example 1.6.1.

1.7.2 2-(3-bromo-5-fluoro-phenyl)-ethylamine

The title compound was prepared by substituting Example 1.7.1 for Example 1.6.1 in Example 1.6.2.

1.7.3 [2-(3-bromo-5-fluoro-phenyl)-ethyl]-carbamic acid tert-butyl ester

Example 1.7.2 (1.40 g) and N,N-dimethylpyridin-4-amine (0.078 g) were dissolved in acetonitrile (50 mL). Di-tert-butyl dicarbonate (1.54 g) was added. The solution was stirred at

room temperature for 30 minutes. The solution was diluted with diethyl ether (150 mL), washed with 0.1 M aqueous HCl (25 mL) twice, washed with brine (50 mL), and dried on anhydrous sodium sulfate. The solution was filtered, concentrated under reduced pressure, and the crude material was purified by flash column chromatography on silica gel, eluting with 5-10% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound.

1.7.4 3-(2-tert-butoxycarbonylamino-ethyl)-5-fluoro-benzoic acid methyl ester

Example 1.7.3 (775 mg) and dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (36 mg) were added to a 50 mL pressure bottle. Methanol (10 mL) and trimethylamine (493 mg) were added. The solution was degassed and flushed with argon three times, followed by degassing and flushing with carbon monoxide. The reaction was heated to 100 °C for 16 hours under 60 psi of carbon monoxide. Additional dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (36 mg) was added and the degassing and flushing procedure was repeated. The reaction was heated to 100 °C for an additional 16 hours under 60 psi of carbon monoxide. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound.

1.7.5 3-(2-amino-ethyl)-5-fluoro-benzoic acid methyl ester

Example 1.7.4 (292 mg) was dissolved in dichloromethane (3 mL). 2,2,2-Trifluoroacetic acid (1680 mg) was added, and the solution was stirred at room temperature for two hours. The solvent was removed under reduced pressure to provide the title compound which was used in the next step without further purification.

1.7.6 3-fluoro-5-[2-(2,2,2-trifluoro-acetylamino)-ethyl]-benzoic acid methyl ester

The title compound was prepared by substituting Example 1.7.5 for Example 1.6.2 in Example 1.6.3.

1.7.7 6-fluoro-2-(2,2,2-trifluoro-acetyl)-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.7.6 for Example 1.6.3 in Example 1.6.4.

1.7.8 6-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.7.7 for Example 1.6.4 in Example 1.6.5.

1.7.9 2-(5-bromo-6-tert-butoxycarbonyl-pyridin-2-yl)-6-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.7.8 for methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride in Example 1.1.12. MS (ESI) m/e 464, 466 $(M+H)^+$.

1.7.10 2-[6-tert-butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-6-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.7.9 for Example 1.1.12 in Example 1.1.13. MS (ESI) m/e 513 $(M+H)^+$, 543 $(M+MeOH-H)^-$.

1.7.11 {2-[5-(4-iodo-5-methyl-pyrazol-1-ylmethyl)-3,7-dimethyl-adamantan-1-yloxy]-ethyl}-di-tert-butyl iminodicarboxylate

Example 1.1.6 (5.000 g) was dissolved in dichloromethane (50 mL). Triethylamine (1.543 g) was added, and the solution was cooled on an ice bath. Methanesulfonyl chloride (1.691 g) was added dropwise. The solution was allowed to warm to room temperature and stir for 30 minutes. Saturated aqueous sodium bicarbonate solution (50 mL) was added. The layers were separated, and the organic layer was washed with brine (50 mL). The aqueous portions were then combined and back extracted with dichloromethane (50 mL). The organic portions were combined, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was dissolved in acetonitrile (50 mL). Di-tert-butyl iminodicarboxylate (2.689 g) and cesium carbonate (7.332 g) were added, and the solution was refluxed for 16 hours. The solution was cooled and added to diethyl ether (100 mL) and water (100 mL). The layers were separated. The organic portion was washed with brine (50 mL). The aqueous portions were then combined and back extracted with diethyl ether (100 mL). The organic portions were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The material was purified by flash column chromatography on silica gel, eluting with 20% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 666 $(M+Na)^+$.

1.7.12 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

The title compound was prepared by substituting Example 1.7.10 for Example 1.1.13 and Example 1.7.11 for Example 1.1.9 in Example 1.1.14. MS (ESI) m/e 902 $(M+H)^+$.

1.7.13 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

The title compound was prepared by substituting Example 1.7.12 for Example 1.1.14 in Example 1.1.15. MS (ESI) m/e 888 $(M+H)^+$, 886 $(M-H)^-$.

1.7.14 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

The title compound was prepared by substituting Example 1.7.13 for Example 1.1.15 in Example 1.1.16.

1.7.15 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

The title compound was prepared by substituting Example 1.7.14 for Example 1.1.16 in Example 1.1.17. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.04 (d, 1H), 7.79 (d, 1H), 7.65 (bs, 3H), 7.50 (m, 2H), 7.40-7.29 (m, 3H), 6.98 (d, 1H), 4.91 (d, 2H), 3.88 (t, 2H), 3.83 (s, 2H), 3.02 (t, 2H), 2.89 (t, 4H), 2.10 (s, 3H), 1.44-1.20 (m, 6H), 1.19-1.00 (m, 6H), 0.86 (bs, 6 H). MS (ESI) m/e 764 $(M+H)^+$, 762 $(M-H)^-$.

1.8 Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (W1.08)

1.8.1 [2-(3-bromo-4-fluoro-phenyl)-ethyl]-carbamic acid tert-butyl ester

The title compound was prepared by substituting 2-(3-bromo-4-fluorophenyl)ethanamine hydrochloride for Example 1.7.2 in Example 1.7.3.

1.8.2 5-(2-tert-butoxycarbonylamino-ethyl)-2-fluoro-benzoic acid methyl ester

The title compound was prepared by substituting Example 1.8.1 for Example 1.7.3 in Example 1.7.4. MS (ESI) m/e 315 $(M+NH_4)^+$.

1.8.3 5-(2-amino-ethyl)-2-fluoro-benzoic acid methyl ester

The title compound was prepared by substituting Example 1.8.2 for Example 1.7.4 in Example 1.7.5.

1.8.4 2-fluoro-5-[2-(2,2,2-trifluoro-acetylamino)-ethyl]-benzoic acid methyl ester

The title compound was prepared by substituting Example 1.8.3 for Example 1.6.2 in Example 1.6.3.

1.8.5 7-fluoro-2-(2,2,2-trifluoro-acetyl)-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.8.4 for Example 1.6.3 in Example 1.6.4. MS (ESI) m/e 323 $(M+NH_4)^+$.

1.8.6 7-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.8.5 for Example 1.6.4 in Example 1.6.5. MS (ESI) m/e 210 $(M+H)^+$, 208 $(M-H)^-$.

1.8.7 2-(5-bromo-6-tert-butoxycarbonyl-pyridin-2-yl)-7-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.8.6 for methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride in Example 1.1.12. MS (ESI) m/e 465,467 $(M+H)^+$.

1.8.8 2-[6-tert-butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-7-fluoro-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

The title compound was prepared by substituting Example 1.8.7 for Example 1.1.12 in Example 1.1.13. MS (ESI) m/e 513 $(M+H)^+$, 543 $(M+MeOH-H)^-$.

1.8.9 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-7-fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

The title compound was prepared by substituting Example 1.8.8 for Example 1.1.13 and Example 1.7.11 for Example 1.1.9 in Example 1.1.14. MS (ESI) m/e 902 $(M+H)^+$, 900 $(M-H)^-$.

1.8.10 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-7-fluoro-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

The title compound was prepared by substituting Example 1.8.9 for Example 1.1.14 in Example 1.1.15. MS (ESI) m/e 788 $(M+H)^+$, 786 $(M-H)^-$.

1.8.11 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

The title compound was prepared by substituting Example 1.8.10 for Example 1.1.15 in Example 1.1.16.

1.8.12 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

The title compound was prepared by substituting Example 1.8.11 for Example 1.1.16 in Example 1.1.17. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 13.08 (bs, 1H), 11.41 (bs, 1H), 8.05 (d, 1H), 7.81 (d, 1H), 7.63 (m, 4H), 7.55-7.22 (m, 6H), 6.95 (d, 1H), 4.78 (s, 2H), 3.86 (m, 4H), 3.50 (m, 2H), 2.97 (m, 2H), 2.90 (m, 2H), 2.09 (s, 3H), 1.48-1.40 (m, 2H), 1.38-1.23 (m, 4H), 1.20-1.01 (m, 6H), 0.88 (bs, 6H). MS (ESI) m/e 764 $(M+H)^+$, 762 $(M-H)^-$.

1.9 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (W1.09)

1.9.1 *tert*-butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-3-[1-({3,5-dimethyl-7-[(2,2,7,7-tetramethyl-10,10-dioxido-3,3-diphenyl-4,9-dioxa-10λ^6-thia-13-aza-3-silapentadecan-15-yl)oxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1*H*-pyrazol-4-yl]pyridine-2-carboxylate

To a solution of Example 1.3.6 (500 mg) in N,N-dimethylformamide (8 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (334 mg). The reaction was stirred at room temperature overnight and methylamine (0.3 mL) was added to quench the reaction. The resulting mixture was stirred for 20 minutes and purified by reverse-phase chromatography using an Analogix system (C18 column), eluting with 50-100% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound.

1.9.2 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

Example 1.9.1 (200 mg) in dichloromethane (5 mL) was treated with trifluoroacetic acid (2.5 mL) overnight. The reaction mixture was concentrated and purified by reverse phase

chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. ^{1}H NMR (500 MHz, dimethylsulfoxide-d_6) δ ppm 12.86 (s, 1H), 8.32 (s, 2H), 8.02 (d, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.51 (d, 1H), 7.40-7.49 (m, 2H), 7.31-7.39 (m, 2H), 7.27 (s, 1H), 6.95 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.15-3.25 (m, 2H), 3.03-3.13 (m, 2H), 3.00 (t, 2H), 2.79 (t, 2H), 2.09 (s, 3H), 1.39 (s, 2H), 1.22-1.34 (m, 4H), 0.94-1.18 (m, 6H), 0.85 (s, 6H). MS (ESI) m/e 854.1 $(M+H)^+$.

Example 2. **Synthesis of Exemplary Synthons**This example provides synthetic methods for exemplary synthons that may be used to make ADCs.

2.1. Synthesis of N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl) carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon E)

2.1.1. (S)-(9H-fluoren-9-yl)methyl (1-((4-(hydroxymethyl) phenyl)amino)-1-oxo-5-ureidopentan-2-yl)carbamate

(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-ureidopentanoic acid (40 g) was dissolved in dichloromethane (1.3L). (4-Aminophenyl)methanol (13.01 g), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (42.1 g) and N,N-diisopropylethylamine (0.035 L) were added to the solution, and the resulting mixture was stirred at room temperature for 16 hours. The product was collected by filtration and rinsed with dichloromethane. The combined solids were dried under vacuum to yield the title compound, which was used in the next step without further purification. MS (ESI) m/e 503.3 $(M+H)^+$.

2.1.2. (S)-2-amino-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide

Example 2.1.1 (44 g) was dissolved in N,N-dimethylformamide (300 mL). The solution was treated with diethylamine (37.2 mL) and stirred for one hour at room temperature. The reaction mixture was filtered, and the solvent was concentrated under reduced pressure. The crude product was purified by basic alumina chromatography eluting with a gradient of 0-30% methanol in ethyl acetate to give the title compound. MS (ESI) m/e 281.2 $(M+H)^+$.

2.1.3. tert-butyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

(S)-2-(Tert-butoxycarbonylamino)-3-methylbutanoic acid (9.69 g) was dissolved in N,N-dimethylformamide (200 mL). To the solution was added 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-

yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (18.65 g), and the reaction was stirred for one hour at room temperature. Example 2.1.2 (12.5 g) and N,N-diisopropylethylamine (15.58 mL) were added and the reaction mixture was stirred for 16 hours at room temperature. The solvent was concentrated under reduced pressure and the residue was purified by silica gel chromatography, eluting with 10% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 480.2 $(M+H)^+$.

2.1.4. (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide

Example 2.1.4 (31.8 g) was dissolved in dichloromethane (650 mL) and to the solution was added trifluoroacetic acid (4.85 mL). The reaction mixture was stirred for three hours at room temperature. The solvent was concentrated under reduced pressure to yield a mixture of the crude title compound and 4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl 2,2,2-trifluoroacetate. The crude material was dissolved in a 1:1 dioxane/water solution (300 mL) and to the solution was added sodium hydroxide (5.55 g). The mixture was stirred for three hours at room temperature. The solvent was concentrated under vacuum, and the crude product was purified by reverse phase HPLC using a CombiFlash system, eluting with a gradient of 5-60% acetonitrile in water containing 0.05% v/v ammonium hydroxide, to give the title compound. MS (ESI) m/e 380.2 $(M+H)^+$.

2.1.5. 1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3,6,9,12-tetraoxapentadecan-15-amide

To a solution of Example 2.1.4 (1.5 g) in N,N-dimethylformamide (50 mL) was added 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate (2.03 g). The mixture was stirred at room temperature for three days. The crude material was added to a reverse phase column (C18, SF65-800g) and was eluted with 20-100% acetonitrile in water with 0.1% trifluoroacetic acid to afford the title compound. MS (ESI) m/e 778.3 $(M+1)^+$.

2.1.6. 4-((2S,5S)-25-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-4,7,23-trioxo-2-(3-ureidopropyl)-10,13,16,19-tetraoxa-3,6,22-triazapentacosanamido)benzyl (4-nitrophenyl) carbonate

To a solution of Example 2.1.5 (2.605 g) and N,N-diisopropylamine (1.8 mL) in N,N-dimethylformamide (20 mL) was added bis(4-nitrophenyl) carbonate (1.23 g). The mixture was stirred at room temperature for 16 hours. The crude material was added to a reverse phase

column (C18, SF65-800g) and was eluted with 20-100% acetonitrile in water with 0.1% trifluoroacetic acid to afford the title compound. MS (ESI) m/e 943.2 $(M+1)^+$.

2.1.7. N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide

To a mixture of Example 2.1.6 (49.6 mg) and Example 1.1.17 (30 mg) in N,N-dimethylformamide (2 mL) at 0 °C was added N,N-diisopropylethylamine (0.018 mL). The reaction mixture was stirred at room temperature overnight, diluted with dimethyl sulfoxide, and purified by RP-HPLC using a Gilson system, eluting with 20-70% acetonitrile in 0.1% trifluoroacetic acid water solution to provide the title compound. MS (ESI) m/e 1563.4 $(M+H)^+$.

2.2. Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon D)

To a solution of 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate (purchased from Synchem, 57 mg) and Example 1.1.17 (57 mg) in N,N-dimethylformamide (6 mL) was added N,N-diisopropylethylamine (0.5 mL). The mixture was stirred overnight and then concentrated under vacuum. The residue was diluted with methanol (3 mL) and acetic acid (0.3 mL) and purified by RP-HPLC (Gilson system, C18 column), eluting with 30-70% acetonitrile in water containing 0.1% trifluoroacetic acid. Lyophilization of the product fractions gave the title compound. ^{1}H NMR (300 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (d, 1H), 9.98 (s, 1H), 7.96-8.10 (m, 2H), 7.74-7.83 (m, 2H), 7.54-7.64 (m, 3H), 7.31-7.52 (m, 6H), 7.24-7.29 (m, 3H), 6.99 (s, 2H), 6.94 (d, 1H), 4.96 (d, 4H), 4.33-4.43 (m, 2H), 4.12-4.24 (m, 2H), 3.22-3.42 (m, 7H), 2.77-3.07 (m, 7H), 1.86-2.32 (m, 7H), 0.92-1.70 (m, 22H), 0.72-0.89 (m, 13H). MS (ESI) m/e 1358.2 $(M+H)^+$.

2.3. Synthesis of N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-

({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy) methyl]phenyl}-L-alaninamide (Synthon J)

2.3.1. (S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)propanamido)propanoic acid

A solution of (S)-2,5-dioxopyrrolidin-1-yl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoate (5 g) in 40 mL dimethoxyethane was added to a solution of L-alanine (1.145 g) and sodium bicarbonate (1.08 g) in water (40 mL). The reaction mixture was stirred at room temperature for 16 hours. Aqueous citric acid (15% v/v, 75 mL) was added to the reaction. The precipitate was filtered, washed with water (2 x 250 mL) and dried under vacuum. The solid was further triturated with diethyl ether (100 mL), filtered, and dried over sodium sulfate to yield the product, which was used in the next step without further purification. MS (ESI) m/e 383.0 $(M+H)^+$.

2.3.2. (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl) phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate

N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (6.21 g) was added to a solution of Example 2.3.1 (3.2 g) and 4-aminobenzyl alcohol (1.546 g) in 50 mL of 2:1 dichloromethane:methanol. The reaction was stirred at room temperature for 2 days. The solvent was concentrated under vacuum. The residue was triturated with 75 mL of ethyl acetate, and the solid was collected by filtration, and dried under vacuum to yield the title compound, which was used in the next step without further purification. MS (ESI) m/e 488.0 $(M+H)^+$.

2.3.3. (S)-2-amino-N-((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)propanamide

Diethylamine (11.75 mL) was added to a solution of Example 2.3.2 (1.58 g) in N,N dimethylformamide (50 mL), and the reaction was allowed to stand at room temperature for 16 hours. The solvent was evaporated under vacuum. The residue was triturated with ethyl acetate (100 mL), and the product was collected by filtration and dried under vacuum to yield the title compound, which was used in the next step without further purification. MS (ESI) m/e 266.0 $(M+H)^+$.

2.3.4. 1-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-N-((S)-1-(((S)-1-((4-

(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3,6,9,12-tetraoxapentadecan-15-amide

Example 2.3.3 (1.033 g) was mixed with 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate (2 g) in N,N-dimethylformamide (19.5 mL) with 1% N,N-diisopropylethylamine for 16 hours. The crude reaction was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (ESI) m/e 664.0 $(M+H)^+$.

2.3.5. 4-((2S,5S)-25-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,5-dimethyl-4,7,23-trioxo-10,13,16,19-tetraoxa-3,6,22-triazapentacosanamido)benzyl (4-nitrophenyl) carbonate

Example 2.3.4 (1.5 g) was mixed with bis(4-nitrophenyl)carbonate (1.38 g) in N,N-dimethylformamide (11.3 mL) with 1% N,N-diisopropylethylamine. The reaction was stirred at room temperature for 16 hours. The crude reaction was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (ESI) m/e 829.0 $(M+H)^+$.

2.3.6. N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide

The trifluoroacetic acid salt of Example 1.1.17 (15 mg) was mixed with Example 2.3.5 (21.3 mg) in N,N-dimethylformamide (1 mL) and N,N-diisopropylethylamine (0.006 mL). The reaction mixture was stirred at room temperature for one hour. The crude reaction was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (ESI) m/e 1450.7 $(M+H)^+$.

2.4.Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-

yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon K)

2.4.1. 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)hexanamide

The title compound was prepared by substituting N-succinimidyl 6-maleimidohexanoate for 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate in Example 2.3.4. MS (ESI) m/e 640.8 $(M+NH_4)^+$.

2.4.2. 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)propanamido)propanamido)benzyl(4-nitrophenyl)carbonate

The title compound was prepared by substituting Example 2.4.1 for Example 2.3.4 in Example 2.3.5.

2.4.3. N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide

The title compound was prepared by substituting Example 2.4.2 for Example 2.3.5 in Example 2.3.6. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.56 (s, 1H), 7.98 (d, 1H), 7.76 (d, 1H), 7.71–7.52 (m, 3H), 7.51–7.21 (m, 4H), 6.97–6.84 (m, 1H), 4.98 (d, 2H), 4.42 (p, 1H), 4.27 (p, 1H), 3.89 (t, 1H), 3.80 (s, 2H), 3.43 (d, 19H), 3.03 (t, 7H), 2.87 (s, 2H), 2.32 (s, 1H), 2.11 (d, 3H), 1.52 (h, 2H), 1.41–0.94 (m, 12H), 0.84 (s, 3H). MS (ESI) m/e 1244.2 $(M+H)^+$.

2.5.Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({(1s,3s)-3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon L)

2.5.1. (3-bromoadamantan-1-yl)methanol

The title compound was prepared by substituting 3-bromoadamantane-1-carboxylic acid for Example 1.1.1 in Example 1.1.2.

2.5.2. 1-((3-bromoadamantan-1-yl)methyl)-1H-pyrazole

The title compound was prepared by substituting Example 2.5.1 for Example 1.1.2 in Example 1.1.3. MS (ESI) m/e 295.2 $(M+H)^+$.

2.5.3. 2-(2-(2-((3-((1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

The title compound was prepared by substituting Example 2.5.2 for Example 1.1.3 and substituting silver sulfate for triethylamine in Example 1.2.1. MS (ESI) m/e 365.1 $(M+H)^+$.

2.5.4. 2-(2-(2-((3-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

The title compound was prepared by substituting Example 2.5.3 for Example 1.2.1 in Example 1.2.2. MS (ESI) m/e 379.1 $(M+H)^+$.

2.5.5. 2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethanol

The title compound was prepared by substituting Example 2.5.4 for Example 1.2.2 in Example 1.2.3. MS (ESI) m/e 504.9 $(M+H)^+$.

2.5.6. 2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)-N-methylethanamine

The title compound was prepared by substituting Example 2.5.5 for Example 1.2.3 in Example 1.2.4. MS (ESI) m/e 518.4 $(M+H)^+$.

2.5.7. tert-butyl (2-(2-(2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethyl)(methyl)carbamate

The title compound was prepared by substituting Example 2.5.6 for Example 1.2.4 in Example 1.2.5. MS (ESI) m/e 617.9 $(M+H)^+$.

2.5.8. tert-butyl methyl(2-(2-(2-((3-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethoxy)ethoxy)ethyl)carbamate

The title compound was prepared by substituting Example 2.5.7 for Example 1.2.5 in Example 1.2.6. MS (ESI) m/e 618.2 $(M+H)^+$.

2.5.9. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(5-methyl-1-((3-((2,2,5-trimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)oxy)adamantan-1-yl)methyl)-1H-pyrazol-4-yl)picolinate

The title compound was prepared by substituting Example 2.5.8 for Example 1.2.6 in Example 1.2.10. MS (ESI) m/e 976.1 $(M+H)^+$.

2.5.10. 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(5-methyl-1-(((1s,3s)-3-(2-(2-(2-(methylamino)ethoxy)ethoxy)ethoxy)adamantan-1-yl)methyl)-1H-pyrazol-4-yl)picolinic acid

The title compound was prepared by substituting Example 2.5.9 for Example 1.2.10 in Example 1.2.11. MS (ESI) m/e 820.3 $(M+H)^+$.

2.5.11. N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide

The title compound was prepared by substituting Example 2.5.10 for Example 1.1.17 in Example 2.2. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.96 (br.s, 1H), 7.96-8.12 (m, 2H), 7.73-7.83 (m, 2H), 7.29-7.66 (m, 9H), 7.17-7.30 (m, 3H), 6.89-7.01 (m, 2H), 4.86-5.01 (m, 4H), 4.28-4.45 (m, 1H), 4.12-4.21 (m, 1H), 3.69-3.92 (m, 3H), 3.27-3.62 (m, 9H), 2.78-3.06 (m, 7H), 2.01-2.23 (m, 7H), 1.87-2.01 (m, 1H), 1.54-1.72 (m, 4H), 1.01-1.54 (m, 22H), 0.72-0.89 (m, 6H). MS (ESI) m/e 1418.4 $(M+H)^+$.

2.6.Synthesis of N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon M)

The title compound was prepared by substituting Example 2.5.10 for Example 1.1.17 in Example 2.1.7. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 9.97 (s, 1H), 8.07-8.13 (m, 1H), 7.97-8.05 (m, 2H), 7.86 (d, 1H), 7.78 (d, 1H), 7.55-7.63 (m, 3H), 7.40-7.51 (m, 3H), 7.32-7.38 (m, 2H), 7.25-7.30 (m, 2H), 6.98 (s, 1H), 6.93 (d, 1H), 4.91-5.01 (m, 4H), 4.31-4.41 (m, 1H), 4.17-4.24 (m, 1H), 3.83-3.91 (m, 2H), 3.76 (s, 2H), 3.30-3.62 (m, 21H), 3.10-3.17 (m, 1H), 2.89-3.05 (m, 4H), 2.81-2.88 (m, 3H), 2.42-2.47 (m, 1H), 2.27-2.40 (m, 3H), 2.04-2.15 (m, 5H), 1.91-2.00 (m, 1H), 1.30-1.72 (m, 16H), 0.76-0.88 (m, 6H). MS (ESI) m/e 1623.3 $(M+H)^+$.

2.7.Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon V)

The title compound was prepared by substituting Example 1.2.11 for Example 1.1.17 in Example 2.2. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 9.61 (s, 1H), 7.97 (d, 1H), 7.76 (d, 1H), 7.67 (d, 1H), 7.61 (d, 1H), 7.51-7.57 (m, 2H), 7.38-7.48 (m, 4H), 7.29-7.36 (m, 2H), 7.23-7.28 (m, 3H), 6.86-6.94 (m, 2H), 4.97 (d, 4H), 4.38-4.45 (m, 1H), 4.12-4.19 (m, 1H), 3.89 (t, 2H), 3.80 (s, 2H), 3.47-3.54 (m, 5H), 3.44 (s, 3H), 3.33-3.41 (m, 6H), 2.93-3.06 (m, 6H), 2.87 (s, 2H), 2.11-2.22 (m, 2H), 2.08 (s, 3H), 1.97-2.05 (m, 1H), 1.70-1.81 (m, 2H), 1.33-1.68 (m, 10H), 0.95-1.32 (m, 14H), 0.80-0.91 (m, 13H). MS (+ESI) m/e 1446.3 $(M+H)^+$.

2.8.Synthesis of N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon DS)

2.8.1. (S)-2-((S)-2-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)acetamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide

The title compound was prepared by substituting 2,5-dioxopyrrolidin-1-yl 2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)acetate for 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate in Example 2.1.5.

2.8.2. 4-((2S,5S)-14-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-4,7-dioxo-2-(3-ureidopropyl)-9,12-dioxa-3,6-diazatetradecanamido)benzyl (4-nitrophenyl) carbonate

The title compound was prepared by substituting Example 2.8.1 for Example 2.3.4 in Example 2.3.5.

2.8.3. N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-

yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide

The title compound was prepared by substituting Example 1.2.11 for Example 1.1.17 and Example 2.8.2 for 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate in Example 2.2. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 9.64 (s, 1H), 7.97 (d, 1H), 7.92 (d, 1H), 7.75 (d, 1H), 7.60 (d, 1H), 7.54 (d, 2H), 7.45 (d, 2H), 7.38-7.43 (m, 1H), 7.29-7.36 (m, 2H), 7.22-7.28 (m, 4H), 6.88-6.93 (m, 2H), 4.98 (d, 4H), 4.39-4.46 (m, 1H), 4.24-4.31 (m, 1H), 3.86-3.93 (m, 4H), 3.80 (s, 2H), 3.46-3.61 (m, 15H), 3.43-3.45 (m, 5H), 3.33-3.38 (m, 4H), 2.87 (s, 3H), 1.99-2.11 (m, 4H), 1.56-1.80 (m, 2H), 1.34-1.50 (m, 4H), 0.94-1.32 (m, 11H), 0.80-0.91 (m, 13H). MS (+ESI) m/e 1478.3 (M+H).

2.9. This paragraph is intentionally left blank.

2.10. Synthesis of N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon BG)

2.10.1. (S)-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide

Example 2.1.4 (3 g) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (1.789 g) were dissolved in methanol (30 mL) and stirred for three hours at room temperature. The solvent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with a gradient of 5-30% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 531.0 $(M+H)^+$.

2.10.2. 4-((S)-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate

Bis(4-nitrophenyl) carbonate (2.293 g), N,N-diisopropylethylamine (1.317 mL) and Example 2.10.1 (2 g) were dissolved in N,N-dimethylformamide (30 mL) and stirred for 16 hours at room temperature. The solvent was concentrated under reduced pressure, and the residue was purified

by silica gel chromatography, eluting with a gradient of 0-10% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 696.9 $(M+H)^+$.

2.10.3. N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide

The title compound was prepared by substituting Example 2.10.2 for Example 2.9.4 in Example 2.9.5. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (bs, 1H), 9.95 (s, 1H), 8.10 (d, 1H), 8.01 (dd, 2H), 7.79 (d, 1H), 7.65-7.56 (m, 3H), 7.55-7.40 (m, 3H), 7.40-7.33 (m, 2H), 7.35-7.24 (m, 3H), 6.99 (s, 2H), 6.95 (d, 1H), 4.42-4.28 (m, 1H), 4.15 (dd, 1H), 3.92-3.85 (m, 2H), 3.83-3.77 (m, 2H), 3.77-3.52 (m, 2H), 3.45-3.38 (m, 2H), 3.30-3.23 (m, 2H), 3.08-2.90 (m, 4H), 2.90-2.81 (m, 3H), 2.09 (s, 3H), 2.02-1.86 (m, 1H), 1.79-1.52 (m, 2H), 1.52-0.92 (m, 15H), 0.91-0.75 (m, 13H). MS (ESI) m/e 1316.1 $(M+H)^+$.

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2.12. Synthesis of N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon BI)

2.12.1. 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)propanamide

A mixture of Example 2.3.3 (9 g) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (9.03 g) in N,N-dimethylformamide (50 mL) was stirred at room temperature for 16 hours. The reaction mixture was diluted with water. The aqueous layer was back extracted with methylene chloride (3 x 100 mL). The organic solvent was concentrated under vacuum. The resulting crude product was absorbed onto silica gel and purified by silica gel chromatography, eluting with 50:1 dichloromethane/methanol, to yield the title compound. MS (ESI) m/e 439.1 $(M+Na)^+$.

2.12.2. 4-((S)-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)propanamido)benzyl (4-nitrophenyl) carbonate

The title compound was prepared by substituting Example 2.12.1 for Example 2.10.1 in Example 2.10.2. The product was purified by silica gel chromatography silica, eluting with 25% tetrahydrofuran /dichloromethane. MS (ESI) m/e 604.0 $(M+H)^+$.

2.12.3. N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide

The title compound was prepared by substituting Example 2.12.2 for Example 2.9.4 in Example 2.9.5. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 9.51 (s, 1H), 7.97 (dd, 1H), 7.90-7.83 (m, 1H), 7.76 (d, 1H), 7.72-7.66 (m, 1H), 7.64-7.57 (m, 1H), 7.60-7.55 (m, 1H), 7.55 (s, 1H), 7.48-7.37 (m, 3H), 7.37-7.29 (m, 2H), 7.29-7.22 (m, 3H), 6.91 (d, 1H), 6.88 (s, 1H), 4.98 (s, 2H), 4.96 (bs, 2H), 4.40 (p, 1H), 4.24 (p, 1H), 3.89 (t, 2H), 3.79 (s, 2H), 3.64 (t, 2H), 3.44 (t, 2H), 3.29-3.14 (m, 2H), 3.02 (t, 2H), 2.86 (s, 3H), 2.08 (s, 3H), 1.36 (bs, 2H), 1.31 (d, 3H), 1.29-0.94 (m, 14H), 0.83 (s, 6H). MS (ESI) m/e 1202.1 $(M+H)^+$.

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2.17. Synthesis of N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon BO)

2.17.1. 3-(1-((3-(2-((((4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-

pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting (9H-fluoren-9-yl)methyl ((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 for Example 2.3.5 in Example 2.3.6. MS (ESI) m/e 1387.3 $(M+H)^+$.

2.17.2. 3-(1-((3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

Example 2.17.1 (15 mg) was mixed with a solution of 30 % diethylamine in N,N-dimethylformamide (0.5 mL), and the reaction mixture was stirred at room temperature overnight. The crude reaction mixture was directly purified by reverse phase HPLC using a C18 column and a gradient of 10-100% acetonitrile in water containing 0.1% trifluoroacetic acid. The fractions containing the product were lyophilized to give the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1165.5 $(M+H)^+$.

2.17.3. 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1-((2,5-dioxopyrrolidin-1-yl)oxy)-1-oxobutane-2-sulfonate

In a 100 mL flask sparged with nitrogen, 1-carboxy-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propane-1-sulfonate was dissolved in dimethylacetamide (20 mL). To this solution N-hydroxysuccinimide (440 mg,) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1000 mg) were added, and the reaction was stirred at room temperature under a nitrogen atmosphere for 16 hours. The solvent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with a gradient of 1-2% methanol in dichloromethane containing 0.1 % v/v acetic acid, to yield the title compound as a mixture of ~ 80% activated ester and 20 % acid, which was used in the next step without further purification. MS (ESI) m/e 360.1 $(M+H)^+$.

2.17.4. N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide

The trifluoroacetic acid salt of Example 2.17.2 (6 mg) was mixed with Example 2.17.3 (16.85 mg) and N,N-diisopropylethylamine (0.025 mL) in N,N-dimethylformamide (0.500 mL), and the reaction mixture was stirred at room temperature overnight. The crude reaction mixture was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give two diastereomers differing in the stereochemistry at the newly-added position deriving from racemic Example 2.17.3. The stereochemistry of the two products at that center was randomly assigned. MS (ESI) m/e 1408.5 $(M-H)^-$.

2.18. Synthesis of N-[(2S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon BP)

The title compound is the second diastereomer isolated during the preparation of Example 2.17.4 as described in Example 2.17.4. MS (ESI) m/e 1408.4 $(M-H)^-$.

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2.21. Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon IQ)

2.21.1. (S)-(9H-fluoren-9-yl)methyl (1-((4-(hydroxymethyl)phenyl) amino-1-oxopropan-2-yl)carbamate

To a solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (50 g) in methanol (400 mL) and dichloromethane (400 mL) was added (4-aminophenyl)methanol (23.73 g) and ethyl 2-ethoxyquinoline-1(2H)-carboxylate (79 g), and the reaction was stirred at room temperature overnight. The solvent was evaporated, and the residue was washed by dichloromethane to give the title compound.

2.21.2. (S)-2-amino-N-(4-(hydroxymethyl)phenyl)propanamide

To a solution of Example 2.21.1 (10 g) in N,N-dimethylformamide (100 mL) was added piperidine (40 mL), and the reaction was stirred for 2 hours. The solvent was evaporated, and the residue was dissolved in methanol. The solids were filtered off, and the filtrate was concentrated to give crude product.

2.21.3. (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl) phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

To a solution of Example 2.21.2 (5 g) in N,N-dimethylformamide (100 mL) was added (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoic acid (10.48 g) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (14.64 g), and the reaction was stirred overnight. The solvent was evaporated, the residue was washed with dichloromethane, and the solids were filtered to give the crude product.

2.21.4. (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxobutan-2-yl)carbamate

The title compound was prepared by substituting Example 2.21.3 for Example 2.10.1 in Example 2.10.2.

2.21.5. 3-(1-((3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido) propanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

A solution of Example 1.3.7 (0.102 g), Example 2.21.4 (0.089 g) and N,N-diisopropylethylamine (0.104 mL) were stirred together in N,N-dimethylformamide (1 mL) at room temperature. After stirring overnight, diethylamine (0.062 mL) was added, and the reaction was stirred for an additional 2 hours. The reaction was diluted with water (1 mL), quenched with trifluoroacetic acid and was purified by Prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

2.21.6. 3-(1-((3-(2-((((4-((S)-2-((S)-2-((R)-2-amino-3-sulfopropanamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (0.028 g) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (0.027 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylethylamine (0.042 mL), and the reaction was stirred for 5 minutes. The mixture was added to Example 2.21.5 (0.050 g), and the mixture was stirred for 1 hour. Diethylamine (0.049 mL) was then added to the reaction and stirring was continued for an additional 1 hour. The reaction was diluted with N,N-dimethylformamide (1 mL) and water (0.5 mL), quenched with trifluoroacetic acid and purified by reverse-phase HPLC using a Gilson system, eluting with 10-88% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. MS (ESI) m/e 1214.4 $(M-H)^-$.

2.21.7. N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide

To a solution of Example 2.21.6 (0.030 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (8.34 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.020 mL), and the reaction was stirred for 1 hour. The reaction was diluted with N,N-dimethylformamide (1 mL) and water (0.5 mL) and was purified by prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.84 (s, 1H), 9.41 (s, 1H), 8.26 (d, 1H), 8.11-7.95 (m, 3H), 7.79 (d, 1H), 7.68 (d, 2H), 7.61 (d, 1H), 7.57-7.27 (m, 6H), 7.24 (d, 2H), 7.12 (t, 1H), 7.02-6.90 (m, 3H), 4.94 (d, 4H), 4.67 (td, 2H), 4.34-4.22 (m, 2H), 4.04-3.94 (m, 2H), 3.88 (t, 2H), 3.82 (s, 2H), 3.42-3.27 (m, 4H), 3.11-2.96 (m, 5H), 2.84 (dd, 1H), 2.30-1.98 (m, 6H), 1.56-1.41 (m, 4H), 1.41-0.79 (m, 28H). MS (ESI) m/e 1409.1 $(M+H)^+$.

2.22. Synthesis of 4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DB)

2.22.1. (E)-tert-butyldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane

To a flask charged with tert-butyldimethyl(prop-2-yn-1-yloxy)silane (5 g) and dichloromethane (14.7 mL) under a nitrogen atmosphere was added dropwise 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.94 g). The mixture was stirred at room temperature for one minute then transferred via cannula to a nitrogen-sparged flask containing Cp_2ZrClH (chloridobis(η5-cyclopentadienyl)hydridozirconium, Schwartz's Reagent) (379 mg). The resulting reaction mixture was stirred at room temperature for 16 hours. The mixture was carefully quenched with water (15 mL), and then extracted with diethyl ether (3x 30 mL). The combined organic phases were washed with water (15 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography, eluting with a gradient from 0-8% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/z 316.0 $(M+NH_4)^+$.

2.22.2. (2S,3R,4S,5S,6S)-2-(4-bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

(2R,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5 g) was dissolved in acetonitrile (100 mL). Ag_2O (2.92 g) was added to the solution, and the reaction was stirred for 5 minutes at room temperature. 4-Bromo-2-nitrophenol (2.74 g) was added, and the reaction mixture was stirred at room temperature for 4 hours. The silver salt residue was filtered through diatomaceous earth, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 10-70% ethyl acetate in heptanes, to give the title compound. MS (ESI+) m/z 550.9 $(M+NH_4)^+$.

2.22.3. (2S,3R,4S,5S,6S)-2-(4-((E)-3-((tert-butyldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.22.2 (1 g), sodium carbonate (0.595 g), tris(dibenzylideneacetone)dipalladium (0.086 g), and 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane (0.055 g) were combined in a 3-neck 50-mL round bottom flask equipped with a reflux condenser, and the system was degassed with nitrogen. Separately, a solution of Example 2.22.1 (0.726 g) in tetrahydrofuran

(15 mL) was degassed with nitrogen for 30 minutes. The latter solution was transferred via cannula into the flask containing the solid reagents, followed by addition of degassed water (3 mL) via syringe. The reaction was heated to 60 °C for two hours. The reaction mixture was partitioned between ethyl acetate (3x 30 mL) and water (30 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient from 0-35% ethyl acetate in heptanes, to provide the title compound. MS (ESI+) m/z 643.1 $(M+NH_4)^+$.

2.22.4. (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A 500-mL three-neck, nitrogen-flushed flask equipped with a pressure-equalizing addition funnel was charged with zinc dust (8.77 g). A degassed solution of Example 2.22.3 (8.39 g) in tetrahydrofuran (67 mL) was added via cannula. The resulting suspension was chilled in an ice bath, and 6N HCl (22.3 mL) was added dropwise via the addition funnel at such a rate that the internal temperature of the reaction did not exceed 35 °C. After the addition was complete, the reaction was stirred for two hours at room temperature, and filtered through a pad of diatomaceous earth, rinsing with water and ethyl acetate. The filtrate was treated with saturated aqueous $NaHCO_3$ solution until the water layer was no longer acidic, and the mixture was filtered to remove the resulting solids. The filtrate was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with ethyl acetate (3x 75 mL), and the combined organic layers were washed with water (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was triturated with diethyl ether and the solid collected by filtration to provide the title compound. MS (ESI+) m/z 482.0 $(M+H)^+$.

2.22.5. (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate

To a solution of 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (5.0 g) in dichloromethane (53.5 mL) was added sulfurous dichloride (0.703 mL). The mixture was stirred at 60 °C for one hour. The mixture was cooled and concentrated to give the title compound, which was used in the next step without further purification.

2.22.6. (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.22.4 (6.78 g) was dissolved in dichloromethane (50 mL), and the solution was chilled to 0 °C in an ice bath. N,N-Diisopropylethylamine (3.64 g) was added, followed by dropwise

addition of a solution of Example 2.22.5 (4.88 g) in dichloromethane (50 mL). The reaction was stirred for 16 hours allowing the ice bath to come to room temperature. Saturated aqueous $NaHCO_3$ solution (100 mL) was added, and the layers were separated. The aqueous layer was further extracted with dichloromethane (2 x 50 mL). The extracts were dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography, eluting with a gradient of 5-95% ethyl acetate/heptane, to give an inseparable mixture of starting aniline and desired product. The mixture was partitioned between 1N aqueous HCl (40 mL) and a 1:1 mixture of diethyl ether and ethyl acetate (40 mL), and then the aqueous phase was further extracted with ethyl acetate (2x 25 mL). The organic phases were combined, washed with water (2x 25 mL), dried over Na_2SO_4, filtered, and concentrated to give the title compound. MS (ESI+) m/z 774.9 $(M+H)^+$.

2.22.7. (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-(((4-nitrophenoxy)carbonyl)oxy)prop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.22.6 (3.57 g) was dissolved in dichloromethane (45 mL) and bis(4-nitrophenyl)carbonate (2.80 g) was added, followed by dropwise addition of N,N-diisopropylethylamine (0.896 g). The reaction mixture was stirred at room temperature for two hours. Silica gel (20 g) was added to the reaction solution, and the mixture was concentrated to dryness under reduced pressure, keeping the bath temperature at or below 25 °C. The silica residue was loaded atop a column, and the product was purified by silica gel chromatography, eluting with a gradient from 0-100% ethyl acetate-heptane, providing partially purified product which was contaminated with nitrophenol. The material was triturated with methyl tert-butyl ether (250 mL), and the resulting slurry was allowed to sit for 1 hour. The product was collected by filtration. Three successive crops were collected in a similar fashion to give the title compound. MS (ESI+) m/z 939.8 $(M+H)^+$.

2.22.8. 3-(1-((3-(2-(((((E)-3-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of the trifluoroacetic acid salt of Example 1.1.17 (77 mg) and Example 2.22.7 (83 mg) in N,N-dimethylformamide (3.5 mL) was added N,N-diisopropylethylamine

(0.074 mL). The reaction was slowly warmed to room temperature and stirred for 16 hours. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous was extracted twice with additional ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield the title compound, which was used in the subsequent step without further purification.

2.22.9. 3-(1-((3-(2-(((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To an ambient solution of Example 2.22.8 (137 mg) in methanol (3 mL) was added 2M lithium hydroxide solution (0.66 mL). The reaction mixture was stirred for two hours at 35 °C and quenched by the addition of acetic acid (0.18 mL). The reaction was concentrated to dryness, and the residue was diluted with methanol. The crude product was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 20-75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1220.3 $(M+Na)^+$.

2.22.10.4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

To a solution of the trifluoroacetic acid salt of Example 2.22.9 (41.9 mg) in N,N-dimethylformamide (1 mL) were added N-succinimidyl 6-maleimidohexanoate (9.84 mg) and N,N-diisopropylethylamine (0.010 mL), and the reaction was stirred at room temperature for 16 hours. The crude reaction was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (bs, 2H), 9.03 (s, 1H), 8.25 (bs, 1H), 8.03 (d, 1H), 7.97-7.85 (m, 1H), 7.79 (d, 1H), 7.64-7.59 (m, 1H), 7.56-7.39 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 7.14-7.06 (m, 1H), 7.04 (d, 1H), 6.98 (s, 2H), 6.95 (d, 1H), 6.60-6.52 (m, 1H), 6.22-6.12 (m, 1H), 4.95 (bs, 2H), 4.90-4.75 (m, 1H), 4.63 (d, 2H), 4.24-4.05 (m, 1H), 4.08-3.62 (m, 8H), 3.50-

3.24 (m, 10H), 3.04-2.97 (m, 2H), 2.92-2.82 (m, 3H), 2.11-2.06 (m, 3H), 2.03 (t, J = 7.4 Hz, 2H), 1.53-1.39 (m, 4H), 1.41-0.73 (m, 23H). MS (ESI) m/e 1413.3 $(M+Na)^+$.

2.23. Synthesis of 4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DM)

2.23.1. 3-(1-((3-(2-(2-(((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)amino)ethoxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of Example 2.22.7 (94 mg) and Example 1.4.10 (90 mg) was added N,N-diisopropylamine (0.054 mL). The reaction was slowly warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with additional ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude material was dissolved in tetrahydrofuran/methanol/H_2O (2:1:1, 8 mL), to which was added lithium hydroxide monohydrate (40 mg). The reaction mixture was stirred overnight. The mixture was concentrated under vacuum, acidified with trifluoroacetic acid and dissolved in dimethyl sulfoxide/methanol. The solution was purified by reverse phase HPLC using a Gilson system and a C18 column, eluting with 10-85% acetonitrile in 0.1% trifluoroacetic acid in water, to give the title compound. MS (ESI) m/e 1228.1 $(M+H)^+$.

2.23.2. 4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.23.1 (20 mg) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (5.5 mg) in N,N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.054 mL). The reaction was stirred overnight. The reaction mixture was diluted with methanol (2 mL) and acidified with trifluoroacetic acid. The solution was purified by reverse phase HPLC using a Gilson system and a C18 column, eluting with 10-85% acetonitrile in 0.1% trifluoroacetic acid in water, to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 9.03 (s, 1H), 8.24 (s, 1H), 7.95-8.11 (m, 2H), 7.79 (d, 1H), 7.61 (d, 1H), 7.32-7.52 (m, 5H), 7.28 (s, 1H), 7.02-7.23 (m, 3H), 6.91-6.96 (m, 3H), 6.57 (d, 1H), 6.05-6.24 (m, 1H), 4.95 (s, 2H), 4.87 (d, 1H), 4.59 (d, 2H), 3.78-3.95 (m, 4H), 3.13 (q, 2H), 3.01 (t, 2H), 2.51-2.57 (m, 2H), 2.27-2.39 (m, 3H), 2.11 (s, 3H), 0.92-1.43 (m, 16H), 0.83 (s, 6H). MS (ESI) m/e 1379.2 $(M+H)^+$.

2.24. Synthesis of 4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DL)

To a solution of Example 2.23.1 (20 mg) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (6.5 mg) in N,N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.054 mL). The reaction mixture was stirred overnight. The reaction mixture was diluted with methanol (2 mL) and acidified with trifluoroacetic acid. The mixture was purified by reverse phase HPLC using a Gilson system and a C18 column, eluting with 10-85% acetonitrile in 0.1% trifluoroacetic acid in water, to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 9.03 (s, 1H), 8.24 (s, 1H), 8.03 (d, 1H), 7.87 (t, 1H), 7.78 (s, 1H), 7.61 (d, 1H), 7.32-7.55 (m, 5H), 6.90-7.19 (m, 5H), 6.56 (d, 1H), 6.08-6.24 (m, 1H), 4.91-4.93 (m, 1H), 4.86 (s, 1H), 4.59 (d, 2H), 3.27-3.46 (m, 14H), 3.13 (q, 3H), 2.96-3.02 (m, 2H), 2.50-2.59 (m, 3H), 2.09 (s, 3H), 2.00-2.05 (m, 3H), 0.94-1.54 (m, 20H), 0.83 (s, 6H). MS (ESI) m/e 1421.2 $(M+H)^+$.

2.25. Synthesis of 4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DR)

2.25.1. 3-(1-((3-(((E)-14-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)-9-methyl-10-oxo-3,6,11-trioxa-9-azatetradec-13-en-1-yl)oxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of Example 2.22.7 (90 mg) and Example 1.2.11 (92 mg) was added N,N-diisopropylamine (0.050 mL). The ice bath was removed, and the reaction was stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous was extracted twice with additional ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to provide the title compound, which was used in the subsequent step without further purification. MS (ESI) m/e 1648.2 $(M+H)^+$.

2.25.2. 3-(1-((3-(((E)-14-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)-9-methyl-10-oxo-3,6,11-trioxa-9-azatetradec-13-en-1-yl)oxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of Example 2.25.1 (158 mg) in methanol (2.0 mL) was added 2M aqueous lithium hydroxide solution (0.783 mL). The reaction was stirred for 4 hours and quenched by the addition of acetic acid (0.1 mL). The reaction was concentrated to dryness, and the residue was chromatographed using a Biotage Isolera One system and a reverse-phase C18 40g column, eluting with 10-85% acetonitrile in 0.1% trifluoroacetic acid in water. The fractions containing the product were lyophilized to give the title compound as a solid. MS (ESI) m/e 1286.2 $(M+H)^+$.

2.25.3. 4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

To an ambient solution of Example 2.25.2 (9.03 mg) in N,N-dimethylformamide (1.0 mL) was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (4 mg) and N,N-diisopropylamine (0.020 mL), and the reaction was stirred overnight. The reaction was diluted with dimethyl sulfoxide and methanol and purified by RP-HPLC on a Biotage Isolera chromatography unit (40g C18 column), eluting with gradient of 10 to 75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The fractions containing the product were concentrated by lyophilization to yield the title compound as a solid. ^{1}H NMR (400MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 8.04 (d, 1H), 7.99 (t, 1H), 7.79 (d, 1H), 7.60 (d, 1H), 7.53-7.41 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 6.99 (s, 2H), 6.98-6.92 (m, 1H), 4.95 (bs, 2H), 3.92-3.85 (m, 1H), 3.81 (s, 2H), 3.63-3.55 (m, 4H), 3.55-3.31 (m, 28H), 3.18-3.10 (m, 2H), 3.05-2.98 (m, 2H), 2.97 (s, 2H), 2.80 (s, 2H), 2.59-2.50 (m, 1H), 2.32 (t, 2H), 2.10 (s, 3H), 1.39-1.34 (m, 2H), 1.31-1.18 (m, 4H), 1.20-0.92 (m, 6H), 0.84 (s, 6H). MS (ESI) m/e 1479.3 $(M+H)^+$.

2.26. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon DZ)

2.26.1. (2S,3R,4S,5S,6S)-2-(4-formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of 2,4-dihydroxybenzaldehyde (15 g) and (2S,3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (10 g) in acetonitrile was added silver carbonate (10 g), and the reaction was heated to 40 °C. After stirring for 4 hours, the reaction was cooled, filtered and concentrated. The crude product was suspended in dichloromethane and filtered through diatomaceous earth and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound.

2.26.2. (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.26.1 (16.12 g) in tetrahydrofuran (200 mL) and methanol (200 mL) was cooled to 0 °C and sodium borohydride (1.476 g) was added portionwise. The reaction was stirred for 20 minutes, then quenched with a 1:1 mixture of water:saturated sodium bicarbonate solution (400 mL). The resulting solids were filtered off and rinsed with ethyl acetate. The phases were separated and the aqueous layer extracted four times with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The crude product was purified via silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 473.9 $(M+NH_4)^+$.

2.26.3. (2S,3R,4S,5S,6S)-2-(4-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To Example 2.26.2 (7.66 g) and *tert*-butyldimethylsilyl chloride (2.78 g) in dichloromethane (168 mL) at -5 °C was added imidazole (2.63 g), and the reaction mixture was stirred overnight allowing the internal temperature of the reaction to warm to 12 °C. The reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted four times with dichloromethane. The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The crude product was purified via silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 593.0 $(M+Na)^+$.

2.26.4. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.26.3 (5.03 g) and triphenylphosphine (4.62 g) in toluene (88 mL) was added di-*tert*-butyl-azodicarboxylate (4.06 g), and the reaction mixture was stirred for 30 minutes. (9H-Fluoren-9-yl)methyl (2-(2-hydroxyethoxy)ethyl)carbamate was added, and the reaction was stirred for an additional 1.5 hours. The reaction was loaded directly onto silica gel, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound.

2.26.5. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.26.4 (4.29 g) was stirred in a 3:1:1 solution of acetic acid:water:tetrahydrofuran (100 mL) overnight. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude product was purified via silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound.

2.26.6. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.26.5 (0.595 g) and bis(4-nitrophenyl)carbonate (0.492 g) in N,N-dimethylformamide (4 mL) was added N,N-diisopropylamine (0.212 mL). After 1.5 hours the reaction was concentrated under high vacuum. The residue was purified by silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 922.9 $(M+Na)^+$.

2.26.7. 3-(1-((3-(2-((((2-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.1.17 (0.106 g) and Example 2.26.6 (0.130 g) in N,N-dimethylformamide (1.5 mL) was added N,N-diisopropylamine (0.049 mL). After 6 hours, additional N,N-diisopropylamine (0.025 mL) was added, and the reaction was stirred overnight. The reaction was diluted with ethyl acetate (50 mL) and washed with water (10 mL) followed by four times with brine (15 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to give the title compound, which was used in the next step without further purification.

2.26.8. 3-(1-((3-(2-((((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

A suspension of Example 2.26.7 (0.215 g) in methanol (2 mL) was treated with 2.0M aqueous lithium hydroxide (1 mL). After stirring for 1 hour, the reaction was quenched by the addition of acetic acid (0.119 mL). The resulting suspension was diluted with dimethyl sulfoxide (1 mL) and was purified by prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

2.26.9. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.26.8 (0.050 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylamine (0.037 mL) followed by 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (0.017 g), and the reaction was stirred at room temperature. After stirring for 1 hour the reaction was diluted with water and was purified by reverse phase HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (s, 1H), 8.03 (d, 1H), 7.82-7.77 (m, 2H), 7.62 (d, 1H), 7.53-7.41 (m, 3H), 7.40-7.33 (m, 2H), 7.28 (s, 1H), 7.19 (d, 1H), 6.98 (s, 2H), 6.95 (d, 1H), 6.66 (s, 1H), 6.60 (d, 1H), 5.06 (t, 1H), 5.00-4.93 (m, 4H), 4.18-4.04 (m, 2H), 3.95-3.85 (m, 2H), 3.85-3.77 (m, 2H), 3.71 (t, 2H), 3.41-3.30 (m, 4H), 3.30-3.23 (m, 4H), 3.19 (q, 2H), 3.01 (t, 2H), 2.85 (d, 3H), 2.09 (s, 3H), 2.02 (t, 2H), 1.53-1.40 (m, 4H), 1.40-0.78 (m, 24H). MS (ESI) m/e 1380.5 $(M-H)^-$.

2.27. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon EA)

To a solution of Example 2.26.8 (0.031 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylamine (0.023 mL) followed by 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (9 mg), and the reaction was stirred at room temperature. After stirring for 1 hour, the reaction was diluted with water and was purified by prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.84 (s, 1H), 8.03 (d, 1H), 8.00 (t, 1H), 7.79 (d, 1H), 7.61 (d, 1H), 7.54-7.41 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 7.19 (d, 1H), 6.97 (s, 2H), 6.95 (d, 1H), 6.66 (s, 1H), 6.60 (d, 1H), 5.11-5.02 (m, 1H), 4.96 (s, 4H), 4.18-4.02 (m, 2H), 3.96-3.84 (m, 2H), 3.80 (s, 2H), 3.71 (t, 2H), 3.43-3.22 (m, 12H), 3.17 (q, 2H), 3.01 (t, 2H), 2.85 (d, 3H), 2.33 (t, 2H), 2.09 (s, 3H), 1.44-0.76 (m, 18H). MS (ESI) m/e 1338.5 $(M-H)^-$.

2.28. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid(Synthon EO)

2.28.1. (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate

A dry 100 mL round bottom flask was nitrogen-sparged and charged with (2S,3R,4S,5S,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (5 g) and capped with a rubber septum under nitrogen atmosphere. Hydrogen bromide solution in glacial acetic acid (33% wt, 11.06 mL) was added, and the reaction was stirred at room temperature for two hours. The reaction mixture was diluted with dichloromethane (75 mL) and poured into 250 mL ice cold water. The layers were separated, and the organic layer was further washed with ice cold water (3 x 100 mL) and saturated aqueous sodium bicarbonate solution (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residual acetic acid was removed by azeotroping it from toluene (3x 50 mL). The solvent was concentrated

under reduced pressure to yield the title compound, which was used in the next step without further purification. MS (ESI) m/e 429.8 $(M+NH_4)^+$.

2.28.2. (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-formyl-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.28.1 (5.13 g) was dissolved in acetonitrile (100 mL). Silver(I) oxide (2.89 g) was added, and the reaction was stirred for 20 minutes. 4-Hydroxy-3-nitrobenzaldehyde (2.085 g) was added, and the reaction mixture was stirred at room temperature for four hours and then vacuum filtered through a Millipore 0.22 μm filter to remove the silver salts. The solvent was concentrated under reduced pressure to yield the title compound, which was used in the next step without further purification. MS (ESI) m/e 514.9 $(M+NH_4)^+$.

2.28.3. (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A dry 1L round bottom flask nitrogen-sparged was charged with a finely ground powder of Example 2.28.2 (5.0 g,) and was kept under a nitrogen atmosphere. Tetrahydrofuran (70 mL) was added, and the solution was sonicated for two minutes to yield a suspension. Methanol (140 mL) was added, and the suspension was sonicated for another 3 minutes. The suspension was set on an ice bath and stirred for 20 minutes under a nitrogen atmosphere to reach equilibrium (0 °C). Sodium borohydride (0.380 g) was added portion wise over 20 minutes, and the cold (0 °C) reaction was stirred for 30 minutes. Ethyl acetate (200 mL) was added to the reaction mixture, and the reaction was quenched while on the ice bath with addition of 300 mL saturated ammonium chloride solution, followed by 200 mL water. The reaction mixture was extracted with ethyl acetate (3x 300 mL), washed with brine (300 mL), dried over $MgSO_4$, and filtered, and the solvent was concentrated under reduced pressure to yield the title compound. MS (ESI) m/e 516.9 $(M+NH_4)^+$.

2.28.4. (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(2-amino-4-(hydroxymethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate

The title compound was prepared by substituting Example 2.28.3 for Example 2.22.2 in Example 2.22.3 and eliminating the trituration step. The product was used in the next step without further purification. MS (ESI) m/e 469.9 $(M+H)^+$.

2.28.5. (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

The title compound was prepared by substituting Example 2.28.4 for Example 2.22.3 in Example 2.22.5. The reaction was quenched by partitioning between dichloromethane and water. The layers were separated, and the aqueous was extracted twice with ethyl acetate. The combined organic layers were washed with 1N aqueous hydrochloric acid and brine, dried over Na_2SO_4, filtered, and concentrated under reduce pressure. The product was purified by silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptane, to yield the title compound. MS (ESI) m/e 762.9 $(M+H)^+$.

2.28.6. (2S,3R,4S,5S,6R)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To an ambient solution of Example 2.28.5 (3.2g) and bis(4-nitrophenyl)carbonate (1.914 g) in N,N-dimethylformamide (20 mL) was added N,N-diisopropylethylamine (1.10 mL,) dropwise. The reaction was stirred for 1.5 hours at room temperature. The solvent was concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with a gradient of 10-100% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/e 927.8 (M+H), 950.1 $(M+Na)^+$.

2.28.7. 3-(1-((3-(2-((((3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.28.6 for Example 2.22.7 in Example 2.22.8. MS (ESI) m/e 1548.3 $(M+H)^+$.

2.28.8. 3-(1-((3-(2-((((3-(3-aminopropanamido)-4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.28.7 for Example 2.22.7 in Example 2.22.8. MS (ESI) m/e 1158.3 $(M+H)^+$.

2.28.9. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

The title compound was prepared by substituting Example 2.28.8 for Example 2.22.8 in Example 2.22.9. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (bs, 1H), 9.13 (bs, 1H), 8.19 (bs, 1H), 8.03 (d, 1H), 7.88 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.55-7.39 (m, 3H), 7.41-7.30 (m, 2H), 7.28 (s, 1H), 7.14 (d, 1H), 7.05-6.88 (m, 4H), 4.96 (bs, 4H), 3.57-3.48 (m, 1H), 3.49-3.09 (m, 11H), 3.08-2.57 (m, 7H), 2.33 (d, 1H), 2.14-1.97 (m, 6H), 1.55-0.90 (m, 20H), 0.86-0.79 (m, 6H). MS (ESI) m/e 1351.3 $(M+H)^+$.

2.29. Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon FB)

2.29.1. 4-(2-(2-bromoethoxy)ethoxy)-2-hydroxybenzaldehyde

A solution of 2,4-dihydroxybenzaldehyde (1.0 g), 1-bromo-2-(2-bromoethoxy)ethane (3.4 g) and potassium carbonate (1.0 g) in acetonitrile (30 mL) was heated to 75 °C for 2 days. The reaction was cooled, diluted with ethyl acetate (100 mL), washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, filtered and concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-30% ethyl acetate in heptane, provided the title compound. MS (ELSD) m/e 290.4 $(M+H)^+$.

2.29.2. 4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde

To a solution of Example 2.29.1 (1.26 g) in N,N-dimethylformamide (10 mL) was added sodium azide (0.43 g), and the reaction was stirred at room temperature overnight. The reaction was diluted with diethyl ether (100 mL), washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-30% ethyl acetate in heptane, gave the title compound. MS (ELSD) m/e 251.4 $(M+H)^+$.

2.29.3. (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.29.2 (0.84 g), (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.99 g) and silver (I) oxide (1.16 g) were stirred together in acetonitrile (15 mL). After stirring overnight, the reaction was diluted with dichloromethane (20 mL). Diatomaceous earth was added, and the reaction filtered and concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-75% ethyl acetate in heptane, gave the title compound.

2.29.4. (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.9.3 (0.695 g) in methanol (5 mL) and tetrahydrofuran (2 mL) was cooled to 0 °C. Sodium borohydride (0.023 g) was added, and the reaction was warmed to room temperature. After stirring for a total of 1 hour, the reaction was poured into a mixture of ethyl acetate (75 mL) and water (25 mL), and saturated aqueous sodium bicarbonate (10 mL) was added. The organic layer was separated, washed with brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-85% ethyl acetate in heptane, gave the title compound. MS (ELSD) m/e 551.8 $(M-H_2O)^-$.

2.29.5. (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To Example 2.29.4 (0.465 g) in tetrahydrofuran (20 mL) was added 5% Pd/C (0.1 g) in a 50 mL pressure bottle, and the mixture was shaken for 16 hours under 30 psi hydrogen. The reaction was filtered and concentrated to give the title compound, which was used without further purification. MS (ELSD) m/e 544.1 $(M+H)^+$.

2.29.6. (2S,3R,4S,5S,6S)-2-(5-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.29.5 (0.443 g) in dichloromethane (8 mL) was cooled to 0°C, then N,N-diisopropylamine (0.214 mL) and (9H-fluoren-9-yl)methyl carbonochloridate (0.190 g) were added. After 1 hour, the reaction was concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-95% ethyl acetate in heptane, gave the title compound. MS (ELSD) m/e 748.15 $(M-OH)^-$.

2.29.7. (2S,3R,4S,5S,6S)-2-(5-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.29.6 (0.444 g) in N,N-dimethylformamide (5 mL) was added N,N-diisopropylamine (0.152 mL) and bis(4-nitrophenyl) carbonate (0.353 g), and the reaction was stirred at room temperature. After 5 hours, the reaction was concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-90% ethyl acetate in heptane, gave the title compound.

2.29.8. 3-(1-((3-(2-((((4-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.1.17 (0.117 g) and Example 2.29.7 (0.143 g) in N,N-dimethylformamide (1.5 m) was added N,N-diisopropylamine (0.134 mL), and the reaction was stirred overnight. The reaction was diluted with ethyl acetate (75 mL) then washed with water (20 mL), followed by brine (4x 20 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated to give the title compound, which was used without further purification.

2.29.9. 3-(1-((3-(2-((((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

A suspension of Example 2.29.8 (0.205 g) in methanol (2 mL) was treated with a solution of lithium hydroxide hydrate (0.083 g) in water (1 mL). After stirring for 1 hour, the reaction was quenched by the addition of acetic acid (0.113 mL), diluted with dimethyl sulfoxide, and purified by prep HPLC using a Gilson system eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

2.29.10.2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.29.9 (0.080 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylamine (0.054 mL) followed by 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (0.025 g), and the reaction was stirred at room temperature. After stirring for 1 hour, the reaction was diluted with water (0.5 mL) and purified by prep HPLC (Gilson system), eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (s, 1H), 8.03 (d, 1H), 7.86-7.81 (m, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.52-7.41 (m, 3H), 7.39-7.32 (m, 2H), 7.28 (s, 1H), 7.19 (d, 1H), 6.99 (s, 2H), 6.95 (d, 1H), 6.68 (d, 1H), 6.59 (d, 1H), 5.09-4.99 (m, 3H), 4.96 (s, 2H), 4.05 (s, 2H), 3.94 (d, 1H), 3.88 (t, 2H), 3.81 (d, 2H), 3.47-3.24 (m, 15H), 3.19 (q, 2H), 3.01 (t, 2H), 2.86 (d, 3H), 2.09 (s, 3H), 2.03 (t, 2H), 1.51-1.41 (m, 4H), 1.41-0.78 (m, 18H), MS (ESI) m/e 1382.2 $(M+H)^+$.

2.30. Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon KX)

2.30.1. 3-(1-((3-(2-((((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.3.7 (0.071 g) and Example 2.29.7 (0.077 g) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylamine (0.072 mL), and the reaction was stirred for 3 hours. The reaction was concentrated, and the resulting oil was dissolved in tetrahydrofuran (0.5 mL) and methanol (0.5 mL) and treated with lithium hydroxide monohydrate

(0.052 g) solution in water (0.5 mL). After stirring for 1 hour, the reaction was diluted with N,N-dimethylformamide (1 mL) and purified by prep HPLC using a Gilson system, eluting with 10-75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. MS (ESI) m/e 1175.2 $(M+H)^+$.

2.30.2. 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.30.1 (0.055 g) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (0.012 g) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylamine (0.022 mL), and the reaction was stirred at room temperature. After stirring for 1 hour, the reaction was diluted with a 1:1 solution of N,N-dimethylformamide and water (2 mL) and purified by prep HPLC using a Gilson system eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 8.07 – 8.00 (m, 2H), 7.79 (d, 1H), 7.62 (d, 1H), 7.55 – 7.41 (m, 3H), 7.40 – 7.32 (m, 2H), 7.28 (s, 1H), 7.20 (d, 1H), 7.11 (t, 1H), 6.98 (s, 2H), 6.95 (d, 1H), 6.66 (s, 1H), 6.60 (dd, 1H), 5.04 (d, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.10 – 4.03 (m, 2H), 3.95 (d, 2H), 3.88 (t, 2H), 3.70 (t, 2H), 3.59 (t, 2H), 3.46 – 3.38 (m, 4H), 3.36 – 3.25 (m, 4H), 3.17 (q, 2H), 3.08 – 2.98 (m, 4H), 2.33 (t, 2H), 2.10 (s, 3H), 1.37 (s, 2H), 1.25 (q, 4H), 1.18 – 0.93 (m, 6H), 0.84 (s, 6H), MS (ESI) m/e 1325.9 $(M+H)^+$.

2.31. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid (Synthon FF)

2.31.1. (2S,3R,4S,5S,6S)-2-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of (9H-fluoren-9-yl)methyl (3-hydroxypropyl)carbamate (0.245 g) and triphenylphosphine (0.216 g) in tetrahydrofuran (2 mL) at 0 °C was added diisopropyl azodicarboxylate (0.160 mL) dropwise. After stirring for 15 minutes, Example 2.26.1 (0.250 g) was added, the ice bath was removed, and the reaction was allowed to warm to room temperature. After 2 hours, the reaction was concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-70% ethyl acetate in heptane, gave the title compound. MS (APCI) m/e 512.0 $(M\text{-}FMOC)^+$.

2.31.2. (2S,3R,4S,5S,6S)-2-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a suspension of Example 2.31.1 (0.233 g) in methanol (3 mL) and tetrahydrofuran (1 mL) was added sodium borohydride (6 mg). After 30 minutes, the reaction was poured into ethyl acetate (50 mL) and water (25 mL) followed by the addition of saturated aqueous sodium bicarbonate solution (5 mL). The organic layer was separated, washed with brine (25 mL), dried over magnesium sulfate, filtered, and concentrated. Purification of the residue by silica gel chromatography, eluting with a gradient of 5-80% ethyl acetate in heptane, gave the title compound. MS (APCI) m/e 718.1 $(M\text{-}OH)^+$.

2.31.3. (2S,3R,4S,5S,6S)-2-(3-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.31.2 (0.140 g) and bis(4-nitrophenyl) carbonate (0.116 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylamine (0.050 mL). After 1.5 hours, the reaction was concentrated under high vacuum. Purification of the residue by silica gel chromatography, eluting with a gradient of 10-70% ethyl acetate in heptane, gave the title compound.

2.31.4. 3-(1-((3-(2-((((2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.1.17 (0.065 g) and Example 2.31.3 (0.067 g) in N,N-dimethylformamide (0.75 mL) was added N,N-diisopropylamine (0.065 mL). After 6 hours, additional N,N-diisopropylamine (0.025 mL) was added, and the reaction mixture was stirred overnight. The reaction was diluted with ethyl acetate (50 mL) and washed with water (20 mL) followed by brine (20 mL). The ethyl acetate layer was dried over magnesium sulfate, filtered, and concentrated to give the title compound, which was used in the next step without further purification.

2.31.5. 3-(1-((3-(2-((((2-(3-aminopropoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

Example 2.31.4 (0.064 g) was dissolved in methanol (0.75 mL) and treated with lithium hydroxide monohydrate (0.031 g) as a solution in water (0.75 mL). After stirring for 2 hours, the reaction was diluted with N,N-dimethylformamide (1 mL) and quenched with trifluoroacetic acid (0.057 mL). The solution was purified by prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

2.31.6. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.31.5 (0.020 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (5.8 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylamine (0.014 mL). After stirring for 2 hours, the reaction was diluted with N,N-dimethylformamide (1.5 mL) and water (0.5 mL). The solution was purified by prep HPLC using a Gilson system, eluting with 10-75% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.83 (s, 1H), 8.03 (d, 1H), 7.83 (t, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.54-7.42 (m, 3H), 7.37 (d, 1H), 7.34 (d, 1H), 7.28 (s, 1H), 7.19 (d, 1H), 6.98

(s, 2H), 6.95 (d, 1H), 6.64 (d, 1H), 6.59 (d, 1H), 5.05 (t, 1H), 4.96 (d, 4H), 4.02-3.94 (m, 2H), 3.88 (t, 2H), 3.46-3.22 (m, 14H), 3.18 (q, 2H), 3.01 (t, 2H), 2.85 (d, 3H), 2.09 (s, 3H), 2.02 (t, 2H), 1.81 (p, 2H), 1.54-1.41 (m, 4H), 1.41-0.78 (m, 18H). MS (ESI) m/e 1350.5 $(M-H)^-$.

2.32. Synthesis of 1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid (Synthon FU)

2.32.1. 2-amino-5-(hydroxymethyl)phenol

Diisobutylaluminum hydride (1M in dichloromethane, 120 mL) was added to methyl 4-amino-3-hydroxybenzoate (10 g) in 50 mL dichloromethane at -78°C over 5 minutes, and the solution was allowed to warm to 0 °C. The reaction mixture was stirred 2 hours. Another 60 mL of diisobutylaluminum hydride (1M in dichloromethane) was added, and the reaction was stirred at 0 °C for one hour more. Methanol (40 mL) was carefully added. Saturated sodium potassium tartrate solution (100 mL) was added, and the mixture was stirred overnight. The mixture was extracted twice with ethyl acetate, the combined extracts were concentrated to a volume of roughly 100 mL, and the mixture was filtered. The solid was collected, and the solution was concentrated to a very small volume and filtered. The combined solids were dried to give the title compound.

2.32.2. 2-(2-azidoethoxy)ethyl 4-methylbenzenesulfonate

To an ambient solution of 2-(2-azidoethoxy)ethanol (4.85 g), triethylamine (5.16 mL), and N,N-dimethylpyridin-4-amine (0.226 g) in dichloromethane (123 mL) was added 4-methylbenzene-1-sulfonyl chloride (7.05 g). The reaction was stirred overnight and quenched by the addition of dichloromethane and saturated aqueous ammonium chloride solution. The layers were separated, and the organic layer was washed twice with brine. The organic layer was dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to provide the title compound, which was used in the subsequent reaction without further purification. MS (ESI) m/e 302.9 $(M+NH_4)^+$.

2.32.3. (4-amino-3-(2-(2-azidoethoxy)ethoxy)phenyl)methanol

To an ambient solution of Example 2.32.1 (0.488 g) in N,N-dimethylformamide (11.68 mL) was added sodium hydride (0.140 g). The mixture was stirred for 0.5 hours, and Example 2.32.2 (1.0 g) was added as a solution in N,N-dimethylformamide (2.0 mL). The reaction was heated to 50

°C overnight. The reaction mixture was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 25-100% ethyl acetate, to give the title compound. MS (ESI) m/e 253.1 $(M+H)^+$.

2.32.4. 2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)aniline

To an ambient solution of Example 2.32.3 (440 mg) and imidazole (178 mg) in tetrahydrofuran (10.6 mL) was added tert-butyldimethylchlorosilane (289 mg). The reaction mixture was stirred for 16 hours and quenched by the addition of ethyl acetate (30 mL) and saturated aqueous sodium bicarbonate (20 mL). The layers were separated, and the aqueous was extracted twice with ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 0 to 50% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/e 366.9 (M+H).

2.32.5. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.32.4 (410 mg) was dried overnight in a 50 mL dry round-bottom flask under high vacuum. To a cold (0 °C bath temperature) solution of Example 2.32.4 (410 mg) and triethylamine (0.234 mL) in toluene (18 mL) was added phosgene (0.798 mL, 1M in dichloromethane). The reaction was slowly warmed to room temperature and stirred for one hour. The reaction was cooled (0 °C bath temperature), and a solution of (3R,4S,5S,6S)-2-hydroxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (411 mg) and triethylamine (0.35 mL) in toluene (5 mL) was added. The reaction was warmed to room temperature and heated to 50 °C for 2 hours. The reaction was quenched by the addition of saturated aqueous bicarbonate solution and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 0-40% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 743.9 $(M+NH_4)^+$.

2.32.6. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(hydroxymethyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.32.5 (700 mg) in methanol (5 mL) was added a solution of p-toluenesulfonic acid monohydrate (18.32 mg) in methanol (2 mL). The reaction was stirred at room temperature for 1 hour. The reaction was quenched by the addition of saturated aqueous sodium bicarbonate solution and dichloromethane. The layers were separated, and the aqueous layer was extracted with additional dichloromethane. The combined organics were dried over $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure to yield the title compound, which was used in the subsequent step without further purification. MS (ESI) m/e 629.8 $(M+ NH_4)^+$.

2.32.7. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

N,N-Diisopropylethylamine (0.227 mL) was added dropwise to an ambient solution of Example 2.32.6 (530 mg) and bis(4-nitrophenyl)carbonate (395 mg) in N,N-dimethylformamide (4.3 mL). The reaction mixture was stirred at ambient temperature for 1.5 hours. The solvent was concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 0-50% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 794.9 $(M+NH_4)^+$.

2.32.8. 3-(1-((3-(2-((((3-(2-(2-azidoethoxy)ethoxy)-4-(((((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)carbonyl)amino)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of the trifluoroacetic acid salt of Example 1.1.17 (111 mg) and Example 2.32.7 (98.5 mg) in N,N-dimethylformamide (3.5 mL) was added N,N-diisopropylethylamine (0.066 mL). The reaction was slowly warmed to room temperature and stirred for 16 hours. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield the title

compound, which was used in the subsequent step without further purification. MS (ESI) m/e 1398.2 $(M+H)^+$.

2.32.9. 3-(1-((3-(2-((((3-(2-(2-azidoethoxy)ethoxy)-4-(((((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)carbonyl)amino)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of Example 2.32.8 (150 mg) in methanol (3.0 mL) was added 2M lithium hydroxide solution (0.804 mL). The reaction was stirred for 1 hour and was quenched by the addition of acetic acid (0.123 mL) while still at 0 °C. The crude reaction solution was purified by reverse phase HPLC using a Gilson system with a C18 column, eluting with a gradient of 10-100% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The fractions containing the product were lyophilized to give the title compound. MS (ESI) m/e 1258.2 $(M+H)^+$.

2.32.10.3-(1-((3-(2-((((3-(2-(2-aminoethoxy)ethoxy)-4-(((((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)carbonyl)amino)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 2.32.9 (45 mg) dissolved in 2:1 tetrahydrofuran:water (0.3 mL) was added a solution of tris(2-carboxyethyl))phosphine hydrochloride (51.3 mg in 0.2 mL water). The reaction was stirred at room temperature for 16 hours. The solvent was partially concentrated under reduced pressure to remove most of the tetrahydrofuran. The crude reaction was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1232.3 $(M+H)^+$.

2.32.11.1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid

To a solution of the trifluoroacetic acid salt of Example 2.32.10 (15 mg) in 1 mL N,N-dimethylformamide were added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (4.12 mg) and N,N-diisopropylethylamine (0.010 mL), and the reaction was stirred at room temperature for 16 hours. The crude reaction mixture was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.84 (s, 1H),8.58 (d, 1H), 8.03 (d, 1H), 7.79 (t, 2H),7.68 (s, 1H), 7.61 (d, 1H), 7.40-7.54 (m, 3H), 7.36 (q, 2H),7.27 (s, 1H), 7.05 (s, 1H), 6.97 (s, 2H), 6.93 (t, 2H), 5.41(d, Hz, 1H), 5.38 (d, 1H), 5.27 (d, 1H),4.85-5.07 (m, 4H), 4.11 (t, 2H), 3.87 (t, 2H), 3.80(s, 2H), 3.71-3.77 (m, 3H), 3.46 (s, 3H), 3.22 (d, 2H), 3.00(t, 2H), 2.86 (d, 3H), 2.08 (s, 3H), 2.01 (t, 2H), 1.44 (dd, 4H), 1.34 (d, 2H), 0.89-1.29(m, 16H), 0.82 (d, 7H), 3.51-3.66 (m, 3H). MS (ESI) m/e 1447.2 $(M+Na)^+$.

2.33. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon GH)

2.33.1. (R)-28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-7,10,26-trioxo-8-(sulfomethyl)-3,13,16,19,22-pentaoxa-6,9,25-triazaoctacosan-1-oic acid

The title compound was synthesized using solid phase peptide synthesis as described herein. 2-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)acetic acid (1543 mg) was dissolved in 10 mL dioxane, and the solvent was concentrated under reduced pressure. (The procedure was repeated twice). The material was lyophilized overnight. The dioxane-dried amino acid was dissolved in 20 mL sieve-dried dichloromethane to which was added N,N-diisopropylethylamine

(4.07 mL). The solution was added to a 2-chlorotrityl solid support resin (8000 mg), which was previously washed (twice) with sieve-dried dichloromethane. The mixture of resin and amino acid was shaken at ambient temperature for 4 hours, drained, washed with 17:2:1 dichloromethane:methanol:N,N-diisopropylethylamine, and washed three times with N,N-dimethylformamide. The mixture was then washed three more times, alternating between sieve-dried dichloromethane and methanol. The loaded resin was dried in a vacuum oven at 40 °C. The resin loading was determined by quantitative Fmoc-loading test measuring absorbance at 301 nm of a solution obtained by deprotecting a known amount of resin by treatment with 20% piperidine in N,N-dimethylformamide. All Fmoc deprotection steps were performed by treatment of the resin with 20% piperidine in N,N-dimethylformamide for 20 minutes followed by a washing step with N,N-dimethylformamide. Coupling of the amino acids (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid and subsequently 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oic acid was done by activation of 4 equivalents of amino acid with 4 equivalents of ((1H-benzo[d][1,2,3]triazol-1-yl)oxy)tri(pyrrolidin-1-yl)phosphonium hexafluorophosphate(V) and 8 equivalents of N,N-diidopropylethylamine in N,N-dimethylformamide for one minute followed by incubation with the resin for one hour. The title compound was cleaved from the resin by treatment with 5 % trifluoroacetic acid in dichloromethane for 30 minutes. The resin was filtered, and the filtrate was concentrated under reduced pressure to yield the title compound which was used in the next step without further purification. MS (ESI) m/e 669.0 $(M+H)^+$.

2.33.2. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

Example 2.33.1 (5.09 mg) was mixed with 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (2.63 mg,) and N,N-diisopropylethylamine (0.004 mL) in 1 mL N,N-dimethylformamide and stirred for two minutes. Example 2.28.8 (8.8 mg) was added, and the reaction mixture was stirred at room temperature for 1.5 hours. The crude reaction mixture was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (ESI) m/e 1806.5 $(M-H)^-$.

2.34. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon FX)

2.34.1. 3-(1-((3-(2-((((2-(3-((R)-2-amino-3-sulfopropanamido)propoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (0.019 g) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (0.019 g) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylamine (7.82 μL). After stirring for 2 minutes, the reaction was added to a solution of Example 2.31.5 (0.057 g) and N,N-diisopropylamine (0.031 mL) in N,N-dimethylformamide (0.5 mL) at room temperature and stirred for 3 hours. Diethylamine (0.023 mL) was added to the reaction and stirring was continued for an additional 2 hours. The reaction was diluted with water (1 mL), quenched with trifluoroacetic acid (0.034 mL), and the solution was purified by prep HPLC using a Gilson system, eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. MS (ESI) m/e 1310.1 $(M+H)^+$.

2.34.2. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.34.1 (0.0277 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (7.82 mg) in N,N-dimethylformamide (0.5 mL) was added

N,N-diisopropylamine (0.018 mL) and the reaction was stirred at room temperature. The reaction was purified by prep HPLC using a Gilson system eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.81 (s, 1H), 8.02 (d, 1H), 7.89-7.81 (m, 2H), 7.78 (d, 1H), 7.60 (d, 1H), 7.53-7.40 (m, 3H), 7.39-7.31 (m, 2H), 7.29 (s, 1H), 7.16 (d, 1H), 6.98-6.92 (m, 3H), 6.63 (s, 1H), 6.56 (d, 1H), 5.08-4.99 (m, 1H), 4.95 (s, 4H), 4.28 (q, 2H), 3.90-3.85 (m, 4H), 3.48-3.06 (m, 12H), 3.00 (t, 2H), 2.88-2.64 (m, 8H), 2.08 (s, 3H), 2.04 (t, 2H), 1.80 (p, 2H), 1.51-1.39 (m, 4H), 1.39-0.75 (m, 18H). MS (ESI) m/e 1501.4 $(M-H)^-$.

2.35. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon H)

2.35.1. (2S,3R,4S,5S,6S)-2-(4-formyl-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of (2R,3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H- pyran-3,4,5-triyl triacetate (4 g) in acetonitrile (100 mL)) was added silver(I) oxide (10.04 g) and 4-hydroxy-3-nitrobenzaldehyde (1.683 g). The reaction mixture was stirred for 4 hours at room temperature and filtered. The filtrate was concentrated, and the residue was purified by silica gel chromatography, eluting with 5-50% ethyl acetate in heptanes, to provide the title compound. MS (ESI) m/e $(M+18)^+$.

2.35.2. (2S,3R,4S,5S,6S)-2-(4-(hydroxymethyl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.35.1 (6 g) in a mixture of chloroform (75 mL) and isopropanol (18.75 mL) was added 0.87 g of silica gel. The resulting mixture was cooled to 0 °C, $NaBH_4$ (0.470 g) was added, and the resulting suspension was stirred at 0 °C for 45 minutes. The reaction mixture was diluted with dichloromethane (100 mL) and filtered through diatomaceous earth. The filtrate was washed with water and brine and concentrated to give the crude product, which was used without further purification. MS (ESI) m/e $(M+NH_4)^+$:

2.35.3. (2S,3R,4S,5S,6S)-2-(2-amino-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A stirred solution of Example 2.35.2 (7 g) in ethyl acetate (81 mL) was hydrogenated at 20 °C under 1 atmosphere H_2, using 10% Pd/C (1.535 g) as a catalyst for 12 hours. The reaction mixture was filtered through diatomaceous earth, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 95/5 dichloromethane/methanol, to give the title compound.

2.35.4. 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid

3-Aminopropanoic acid (4.99 g) was dissolved in 10% aqueous Na_2CO_3 solution (120 mL) in a 500 mL flask and cooled with an ice bath. To the resulting solution, (9H-fluoren-9-yl)methyl carbonochloridate (14.5 g) in 1,4-dioxane (100 mL) was gradually added. The reaction mixture was stirred at room temperature for 4 hours, and water (800 mL) was then added. The aqueous phase layer was separated from the reaction mixture and washed with diethyl ether (3 x 750 mL). The aqueous layer was acidified with 2N HCl aqueous solution to a pH value of 2 and extracted with ethyl acetate (3 x 750 mL). The organic layers were combined and concentrated to obtain crude product. The crude product was recrystallized in a mixed solvent of ethyl acetate: hexane 1:2 (300 mL) to give the title compound.

2.35.5. (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate

To a solution of Example 2.35.4 in dichloromethane (160 mL) was added sulfurous dichloride (50 mL). The mixture was stirred at 60 °C for 1 hour. The mixture was cooled and concentrated to give the title compound.

2.35.6. (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.35.3 (6 g) in dichloromethane (480 mL) was added N,N-diisopropylethylamine (4.60 mL). Example 2.35.5 (5.34 g) was added, and the mixture was stirred at room temperature for 30 minutes. The mixture was poured into saturated aqueous sodium bicarbonate and was extracted with ethyl acetate. The combined extracts were washed with water and brine and were dried over sodium sulfate. Filtration and concentration gave a residue that was purified via radial chromatography, using 0-100% ethyl acetate in petroleum ether as mobile phase, to give the title compound.

2.35.7. (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a mixture of Example 2.35.6 (5.1 g) in N,N-dimethylformamide (200 mL) was added bis(4-nitrophenyl) carbonate (4.14 g) and N,N-diisopropylethylamine (1.784 mL). The mixture was stirred for 16 hours at room temperature and concentrated under reduced pressure. The crude material was dissolved in dichloromethane and aspirated directly onto a 1 mm radial Chromatotron plate and eluted with 50-100% ethyl acetate in hexanes to give the title compound. MS (ESI) m/e $(M+H)^+$.

2.35.8. 3-(1-((3-(2-((((3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.1.17 (325 mg) and Example 2.35.7 (382 mg) in N,N-dimethylformamide (9 mL) at 0 °C was added N,N-diisopropylamine (49.1 mg). The reaction mixture was stirred at 0 °C for 5 hours, and acetic acid (22.8 mg) was added. The resulting mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was dissolved in a mixture of tetrahydrofuran (10 mL) and methanol (5 mL). To this solution at 0 °C was added 1 M aqueous lithium hydroxide solution (3.8 mL). The resulting mixture was stirred at 0 °C for 1 hour, acidified with acetic acid and concentrated. The concentrate was lyophilized to provide a powder. The powder was dissolved in N,N-dimethylformamide (10 mL), cooled in an ice-bath, and piperidine (1 mL) at 0 °C was added. The mixture was stirred at 0 °C for 15 minutes and 1.5 mL of acetic acid was added. The solution was purified by reverse-phase HPLC using a Gilson system, eluting with 30-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1172.2 $(M+H)^+$.

2.35.9. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-

dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

To Example 2.35.8 (200 mg) in N,N-dimethylformamide (5 mL) at 0 °C was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (105 mg) and N,N-diisopropylethylamine (0.12 mL). The mixture was stirred at 0 °C for 15 minutes, warmed to room temperature and purified by reverse-phase HPLC on a Gilson system using a 100g C18 column, eluting with 30-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 2H) 9.07 (s, 1H) 8.18 (s, 1H) 8.03 (d, 1H) 7.87 (t, 1H) 7.79 (d, 1H) 7.61 (d, 1H) 7.41-7.53 (m, 3H) 7.36 (q, 2H) 7.28 (s, 1H) 7.03-7.09 (m, 1H) 6.96-7.03 (m, 3H) 6.94 (d, 1H) 4.95 (s, 4H) 4.82 (t, 1H) 3.88 (t, 3H) 3.80 (d, 2H) 3.01 (t, 2H) 2.86 (d, 3H) 2.54 (t, 2H) 2.08 (s, 3H) 2.03 (t, 2H) 1.40-1.53 (m, 4H) 1.34 (d, 2H) 0.90-1.28 (m, 12H) 0.82 (d, 6H). MS (ESI) m/e 1365.3 $(M+H)^+$.

2.36. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon I)

The title compound was prepared using the procedure in Example 2.35.9, replacing 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate with 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 8.95 (s, 1H) 8.16 (s, 1H) 7.99 (d, 1H) 7.57-7.81 (m, 4H) 7.38-7.50 (m, 3H) 7.34 (q, 2H) 7.27 (s, 1H) 7.10 (d, 1H) 7.00 (d, 1H) 6.88-6.95 (m, 2H) 4.97 (d, 4H) 4.76 (d, 2H) 3.89 (t, 2H) 3.84 (d, 2H) 3.80 (s, 2H) 3.57-3.63 (m, 4H) 3.44-3.50 (m, 4H) 3.32-3.43 (m, 6H) 3.29 (t, 2H) 3.16 (q, 2H) 3.02 (t, 2H) 2.87 (s, 3H) 2.52-2.60 (m, 2H) 2.29-2.39 (m, 3H) 2.09 (s, 3H) 1.37 (s, 2H) 1.20-1.29 (m, 4H) 1.06-1.18 (m, 4H) 0.92-1.05 (m, 2H) 0.83 (s, 6H). MS (ESI) m/e 1568.6 $(M-H)^-$.

2.37. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon KQ)

The title compound was prepared using the procedure in Example 2.35.9, replacing 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate with 2,5-dioxopyrrolidin-1-yl 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoate. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.86 (s, 3H) 9.08 (s, 2H) 8.17 (s, 1H) 8.03 (d, 1H) 7.89 (t, 1H) 7.79 (d, 1H) 7.61 (d, 1H) 7.46-7.53 (m, 1H) 7.41-7.46 (m, 1H) 7.31-7.40 (m, 1H) 7.28 (s, 1H) 7.03-7.10 (m, 1H) 6.91-7.03 (m, 2H) 4.69-5.08 (m, 4H) 3.83-3.95 (m, 2H) 3.74-3.83 (m, 2H) 3.21-3.47 (m, 12H) 2.95-3.08 (m, 1H) 2.86 (d, 2H) 1.98-2.12 (m, 3H) 1.62-1.79 (m, 2H) 0.90-1.43 (m, 8H) 0.82 (d, 3H). MS (ESI) m/e 1337.2 $(M+H)^+$.

2.38. Synthesis of 4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid (Synthon KP)

2.38.1. 3-(1-((-((1-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodecan-12-yl)oxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 1.2.11 for Example 1.1.17 in Example 2.35.8.

2.38.2. 4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid

The title compound was prepared by substituting Example 2.38.1 for Example 2.35.8 and 2,5-dioxopyrrolidin-1-yl 2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)acetate for 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate in Example 2.35.9. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 8.92 (s, 1H), 8.12-8.15 (m, 1H), 7.97 (d, 1H), 7.76 (d, 1H), 7.61 (d, 1H), 7.28-7.49 (m, 6H), 7.25 (s, 1H), 7.09 (d, 1H), 6.97-7.02 (m, 1H), 6.88-

6.94 (m, 2H), 4.97 (d, 4H), 4.75 (d, 1H), 3.76-3.93 (m, 9H), 3.47-3.60 (m, 16H), 3.32-3.47 (m, 15H), 2.88 (s, 3H), 2.59 (t, 2H), 2.08 (s, 3H), 1.38 (s, 2H), 0.93-1.32 (m, 11H), 0.84 (s, 6H). MS (ESI) m/e 1485.2 $(M+H)^+$.

2.39. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid (Synthon HA)

2.39.1. methyl 6-(vinylsulfonamido)hexanoate

To a solution of 6-methoxy-6-oxohexan-1-aminium chloride (0.3 g) and triethylamine (1.15 mL) in dichloromethane at 0 °C was dropwise added ethenesulfonyl chloride (0.209 g). The reaction mixture was warmed to room temperature and stirred for 1 hour. The mixture was diluted with dichloromethane and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound. MS (ESI) m/e 471.0 $(2M+H)^+$.

2.39.2. 6-(vinylsulfonamido)hexanoic acid

A solution of Example 2.39.1 (80 mg) and lithium hydroxide monohydrate (81 mg) in a mixture of tetrahydrofuran (1 mL) and water (1 mL) was stirred for 2 hours, then diluted with water (20 mL), and washed with diethyl ether (10 mL). The aqueous layer was acidified to pH 4 with 1N aqueous HCl and extracted with dichloromethane (3x 10 mL). The organic layer was washed with brine (5 mL), dried over sodium sulfate, filtered and concentrated to provide the title compound.

2.39.3. 2,5-dioxopyrrolidin-1-yl 6-(vinylsulfonamido)hexanoate

A mixture of Example 2.39.2 (25 mg), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (43.3 mg) and 1-hydroxypyrrolidine-2,5-dione (15.6 mg) in dichloromethane (8 mL) was stirred overnight, washed with saturated aqueous ammonium chloride solution and brine, and concentrated to provide the title compound.

2.39.4. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid

The title compound was prepared using the procedure in Example 2.35.9, replacing 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate with Example 2.39.3. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 2H) 9.07 (s, 1H) 8.18 (s, 1H) 8.03 (d, 1H) 7.87 (t, 1H) 7.79 (d, 1H) 7.61 (d, 1H) 7.41-7.53 (m, 3H) 7.33-7.39 (m, 2H) 7.28 (s, 1H) 7.17 (t, 1H) 7.04-7.08 (m, 1H) 6.98-7.03 (m, 1H) 6.95 (d, 1H) 6.65 (dd, 1H) 5.91-6.04 (m, 2H) 4.96 (s, 4H) 4.82 (s, 1H) 3.22-3.48 (m, 11H) 3.01 (t, 2H) 2.86 (d, 3H) 2.73-2.80 (m, 2H) 2.51-2.57 (m, 2H) 1.99-2.12 (m, 5H) 1.29-1.52 (m, 6H) 0.90-1.29 (m, 12H) 0.82 (d, 6H). MS (ESI) m/e 1375.3 $(M+H)^+$.

2.40. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon HB)

2.40.1. ethyl 6-((2-hydroxyethyl)thio)hexanoate

A mixture of ethyl 6-bromohexanoate (3 g), 2-mercaptoethanol (0.947 mL) and K_2CO_3 (12 g) in ethanol (100 mL) was stirred overnight and filtered. The filtrate was concentrated. The residue was dissolved in dichloromethane (100 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to provide the title compound.

2.40.2. 6-((2-hydroxyethyl)thio)hexanoic acid

The title compound was prepared using the procedure in Example 2.39.2, replacing Example 2.39.2 with Example 2.40.1. MS (ESI) m/e 175.1 $(M-H_2O)^-$.

2.40.3. 6-((2-hydroxyethyl)sulfonyl)hexanoic acid

To a stirred solution of Example 2.40.2 (4 g) in a mixture of water (40 mL) and 1,4-dioxane (160 mL) was added Oxone® (38.4 g). The mixture was stirred overnight. The mixture was filtered and the filtrate was concentrated. The residual aqueous layer was extracted with dichloromethane. The extracts were combined and dried over Na_2SO_4, filtered, and concentrated to provide the title compound.

2.40.4. 6-(vinylsulfonyl)hexanoic acid

To a stirred solution of Example 2.40.3 (1 g) in dichloromethane (10 mL) under argon was added triethylamine (2.8 mL), followed by the addition of methanesulfonyl chloride (1.1 mL) at 0 °C. The mixture was stirred overnight and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated to provide the title compound.

2.40.5. 2,5-dioxopyrrolidin-1-yl 6-(vinylsulfonyl)hexanoate

To a stirred solution of Example 2.40.4 (0.88 g) in dichloromethane (10 mL) was added 1-hydroxypyrrolidine-2,5-dione (0.54 g) and N,N'-methanediylidenedicyclohexanamine (0.92 g). The mixture was stirred overnight and filtered. The filtrate was concentrated and purified by flash chromatography, eluting with 10-25% ethyl acetate in petroleum to provide the title compound. MS (ESI) m/e 304.1 $(M+H)^+$.

2.40.6. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

The title compound was prepared using the procedure in Example 2.35.9, replacing 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate with Example 2.40.5. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.84 (s, 2H) 9.07 (s, 1H) 8.18 (s, 1H) 8.03 (d, 1H) 7.89 (t, 1H) 7.79 (d, 1H) 7.61 (d, 1H) 7.41-7.53 (m, 3H) 7.32-7.40 (m, 2H) 7.28 (s, 1H) 7.04-7.11 (m, 1H) 6.98-7.03 (m, 1H) 6.88-6.97 (m, 2H) 6.17-6.26 (m, 2H) 4.95 (s, 4H) 4.82 (s, 1H) 3.74-3.99 (m, 8H) 3.41-3.46 (m, 8H) 3.24-3.41 (m, 8H) 2.97-3.08 (m, 4H) 2.86 (d, 3H) 2.54 (t, 2H) 2.00-2.13 (m, 5H) 1.43-1.64 (m, 4H) 0.89-1.40 (m, 15H) 0.82 (d, 6H). MS (ESI) m/e 1360.2 $(M+H)^+$.

2.41. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon LB)

2.41.1. 3-(1-((3-(2-((((2-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 1.6.13 for Example 1.1.17 in Example 2.26.7.

2.41.2. 3-(1-((3-(2-((((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.41.1 for Example 2.26.7 in Example 2.26.8. MS (ESI) m/e 1193 $(M+H)^+$, 1191 $(M-H)^-$.

2.41.3. 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

The title compound was prepared by substituting Example 2.41.2 for Example 2.26.8 in Example 2.27. ^{1}H NMR (400MHz, dimethyl sulfoxide-d_6) δ ppm 12.88 (bs, 1H), 8.03 (d, 1H), 8.02 (t, 1H), 7.78 (d, 1H), 7.73 (1H), 7.53 (d, 1H), 7.47 (td, 1H), 7.35 (td, 1H), 7.29 (s, 1H), 7.26 (t, 1H), 7.26 (t, 1H), 7.19 (d, 1H), 7.02 (d, 1H), 6.98 (s, 1H), 6.65 (d, 1H), 6.59 (dd, 1H), 5.07 (d, 1H), 5.01 (s, 1H), 4.92 (1H), 4.08 (m, 2H), 3.94 (t, 2H), 3.90 (d, 2H), 3.87 (s, 2H), 3.70 (m, 6H), 3.60 (m, 6H), 3.44 (t, 2H), 3.39 (t, 2H), 3.32 (t, 1H), 3.28 (dd, 1H), 3.17 (q, 2H), 3.03 (q, 2H), 2.92 (t, 2H), 2.33 (t, 2H), 2.10 (s, 3H), 1.37 (s, 2H), 1.25 (q, 4H), 1.11 (q, 4H), 1.00 (dd, 2H), 0.83 (s, 6H). MS (ESI) m/e 1366 $(M+Na)^+$, 1342 $(M-H)^-$.

2.42. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon NF)

2.42.1. (2S,3R,4S,5S,6S)-2-(4-formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

2,4-Dihydroxybenzaldehyde (15 g) and (2S,3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (10 g) were dissolved in acetonitrile followed by the addition of silver carbonate (10 g) and the reaction was heated to 49°C. After stirring for 4 hours, the reaction was cooled, filtered and concentrated. The crude title compound was suspended in dichloromethane and was filtered through diatomaceous earth and concentrated. The residue was purified by silica gel chromatography eluting with 1-100% ethyl acetate/heptane to provide the title compound.

2.42.2. (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.42.1 (16.12 g) in tetrahydrofuran (200 mL) and methanol (200 mL) was cooled to 0°C and sodium borohydride (1.476 g) was added portionwise. The reaction was stirred for 20 minutes and was quenched with a 1:1 mixture of water:aqueous saturated sodium bicarbonate solution (400 mL). The resulting solids were filtered off and rinsed with ethyl acetate. The phases were separated and the aqueous layer was extracted four times with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The crude title compound was purified via silica gel chromatography eluting with 1-100% ethyl acetate/heptanes to provide the title compound. MS (ESI) m/e 473.9 $(M+NH_4)^+$.

2.42.3. (2S,3R,4S,5S,6S)-2-(4-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To Example 2.42.2 (7.66 g) and *tert*-butyldimethylsilyl chloride (2.78 g) in dichloromethane (168 mL) at -5°C was added imidazole (2.63 g) and the reaction was stirred overnight allowing the internal temperature of the reaction to warm to 12°C. The reaction mixture was poured into saturated aqueous ammonium chloride and extracted four times with dichloromethane. The combined organics were washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude title compound was purified via silica gel chromatography eluting with 1-50% ethyl acetate/heptanes to provide the title compound. MS (ESI) m/e 593.0 $(M+Na)^+$.

2.42.4. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)-6-

(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To Example 2.42.3 (5.03 g) and triphenylphosphine (4.62 g) in toluene (88 mL) was added di-*tert*-butyl-azodicarboxylate (4.06 g) and the reaction was stirred for 30 minutes. (9H-Fluoren-9-yl)methyl (2-(2-hydroxyethoxy)ethyl)carbamate was added and the reaction was stirred for an addition 1.5 hours. The reaction was loaded directly onto silica gel and was eluted with 1-50% ethyl acetate/heptanes to provide the title compound.

2.42.5. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.42.4 (4.29 g) was stirred in a 3:1:1 solution of acetic acid:water:tetrahydrofuran (100 mL) overnight. The reaction was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude title compound was purified via silica gel chromatography eluting with 1-50% ethyl acetate/heptanes to provide the title compound.

2.42.6. (2S,3R,4S,5S,6S)-2-(3-(2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.42.5 (0.595 g) and bis(4-nitrophenyl) carbonate (0.492 g) in N,N-dimethylformamide (4 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.212 mL). After 1.5 hours, the reaction was concentrated under high vacuum. The reaction was loaded directly onto silica gel and eluted using 1-50% ethyl acetate/heptanes to provide the title compound. MS (ESI) m/e 922.9 $(M+Na)^+$.

2.42.7. 3-(1-((3-(2-((((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

Example 1.1.17 (92 mg) was dissolved in dimethylformamide (0.6 mL). Example 2.42.6 (129 mg) and N-ethyl-N-isopropylpropan-2-amine (0.18 mL) were added. The reaction was stirred at room temperature for one hour. The reaction was then concentrated and the residue was

dissolved in tetrahydrofuran (0.6 mL) and methanol (0.6 mL). Aqueous LiOH (1.94*N*, 0.55 mL) was added and the mixture stirred at room temperature for one hour. Purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA water, provided the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1187.4 $(M-H)^-$.

2.42.8. 3-(1-((3-(2-((((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.26.8 for Example 2.31.5 in Example 2.34.1. MS (ESI) m/e 1338.4 $(M-H)^-$.

2.42.9. 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-((R)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-sulfopropanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

The title compound was prepared by substituting Example 2.42.2 for Example 2.34.1 and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate for 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate in Example 2.34.2. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.06 (d, 1H), 8.02 (d, 1H), 7.80 (m, 2H), 7.61 (d,1H), 7.52 (d, 1H), 7.45 (m, 2H), 7.36 (m, 2H), 7.30 (s, 1H), 7.18 (d, 1H), 6.97 (s, 2H), 6.96 (m,2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 4.96 (s, 4H), 4.31 (m, 1H), 4.09 (m, 2H), 3.88 (m, 3H), 3.80 (m, 2H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44 (m, 6H), 3.28 (m, 4H), 3.19 (m, 2H), 3.01 (m, 2H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.33 (m, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI-) m/e 1489.5 (M-1).

2.43. Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-

alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon NG)

The title compound was prepared by substituting Example 2.42.1 for Example 2.34.1 in Example 2.34.2. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.02 (d, 1H), 7.87 (d, 1H), 7.80 (m, 2H), 7.61 (d,1H), 7.52 (d, 1H), 7.45 (m, 2H), 7.36 (m, 2H), 7.30 (s, 1H), 7.18 (d, 1H), 6.97 (s, 2H), 6.96 (m,2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 4.96 (s, 4H), 4.31 (m, 1H), 4.09 (m, 2H), 3.88 (m, 3H), 3.80 (m, 2H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44 (m, 6H), 3.28 (m, 4H), 3.19 (m, 2H), 3.01 (m, 2H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.09 (s, 3H), 2.05 (t, 2H), 1.46 (br m, 4H), 1.33 (br m, 2H), 1.28-0.90 (m, 12H), 0.84, 0.81 (both s, total 6H). MS (ESI-) m/e 1531.5 (M-1).

2.44. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,20-dioxo-7,10,13,16-tetraoxa-3,19-diazadocos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon AS)

To a solution of Example 1.1.17 (56.9 mg) and N,N-diisopropylethylamine (0.065 mL) in N,N-dimethylformamide (1.0 mL) was added 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate (50 mg). The reaction was stirred overnight, and the solution was purified by reverse phase HPLC using a Gilson system, eluting with 20-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 8.08-7.95 (m, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.55-7.40 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 7.01-6.89 (m, 3H), 4.95 (s, 2H), 3.89 (s, 2H), 3.81 (s, 2H), 3.55-3.25 (m, 23H), 3.14 (d, 2H), 2.97 (t, 4H), 2.76 (d, 2H), 2.57 (s, 1H), 2.31 (d, 1H), 2.09 (s, 3H), 1.35 (s, 2H), 1.30-0.93 (m, 12H), 0.85 (d, 6H). MS (ESI) m/e 1180.3 $(M+Na)^+$.

2.45. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-methyl-10,26-dioxo-3,6,13,16,19,22-hexaoxa-9,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon AT)

To a solution of Example 1.2.11 (50 mg) and N,N-diisopropylethylamine (0.051 mL) in N,N-dimethylformamide (1.0 mL) was added 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate (39 mg). The reaction was stirred overnight and purified by reverse phase HPLC using a Gilson system, eluting with 20-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were

combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz dimethyl sulfoxide-d_6) δ ppm 12.85 (s, 1H), 8.04 (d, 1H), 7.99 (t, 1H), 7.79 (d, 1H), 7.60 (d, 1H), 7.53-7.41 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 6.99 (s, 2H), 6.98-6.92 (m, 1H), 4.95 (bs, 2H), 3.92-3.85 (m, 1H), 3.81 (s, 2H), 3.63-3.55 (m, 4H), 3.55-3.31 (m, 28H), 3.18-3.10 (m, 2H), 3.05-2.98 (m, 2H), 2.97 (s, 2H), 2.80 (s, 2H), 2.59-2.50 (m, 1H), 2.32 (t, 2H), 2.10 (s, 3H), 1.39-1.34 (m, 2H), 1.31-1.18 (m, 4H), 1.20-0.92 (m, 6H), 0.84 (s, 6H). MS (ESI) m/e 1268.4 $(M+Na)^+$.

2.46. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl](methyl)amino}ethoxy)ethoxy]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon AU)

To a solution of Example 1.2.11 (50 mg) and N,N-diisopropylethylamine (0.051 mL) in N,N-dimethylformamide (1.0 mL) was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (18 mg). The reaction was stirred overnight and purified by reverse phase HPLC using a Gilson system, eluting with 20-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 12.92-12.82 (m, 1H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.53-7.41 (m, 3H), 7.40-7.32 (m, 2H), 7.28 (s, 1H), 7.01-6.97 (m, 2H), 6.98-6.92 (m, 1H), 4.95 (bs, 2H), 4.04-3.84 (m, 3H), 3.86-3.75 (m, 3H), 3.49-3.32 (m, 10H), 3.01 (s, 2H), 2.95 (s, 2H), 2.79 (s, 2H), 2.31-2.19 (m, 2H), 2.10 (s, 3H), 1.52-1.40 (m, 4H), 1.36 (s, 2H), 1.31-0.94 (m, 14H), 0.84 (s, 6H). MS (ESI) m/e 1041.3 $(M+H)^+$.

2.47. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon BK)

2.47.1. 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1-((2,5-dioxopyrrolidin-1-yl)oxy)-1-oxobutane-2-sulfonate

In a 100 mL flask sparged with nitrogen, 1-carboxy-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propane-1-sulfonate was dissolved in dimethylacetamide (20 mL). To this solution N-hydroxysuccinimide (440 mg,) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1000 mg) were added, and the reaction was stirred at room temperature under a nitrogen atmosphere for 16 hours. The solvent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography running a gradient of 1-2% methanol in dichloromethane with 0.1 % acetic acid v/v included in the solvents to yield the title compound as

a mixture of ~ 80% activated ester and 20 % acid, which was used in the next step without further purification. MS (ESI) m/e 360.1 $(M+H)^+$.

2.47.2. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

To a solution of Example 1.1.17 (5 mg) and Example 2.47.1 (20.55 mg) in N,N-dimethylformamide (0.25 mL) was added N,N-diisopropylethylamine (0.002 mL) and the reaction was stirred at room temperature for 16 hours. The crude reaction mixture was purified by reverse phase HPLC using a Gilson system and a C18 25 x 100 mm column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 8.01-7.95 (m, 1H), 7.76 (d, 1H), 7.60 (dd, 1H), 7.49-7.37 (m, 3H), 7.37-7.29 (m, 2H), 7.28-7.22 (m, 1H), 6.92 (d, 1H), 6.85 (s, 1H), 4.96 (bs, 2H), 3.89 (t, 2H), 3.80 (s, 2H), 3.35 (bs, 5H), 3.08-2.96 (m, 3H), 2.97-2.74 (m, 2H), 2.21 (bs, 1H), 2.08 (s, 4H), 1.42-1.38 (m, 2H), 1.31-1.23 (m, 4H), 1.23-1.01 (m, 6H), 0.97 (d, 1H), 0.89-0.79 (m, 6H). MS (ESI) m/e 1005.2 $(M+H)^+$.

2.48. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetratriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon BQ)

The title compound was prepared as described in Example 2.44, replacing 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate with 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22,25,28-octaoxa-4-azahentriacontan-31-oate (MAL-dPEG8-NHS-Ester). MS (ESI) m/e 1334.3 $(M+H)^+$.

2.49. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,26-dioxo-7,10,13,16,19,22-hexaoxa-3,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon BR)

The title compound was prepared as described in Example 2.44, replacing 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate with 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22-hexaoxa-4-azapentacosan-25-oate (MAL-dPEG6-NHS-Ester). MS (ESI) m/e 1246.3 $(M+H)^+$.

2.50 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OI)

2.50.1 3-(1-((3-(2-((((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 1.1.17 for Example 1.3.7 in Example 2.30.1. MS (ESI) m/e 1189.5 $(M+H)^+$.

2.50.2 3-(1-((3-(2-((((4-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.50.1 for Example 2.31.5 in Example 2.34.1. MS (ESI) m/e 1339.5 $(M+H)^+$.

2.50.3 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid

The title compound was prepared by substituting Example 2.50.2 for Example 2.34.1 in Example 2.34.2. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.83 (s, 2H); 8.01 (dd, 1H), 7.86 (d, 1H), 7.80 – 7.71 (m, 2H), 7.60 (dd, 1H), 7.52 – 7.26 (m, 7H), 7.16 (d, 1H), 6.94 (d, 3H), 6.69 (d, 1H), 6.61 – 6.53 (m, 1H), 5.09 – 4.91 (m, 5H), 3.46 – 3.08 (m, 14H), 2.99 (t, 2H), 2.88 – 2.63 (m, 5H), 2.13 – 1.94 (m, 5H), 1.52 – 0.73 (m, 27H). MS (ESI) m/e 1531.4 $(M-H)^-$.

2.51 Synthesis of N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon NX)

2.51.1 (S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanoic acid

To a cold (ice bath) solution of (S)-6-amino-2-((tert-butoxycarbonyl)amino)hexanoic acid (8.5 g) in a mixture of 5% aqueous $NaHCO_3$ solution (300 mL) and 1,4-dioxane (40 mL) was added dropwise a solution of (9H-fluoren-9-yl)methyl pyrrolidin-1-yl carbonate (11.7 g) in 1,4-dioxane (40 mL). The reaction mixture was allowed to warm to room temperature and was stirred for 24 hours. Three additional vials were set up as described above. After the reactions were complete, the four reaction mixtures were combined, and the organic solvent was removed under vacuum. The aqueous layer was acidified to pH 3 with aqueous hydrochloric acid solution (1N) and then extracted with ethyl acetate (3 × 500 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated under vacuum to give a crude compound, which was recrystallized from methyl tert-butyl ether to afford the title compound. ^{1}H NMR (400MHz, chloroform-*d*) δ 11.05 (br. s., 1H), 7.76 (d, 2H), 7.59 (d, 2H), 7.45 - 7.27 (m, 4H), 6.52 - 6.17 (m, 1H), 5.16 - 4.87 (m, 1H), 4.54 - 4.17 (m, 4H), 3.26 - 2.98 (m, 2H), 1.76 - 1.64 (m, 1H), 1.62 - 1.31 (m, 14H).

2.51.2 tert-butyl 17-hydroxy-3,6,9,12,15-pentaoxaheptadecan-1-oate

To a solution of 3,6,9,12-tetraoxatetradecane-1,14-diol (40 g) in toluene (800 mL) was added portion-wise potassium tert-butoxide (20.7 g). The mixture was stirred at room temperature for 30 minutes. Tert-butyl 2-bromoacetate (36 g) was added dropwise to the mixture. The reaction was stirred at room temperature for 16 hours. Two additional vials were set up as described above. After the reactions were complete, the three reaction mixtures were combined. Water (500 mL) was added to the combined mixture, and the volume was concentrated to 1 liter. The mixture was extracted with dichloromethane and was washed with aqueous 1N potassium tert-butoxide solution (1 L). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography, eluting with dichloromethane:methanol 50:1, to obtain the title compound. ^{1}H NMR (400MHz, chloroform-*d*) δ 4.01 (s, 2H), 3.75 - 3.58 (m, 21H), 1.46 (s, 9H).

2.51.3 tert-butyl 17-(tosyloxy)-3,6,9,12,15-pentaoxaheptadecan-1-oate

To a solution of Example 2.51.2 (30 g) in dichloromethane (500 mL) was added dropwise a solution of 4-methylbenzene-1-sulfonyl chloride (19.5 g) and triethylamine (10.3 g) in dichloromethane (500 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred at room temperature for 18 hours and was poured into water (100 mL). The solution was extracted with dichloromethane (3 × 150 mL), and the organic layer was washed with hydrochloric acid (6N, 15 mL) then $NaHCO_3$ (5% aqueous solution, 15 mL) followed by water (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to obtain a residue, which was purified by silica gel column chromatography, eluting with petroleum ether:ethyl acetate 10:1 to dichloromethane:methanol 5:1, to obtain the title compound. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.79 (d, 2H), 7.34 (d, 2H), 4.18 - 4.13 (m, 2H), 4.01 (s, 2H), 3.72 - 3.56 (m, 18H), 2.44 (s, 3H), 1.47 (s, 9H).

2.51.4 2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-oic acid

To a solution of Example 2.51.3 (16 g) in tetrahydrofuran (300 mL) was added sodium hydride (1.6 g) at 0 °C. The mixture was stirred at room temperature for 4 hours. A solution of 2,5,8,11,14,17-hexaoxanonadecan-19-ol (32.8 g) in tetrahydrofuran (300 mL) was added dropwise at room temperature to the reaction mixture. The resulted reaction mixture was stirred at room temperature for 16 hours, and water (20 mL) was added. The mixture was stirred at room temperature for another 3 hours to complete the tert-butyl ester hydrolysis. The final reaction mixture was concentrated under reduced pressure to remove the organic solvent. The aqueous residue was extracted with dichloromethane (2 × 150 mL). The aqueous layer was acidified to pH 3 and then extracted with ethyl acetate (2 × 150 mL). Finally, the aqueous layer was concentrated to obtain crude product, which was purified by silica gel column chromatography, eluting with a gradient of petroleum ether:ethyl acetate 1:1 to dichloromethane:methanol 5:1, to obtain the title compound. ^{1}H NMR (400MHz, chloroform-*d*) δ 4.19 (s, 2H), 3.80 - 3.75 (m, 2H), 3.73 - 3.62 (m, 40H), 3.57 (dd, 2H), 3.40 (s, 3H)

2.51.5 (43S,46S)-43-((tert-butoxycarbonyl)amino)-46-methyl-37,44-dioxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-38,45-diazaheptatetracontan-47-oic acid

Example 2.51.5 was synthesized using standard Fmoc solid phase peptide synthesis procedures and a 2-chlorotrytil resin. Specifically, 2-chlorotrytil resin (12 g), (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (10 g) and N,N-diisopropylethylamine (44.9 mL) in anhydrous, sieve-dried dichloromethane (100 mL) was shaken at 14 °C for 24 hours. The

mixture was filtered, and the cake was washed with dichloromethane (3 × 500 mL), N,N-dimethylformamide (2 × 250 mL) and methanol (2 × 250 mL) (5 minutes each step). To the above resin was added 20% piperidine/N,N-dimethylformamide (100 mL) to remove the Fmoc group. The mixture was bubbled with nitrogen gas for 15 minutes and filtered. The resin was washed with 20% piperidine/N,N-dimethylformamide (100 mL) another five times (5 minutes each washing step), and washed with N,N-dimethylformamide (5 × 100 mL) to give the deprotected, L-Ala loaded resin.

To a solution of Example 2.51.1 (9.0 g) in N,N-dimethylformamide (50 mL) was added hydroxybenzotriazole (3.5 g), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (9.3 g) and N,N-diisopropylethylamine (8.4 mL). The mixture was stirred at 20 °C for 30 minutes. The above mixture was added to the L-Ala loaded resin and mixed by bubbling with nitrogen gas at room temperature for 90 minutes. The mixture was filtered, and the resin was washed with N,N-dimethylformamide (5 minutes each step). To the above resin was added approximately 20% piperidine/ N,N-dimethylformamide (100 mL) to remove the Fmoc group. The mixture was bubbled with nitrogen gas for 15 minutes and filtered. The resin was washed with 20% piperidine/N,N-dimethylformamide (100 mL x 5) and N,N-dimethylformamide (100 mL x 5) (5 minutes each washing step).

To a solution of Example 2.51.4 (11.0 g) in N,N-dimethylformamide (50 mL) was added hydroxybenzotriazole (3.5 g), 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (9.3 g) and N,N-diisopropylethylamine (8.4 mL), and the mixture was added to the resin and mixed by bubbling with nitrogen gas at room temperature for 3 hours. The mixture was filtered and the residue was washed with N,N-dimethylformamide (5 × 100 mL), dichloromethane (8 × 100 mL) (5 minutes each step).

To the final resin was added 1% trifluoroacetic acid/dichloromethane (100 mL) and mixed by bubbling with nitrogen gas for 5 minutes. The mixture was filtered, and the filtrate was collected. The cleavage operation was repeated four times. The combined filtrate was brought to pH 7 with $NaHCO_3$ and washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to obtain the title compound. ^{1}H NMR (400MHz, methanol-d_4) δ 4.44 - 4.33 (m, 1H), 4.08 - 4.00 (m, 1H), 3.98 (s, 2H), 3.77 - 3.57 (m, 42H), 3.57 - 3.51 (m, 2H), 3.36 (s, 3H), 3.25 (t, 2H), 1.77 (br. s., 1H), 1.70 - 1.51 (m, 4H), 1.44 (s, 9H), 1.42 - 1.39 (m, 3H).

2.51.6 3-(1-((3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

A solution of the trifluoroacetic acid salt of Example 1.3.7 (0.102 g), Example 2.21.4 (0.089 g) and N,N-diisopropylethylamine (0.104 mL) were stirred in N,N-dimethylformamide (1 mL) at room temperature for 16 hours. Diethylamine (0.062 mL) was added, and the reaction was stirred for 2 hours at room temperature. The reaction was diluted with water (1 mL), quenched with trifluoroacetic acid (0.050 mL) and purified by reverse-phase HPLC using a Gilson system and a C18 column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (LC-MS) m/e 1066.5 $(M+H)^+$.

2.51.7 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((4-((43S,46S,49S,52S)-43-((tert-butoxycarbonyl)amino)-49-isopropyl-46,52-dimethyl-37,44,47,50-tetraoxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-38,45,48,51-tetraazatripentacontanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

Example 2.51.5 (16.68 mg), was mixed with 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (7.25 mg) and N,N-diisopropylethylamine (0.015 mL) in N-methylpyrrolidone (1 mL) for 10 minutes and was added to a solution of Example 2.51.6 (25 mg) and N,N-diisopropylethylamine (0.015 mL) in N-methylpyrrolidinone (1.5 mL). The reaction mixture was stirred at room temperature for two hours. The reaction mixture was purified by reverse-phase HPLC using a Gilson system and a C18 column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. MS (ESI) m/e 961.33 $(2M+H)^{2+}$.

2.51.8 3-(1-((3-(2-((((4-((43S,46S,49S,52S)-43-amino-49-isopropyl-46,52-dimethyl-37,44,47,50-tetraoxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-38,45,48,51-tetraazatripentacontanamido)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

Example 2.51.7 (25 mg) was treated with 1 mL trifluoroacetic acid for 5 minutes. The solvent was removed by a gentle flow of nitrogen. The residue was lyophilized from 1:1 acetonitrile: water to give the title compound, which was used in the next step without further purification. MS (LC-MS) m/e 1822.0 $(M+H)^+$.

2.51.9 N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide

To a solution of Example 2.51.8, (23 mg), N-succinimidyl 6-maleimidohexanoate (4.40 mg) and hydroxybenzotriazole (0.321 mg) in N-methylpyrrolidone (1.5 mL) was added N,N-diisopropylethylamine (8.28 µL). The reaction mixture was stirred for 16 hours at room temperature. The reaction mixture was purified by reverse-phase HPLC using a Gilson system and a C18 column, eluting with 5-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The product fractions were lyophilized to give the title compound. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ ppm 7.76 (dq, 3H), 7.64 – 7.51 (m, 5H), 7.45 (dd, 4H), 7.35 (td, Hz, 3H), 4.97 (d, 5H), 3.95 – 3.79 (m, 8H), 3.57 (d, 46H), 3.50 – 3.30 (m, 14H), 1.58 – 0.82 (m, 59H). MS (LC-MS) m/e 1007.8 $(2M+H)^{2+}$.

2.52 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon OJ)

The title compound was prepared by substituting Example 2.50.1 for Example 2.30.1 in Example 2.30.2. ^{1}H NMR (500 MHz, dimethyl sulfoxide-d_6) δ ppm 12.87 (s, 2H); 8.06 – 7.98 (m, 1H), 7.78 (d, 1H), 7.61 (dd, 1H), 7.52 – 7.41 (m, 2H), 7.39 – 7.26 (m, 2H), 7.18 (d, 1H), 7.01 – 6.91 (m, 2H), 6.68 (d, 1H), 6.59 (d, 1H), 5.08 – 4.98 (m, 2H), 4.95 (s, 1H), 3.59 (t, 1H), 3.46 – 3.36 (m, 3H), 3.34 – 3.22 (m, 2H), 3.16 (q, 1H), 3.01 (t, 1H), 2.85 (d, 2H), 2.32 (t, 1H), 2.09 (s, 2H), 1.44 – 0.71 (m, 10H). MS (ESI) m/e 1338.4 $(M-H)^-$.

2.53 Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon XY)

The title compound was prepared as described in Example 2.34.2, substituting 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate for 2,5-

dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate and N-methyl-2-pyrrolidone for N,N-dimethylformamide. MS (ESI) m/e 1458.0 $(M-H)^-$.

2.54 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide (Synthon LX)

2.54.1 methyl 4-((tert-butoxycarbonyl)amino)-2-iodobenzoate

To a solution of 3-iodo-4-(methoxycarbonyl)benzoic acid (9 g) in tert-butanol (100 mL) was added diphenyl phosphorazidate (7.6 mL) and triethylamine (4.9 mL). The mixture was heated to 83 °C (internal temperature) overnight. The mixture was concentrated under reduced pressure to dryness and purified by flash chromatography, eluting with a gradient of 0% to 20% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 377.9 $(M+H)^+$.

2.54.2 methyl 4-amino-2-iodobenzoate

Example 2.54.1 (3 g) was dissolved in dichloromethane (30 mL) and trifluoroacetic acid (10 mL) and stirred at room temperature for 1.5 hours. The mixture was concentrated under reduced pressure to dryness and partitioned between water (adjusted to pH 1 with hydrochloric acid) and ether. The layers were separated, and the organic layer was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate, filtered and concentrated under reduced pressure to dryness. The resulting solid was triturated with toluene to give the title compound. MS (ESI) m/e 278.0 $(M+H)^+$.

2.54.3 methyl 4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-iodobenzoate

A flask was charged with Example 2.54.2 (337 mg) and Fmoc-Val-Ala-OH (500 mg). Ethyl acetate (18 mL) was added followed by pyridine (0.296 mL). The resulting suspension was chilled in an ice bath and T3P (50% solution in ethyl acetate, 1.4 mL) was added dropwise. Stirring was continued at 0 °C for 45 minutes, and the reaction was placed in a -20 °C freezer overnight. The reaction was allowed to warm to room temperature and then quenched with water. The layers were separated, and the aqueous was extracted twice more with ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane then treated with diethyl ether to precipitate the title compound, which was collected by filtration. MS (ESI) m/e 669.7 $(M+H)^+$.

2.54.4 (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(hydroxymethyl)-3-iodophenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

Example 2.54.3 (1 g) was dissolved in tetrahydrofuran (15 mL), and the solution was chilled to -15 °C in an ice-acetone bath. Lithium aluminum hydride (1N in tetrahydrofuran, 3 mL) was then added dropwise, keeping the temperature below -10 °C. The reaction was stirred for 1 hour and then carefully quenched with 10% citric acid (25 mL). The reaction was partitioned between water and ethyl acetate. The layers were separated, and the organic extracted twice with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 5% to 6% methanol/dichloromethane, to give the title compound. MS (ESI) m/e 664.1 $(M+H)^+$.

2.54.5 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl 3-(prop-2-yn-1-yloxy)propane-1-sulfonate

4-((Tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutan-1-ol (1.8 g) and 3-(prop-2-yn-1-yloxy)propane-1-sulfonyl chloride (2.1 g) were combined in dichloromethane (50.0 mL). The mixture was chilled in an ice bath and triethylamine (3.5 mL) was added dropwise. The reaction was stirred at room temperature for 3 hours and quenched by the addition of water. The layers were separated, and the aqueous was extracted thrice with dichloromethane. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 0% to 25% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 534.0 $(M+NH4)^+$.

2.54.6 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl 3-((3-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenyl)prop-2-yn-1-yl)oxy)propane-1-sulfonate

Example 2.54.4 (1.5 g), copper(I) iodide (0.045 g) and bis(triphenylphosphine)palladium(II) dichloride (0.164 g) were combined in a flask, and the system was degassed with N_2 for 45 minutes. Separately, Example 2.54.5 (2.38 g) was dissolved in N,N-dimethylformamide (12 mL), and the solution was degassed with nitrogen for 45 minutes. The N,N-dimethylformamide solution was transferred via syringe to the dried reagents. N,N-Diisopropylethylamine (1.2 mL) was added, and the reaction was stirred overnight. The reaction mixture was diluted with water (400 mL) and extracted with dichloromethane (4 x 200 mL). The combined extracts were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 0% to 5% methanol/dichloromethane, to give the title compound. MS (ESI) m/e 1012.1 $(M-H2O)^+$.

2.54.7 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl 3-((3-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)prop-2-yn-1-yl)oxy)propane-1-sulfonate

To a solution of Example 2.54.6 (700 mg) and bis(4-nitrophenyl) carbonate (207 mg) in N,N-dimethylformamide (3 mL) was added N,N-diisopropylethylamine (0.129 mL). The reaction was stirred at room temperature for 2 hours then concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 0% to 60% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 1211.9 $(M+NH_4)^+$.

2.54.8 3-(1-(((1r,3r)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(3-(3-sulfopropoxy)prop-1-yn-1-yl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

A solution of Example 1.1.17 (0.026 g) and Example 2.54.7 (0.033 g) in N,N-dimethylformamide (0.4 mL) was added N,N-diisopropylethylamine (0.024 mL), and the reaction was stirred for 5 hours. The reaction was concentrated under reduced pressure to an oil. The oil was dissolved in tetrahydrofuran (0.2 mL) and treated with tetrabutylammonium fluoride (1.0M in tetrahydrofuran, 0.27 mL), and the reaction stirred overnight. The reaction was diluted with N,N-dimethylformamide (1.3 mL), water (0.7 mL) and purified by preparatory reverse-phase HPLC on a Gilson system (Luna column, 250 x 50, flow 60 mL/min) using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. MS (ESI) m/e 1255.8 $(M+H)^+$.

2.54.9 N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide

To a solution Example 2.54.8 (0.022 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (7.02 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.015 mL), and the reaction stirred at room temperature for 3 hours. The reaction was diluted with N,N-dimethylformamide (1.3 mL), water (0.7 mL) and purified by preparatory reverse-phase HPLC on a Gilson system (Luna column, 250 x 50, flow 60 mL/min)

using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. 1H NMR (400 MHz, DMSO-d6) δ 8.14 (d, 1H), 8.02 (d, 1H), 7.77 (d, 3H), 7.59 (t, 2H), 7.51 – 7.39 (m, 3H), 7.34 (td, 3H), 7.26 (s, 1H), 6.97 (s, 2H), 6.93 (d, 1H), 5.05 (s, 2H), 4.94 (s, 2H), 4.34 (s, 3H), 4.21 – 4.10 (m, 2H), 3.87 (t, 2H), 3.78 (d, 2H), 3.53 (t, 4H), 3.24 (s, 4H), 2.99 (t, 2H), 2.84 (d, 4H), 2.46 – 2.38 (m, 2H), 2.25 – 2.02 (m, 5H), 1.92 (dt, 2H), 1.87 – 1.75 (m, 2H), 1.45 (dt, 4H), 1.38 – 0.87 (m, 18H), 0.87 – 0.71 (m, 10H). .MS (ESI) m/e 1448.8 (M+H)+.

2.55 Synthesis of (6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid (Synthon MJ)

2.55.1 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydropyran-2-one

To a solution of (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (75 g) in dimethyl sulfoxide (400 mL) at 0 °C was added Ac2O (225 mL). The mixture was stirred for 16 hours at room temperature before cooled to 0 °C. A large volume of water was added, and stirring was stopped so that the reaction mixture was allowed to settle for 3 hours (the crude lactone lies at the bottom of the flask). The supernatant was removed, and the crude mixture was diluted with ethyl acetate and washed 3 times with water, neutralized with saturated aqueous solution of NaHCO3 and washed again twice with water. The organic layer was then dried over magnesium sulfate, filtered and concentrated to give the title compound. MS (ESI) m/e 561 (M+Na)+.

2.55.2 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-2-ethynyl-tetrahydro-2H-pyran-2-ol

To a solution of ethynyltrimethylsilane (18.23 g) in tetrahydrofuran (400 mL) under nitrogen and chilled in a dry ice/acetone bath (internal temp -65 oC) was added 2.5M BuLi in hexane (55.7 mL) dropwise, keeping the temperature below -60 °C. The mixture was stirred in a cold bath for 40 minutes, followed by an ice-water bath (internal temp rose to 0.4oC) for 40 minutes, and finally cooled to -75oC again. A solution of Example 2.55.1 (50 g) in tetrahydrofuran (50 mL) was added dropwise, keeping the internal temperature below -70 °C. The mixture was stirred in a dry ice/acetone bath for additional 3 hours. The reaction was quenched with saturated aqueous NaHCO3 solution (250 mL). The mixture was allowed to warm to room temperature, extracted with ethyl acetate (3x 300 mL), dried over MgSO4 and concentrated in vacuo to give the title compound. MS (ESI) m/e 659 (M+Na)+.

2.55.3 trimethyl(((3S,4R,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)ethynyl)silane

To a mixed solution of Example 2.55.2 (60 g) in acetonitrile (450 mL) and dichloromethane (150 mL) at -15 oC in an ice-salt bath was added triethylsilane (81 mL) dropwise, followed by addition of BF3.OEt2 (40.6 mL) at such a rate that the internal temperature did not exceed -10 °C. The mixture was then stirred at -15 oC to -10 oC for 2 hours. The reaction was quenched with saturated aqueous NaHCO3 solution (275 mL) and stirred for 1 hour at room temperature. The mixture was then extracted with ethyl acetate (3 x 550 mL). The extracts were dried over MgSO4 and concentrated. The residue was purified by flash chromatography eluting with a gradient of 0% to 7% ethyl acetate/petroleum ether to give the title compound. MS (ESI) m/e 643 $(M+Na)^+$.

2.55.4 (2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-(benzyloxymethyl)-6-ethynyl-tetrahydro-2H-pyran

To a mixed solution of Example 2.55.3 (80 g) in dichloromethane (200 mL) and methanol (1000 mL) was added 1N aqueous NaOH solution (258 mL). The mixture was stirred at room temperature for 2 hours. The solvent was removed. The residue was then partitioned between water and dichloromethane. The extracts were washed with brine, dried over Na_2SO_4 and concentrated to give the title compound. MS (ESI) m/e 571 $(M+Na)^+$.

2.55.5 (2R,3R,4R,5S)-2-(acetoxymethyl)-6-ethynyl-tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.55.4 (66 g) in acetic anhydride (500 mL) cooled by an ice/water bath was added $BF_3{\cdot}OEt_2$ (152 mL) dropwise. The mixture was stirred at room temperature for 16 hours, cooled with an ice/water bath and neutralized with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (3x500 mL), dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by flash chromatography eluting with a gradient of 0% to 30% ethyl acetate/petroleum ether to give the title compound. MS (ESI) m/e 357 $(M+H)^+$.

2.55.6 (3R,4R,5S,6R)-2-ethynyl-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol

To a solution of Example 2.55.5 (25 g) in methanol (440 mL) was added sodium methanolate (2.1 g). The mixture was stirred at room temperature for 2 hours, then neutralized with 4M HCl in dioxane. The solvent was removed, and the residue was adsorbed onto silica gel and loaded onto a silica gel column. The column was eluted with a gradient of 0 to 100% ethyl acetate/petroleum ether then 0% to 12% methanol/ethyl acetate to give the title compound. MS (ESI) m/e 211 $(M+Na)^+$.

2.55.7 (2S,3S,4R,5R)-6-ethynyl-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-carboxylic acid

A three-necked RBF was charged with Example 2.55.6 (6.00 g), KBr (0.30 g), tetrabutylammonium bromide (0.41 g) and 60 mL of saturated aqueous $NaHCO_3$ solution. TEMPO (0.15 g) in 60 mL dichloromethane was added. The mixture was stirred vigorously and cooled in an ice-salt bath to -2 °C internal temperature. A solution of brine (12 mL), aqueous $NaHCO_3$ solution (24 mL) and NaOCl (154 mL) was added dropwise such that the internal temperature was maintained below 2 °C. The pH of the reaction mixture was maintained in the 8.2-8.4 range with the addition of solid Na_2CO_3. After a total of 6 hours the reaction was cooled to 3 °C internal temperature and EtOH (~20 mL) was added dropwise and stirred for ~ 30 minutes. The mixture was transferred to a separatory funnel, and the dichloromethane layer was discarded. The pH of the aqueous layer was adjusted to 2-3 using 1 M HCl. The aqueous layer was then concentrated to dryness to afford an off-white solid. Methanol (100 mL was) added to the dry solid, and the slurry was stirred for ~30 minutes. The mixture was filtered over a pad of Celite, and the residue in the funnel was washed with ~100 mL of methanol. The filtrate was concentrated under reduced pressure to obtain the title compound.

2.55.8 (2S,3S,4R,5R)-methyl 6-ethynyl-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate

A 500 mL three-necked RBF was charged with a suspension of Example 2.55.7 (6.45 g) in methanol (96 mL) and was cooled in an ice-salt-bath with internal temperature of -1 °C. Neat thionyl chloride (2.79 mL) was carefully added. The internal temperature kept rising throughout the addition but did not exceed 10 °C. The reaction was allowed to slowly warm up to 15-20 °C over 2.5 hours. After 2.5 hours, the reaction was concentrated to give the title compound.

2.55.9 (3S,4R,5S,6S)-2-ethynyl-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To Example 2.55.8 (6.9 g) as a solution in N,N-dimethylformamide (75 mL) was added DMAP (0.17 g) and acetic anhydride (36.1 mL). The suspension was cooled in an ice-bath and pyridine (18.04 mL) was added via syringe over 15 minutes. The reaction was allowed to warm to room temperature overnight. Additional acetic anhydride (12 mL) and pyridine (6 mL) were added and stirring was continued for an additional 6 hours. The reaction was cooled in an ice-bath and 250 mL of saturated aqueous NaHCO3 solution was added and stirred for 1 hour. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The organic extract was washed twice with saturated $CuSO_4$ solution, dried and concentrated. The residue was purified by flash chromatography, eluting with 50% ethyl acetate/petroleum ether to give the title compound. ^1H NMR (500 MHz, methanol-d_4) δ ppm 5.29 (t, 1H), 5.08 (td, 2H), 4.48 (dd, 1H), 4.23 (d, 1H), 3.71 (s, 3H), 3.04 (d, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 4H).

2.55.10 (2S,3S,4R,5S,6S)-2-((5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenyl)ethynyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.55.9 (32.0 mg), Example 2.54.4 (50 mg), copper(I) iodide (1.5 mg) and bis(triphenylphosphine)palladium(II) dichloride (5.5 mg) were combined in a septum-capped vial and sparged. Separately, N,N-diisopropylethylamine (27.0 μL) and N,N-dimethylformamide (390 μL) were combined and sparged for 1 hour and cannulated into the dry reagents. The reaction was stirred at room temperature overnight. The reaction was partitioned between ethyl acetate and water. The combined organics were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 0% to 20% methanol/dichloromethane, to give the title compound. MS (ESI) m/e 838.1 $(M-H_2O)^+$.

2.55.11 (2S,3S,4R,5S,6S)-2-((5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)ethynyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.55.10 (51 mg) and bis(4-nitrophenyl) carbonate (36.3 mg) were combined in N,N-dimethylformamide (298 μL) and N,N-diisopropylethylamine (11.55 mg) was added. The reaction was stirred at room temperature for 2 hours and then concentrated under a stream of nitrogen. The residue was purified by flash chromatography, eluting with a gradient of 0% to 70% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 1037.9 $(M+NH_4)^+$.

2.55.12 3-(1-(((1r,3r)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethynyl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid, Trifluoroacetic Acid

To a solution of Example 1.1.17 (0.044 g) and Example 2.55.11 (0.047 g) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.040 mL), and the reaction was stirred for 4 hours. The reaction was concentrated under reduced pressure. The residue was dissolved in methanol (0.5 mL) and tetrahydrofuran (0.5 mL) and treated with lithium hydroxide hydrate (0.029 g) as a solution in water (0.5 mL). The reaction was stirred for 1.5 hours, diluted with N,N-dimethylformamide (1 mL) and purified by preparatory reverse-phase HPLC on a

Gilson system using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. MS (ESI) m/e 1279.9 $(M+H)^+$

2.55.13 (6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid

To a solution of Example 2.55.12 (0.025 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (7.19 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.016 mL), and the reaction was stirred for 3 hours. The reaction was diluted with a 1:1 mixture of N,N-dimethylformamide (1.3 mL) and water (0.7 mL) and purified by preparatory reverse-phase HPLC on a Gilson system using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85 (s, 2H), 10.03 (s, 1H), 8.17 (d, 1H), 8.03 (d, 1H), 7.78 (q, 3H), 7.62 (d, 1H), 7.55 (d, 1H), 7.54 – 7.40 (m, 3H), 7.36 (td, 3H), 7.28 (s, 1H), 6.99 (s, 2H), 6.95 (d, 1H), 5.11 (s, 2H), 4.96 (s, 2H), 4.36 (q, 1H), 4.25 – 4.13 (m, 2H), 3.88 (t, 2H), 3.80 (d, 2H), 3.69 (d, 2H), 3.44 (s, 2H), 3.36 (td, 2H), 3.32 – 3.16 (m, 4H), 3.01 (t, 2H), 2.90 (s, 2H), 2.84 (s, 2H), 2.16 (td, 2H), 2.09 (s, 4H), 1.95 (q, 1H), 1.47 (p, 4H), 1.29 (d, 6H), 1.24 (s, 1H), 1.16 (q, 4H), 1.08 (d, 3H), 0.83 (dt, 12H). MS (ESI) m/e 1472.3 $(M+H)^+$.

2.56 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide (Synthon NH)

2.56.1 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl 3-(3-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenyl)propoxy)propane-1-sulfonate

To a solution of Example 2.54.6.(900 mg) in tetrahydrofuran (20 mL) and methanol (10 mL) was added to 10% Pd/C (200 mg, dry) in a 50 mL pressure bottle and shaken for 16 hours under 30 psi H_2 at room temperature. The reaction was filtered and concentrated under reduced pressure to give the title compound. MS (ESI) m/e 1016.1 $(M-H_2O)^+$.

2.56.2 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl 3-(3-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)propoxy)propane-1-sulfonate

To a solution of Example 2.56.1 (846 mg) and bis(4-nitrophenyl) carbonate (249 mg) in N,N-dimethylformamide (4 mL) was added N,N-diisopropylethylamine (116 mg). The reaction was stirred at room temperature for 2 hours and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with a gradient of 0% to 60% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 1216.0 $(M+NH_4)^+$.

2.56.3 3-(1-(((1r,3r)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(3-(3-sulfopropoxy)propyl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a solution of Example 1.1.17 (0.018 g) and Example 2.56.2 (0.022 g) in N,N-dimethylformamide (0.4 mL) was added N,N-diisopropylethylamine (0.016 mL), and the reaction was stirred for 5 hours. The reaction was concentrated under reduced pressure, dissolved in tetrahydrofuran (0.2 mL) and treated with tetrabutylammonium fluoride (1.0M in tetrahydrofuran, 0.367 mL) overnight. The reaction was diluted with a mixture of N,N-dimethylformamide :water 2:1 (2 mL) and purified by preparatory reverse-phase HPLC on a Gilson system using a gradient of 10% to 85% acetonitrile/water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. MS (ESI) m/e 1255.8 $(M+H)^+$.

2.56.4 N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide

To a solution of Example 2.56.3 (0.016 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (5.4 mg) in N,N-dimethylformamide (0.4 mL) was added N,N-diisopropylethylamine (10.17 µL), and the reaction was stirred for 5 hours. The reaction was diluted with a 1:1 mixture of N,N-dimethylformamide (1.3 mL) and water (0.7 mL) and purified by preparatory reverse-phase HPLC on a Gilson system using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 2H), 9.87 (s, 1H), 8.07 (d, 1H), 7.76

(dd, 2H), 7.61 – 7.50 (m, 2H), 7.50 – 7.37 (m, 3H), 7.36 – 7.28 (m, 3H), 7.24 (s, 1H), 7.18 (d, 1H), 6.95 (s, 1H), 6.91 (d, 1H), 4.97 (s, 2H), 4.92 (s, 2H), 4.35 (p, 2H), 4.13 (dd, 2H), 3.85 (t, 2H), 3.76 (d, 2H), 3.41 – 3.25 (m, 8H), 3.21 (d, 2H), 2.97 (t, 2H), 2.80 (s, 3H), 2.60 (t, 2H), 2.23 – 2.01 (m, 5H), 1.93 (dq, 2H), 1.73 (dp, 4H), 1.44 (h, 4H), 1.37 – 0.86 (m, 18H), 0.80 (dd, 12H). MS (ESI) m/e 1452.4 $(M+H)^+$.

2.57 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid (Synthon OV)

2.57.1 4-(5-chloropent-1-yn-1-yl)-2-hydroxybenzaldehyde

4-bromo-2-hydroxybenzaldehyde (2.000 g), bis(triphenylphosphine)palladium(II) dichloride (0.349 g) and copper(I) iodide (0.095 g) were weighed into a 100 mL RBF, and the vial was flushed with a stream of nitrogen. N,N-Diisopropylethylamine (3.48 mL), 5-chloropent-1-yne (2.041 g) and N,N-dimethylformamide (40 mL) were added, and the reaction heated to 50°C overnight. The reaction was cooled, diluted with ethyl acetate (100 mL) and washed with 1N hydrochloric acid (75 mL) and brine (75 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 1% to 5% ethyl acetate/heptane, to give the title compound. +H NMR (400 MHz, Chloroform-d) δ 9.87 (s, 1H), 7.48 (d, 1H), 7.04 - 7.00 (m, 2H), 3.72 (t, 2H), 2.66 (t, 2H), 2.16 - 2.03 (m, 2H).

2.57.2 4-(5-azidopent-1-yn-1-yl)-2-hydroxybenzaldehyde

To a solution of Example 2.57.1 (2.15 g) in N,N-dimethylformamide (40 mL) was added sodium azide (0.942 g), and the reaction was heated to 75 °C for 1 hour. The reaction was cooled, diluted with diethyl ether (100 mL), washed with water (50 mL), brine (50 mL), dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 1% to 7% ethyl acetate/heptane, to give the desired product. ^{1}H NMR (400 MHz, Chloroform-d) δ 11.04 (s, 1H), 9.89 (s, 1H), 7.50 (d, 1H), 7.07 - 7.01 (m, 2H), 3.50 (t, 2H), 2.60 (t, 2H), 1.92 (p, 2H).

2.57.3 (2S,3R,4S,5S,6S)-2-(5-(5-azidopent-1-yn-1-yl)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.57.2 (1.28 g), (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (3.33 g) and silver oxide (1.94 g) were stirred in acetonitrile (25 mL). After stirring overnight, the reaction was diluted with dichloromethane (50 mL), filtered through a plug of Celite and concentrated under reduced pressure. The residue was purified by silica gel

chromatography, eluting with a gradient of 5% to 40% ethyl acetate/heptane, to give the title compound.

2.57.4 (2S,3R,4S,5S,6S)-2-(5-(5-azidopent-1-yn-1-yl)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.57.3 (1.82 g) in tetrahydrofuran (6 mL) and methanol (6 mL) was cooled to 0 °C, and sodium borohydride (0.063 g) was added in one portion. After stirring for 30 minutes, the reaction was diluted with diethyl ether (100 mL) and washed with sodium bicarbonate solution (100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 10% to 55% ethyl acetate/heptanes over 40 minutes, to give the title compound. ^{1}H NMR (501 MHz, Chloroform-d) δ 7.31 (d, 1H), 7.18 (dd, 1H), 7.05 (d, 1H), 5.43 - 5.29 (m, 3H), 5.17 (d, 1H), 4.76 (dd, 1H), 4.48 (dd, 1H), 4.17 (d, 1H), 3.74 (s, 3H), 3.51 (t, 2H), 2.72 (dd, 1H), 2.57 (t, 2H), 2.13 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 1.91 (p, 2H).

2.57.5 (2S,3R,4S,5S,6S)-2-(5-(5-aminopentyl)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.57.4 (1.33 g) and tetrahydrofuran (20 mL) were added to 10% palladium/C (0.14 g) in a 50 mL pressure bottle and stirred at room temperature for 6 hours under 30 psi H_2. After 16 hours the reaction was filtered and concentrated under reduced pressure to give the title compound. MS (ESI) m/e 526.3 $(M+H)^+$.

2.57.6 (2S,3R,4S,5S,6S)-2-(5-(5-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pentyl)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.57.5 (1.277 g) in dichloromethane (10 mL) was cooled to 0 °C. N,N-diisopropylethylamine (0.637 mL) and (9H-fluoren-9-yl)methyl carbonochloridate (0.566 g) were added, and the reaction was stirred for 1 hour. The reaction was purified by silica gel chromatography, eluting with a gradient of 10% to 75% ethyl acetate/heptane, to give the title compound. MS (ESI) m/e 748.4 $(M+H)^+$.

2.57.7 (2S,3R,4S,5S,6S)-2-(5-(5-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pentyl)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.57.6 (0.200 g) in N,N-dimethylformamide (1 mL) were added N,N-diisopropylethylamine (0.070 mL) and bis(4-nitrophenyl) carbonate (0.163 g), and the reaction was stirred for 4 hours at room temperature. The reaction was concentrated under reduced

pressure and purified via silica gel chromatography, eluting with a gradient of 10% to 65% heptanes/ethyl acetate, to give the title compound. MS (ESI) m/e 913.3 $(M+H)^+$.

2.57.8 3-(1-(((1S,3r)-3-(2-((((4-(5-aminopentyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid, Trifluoroacetic Acid

To a solution of Example 1.1.17 (0.075 g) and Example 2.57.7 (0.078 g) in N,N-dimethylformamide (0.5 m) was added N,N-diisopropylethylamine (0.075 mL), and the reaction was stirred for 3 hours. The reaction was concentrated under reduced pressure, dissolved in tetrahydrofuran (0.5 mL), methanol (0.5 mL) and treated with lithium hydroxide hydrate (0.054 g) as a solution in water (1 mL). After 1 hour, the reaction was quenched with 2,2,2-trifluoroacetic acid (0.099 mL), diluted with N,N-dimethylformamide (0.5 mL) and purified by preparatory reverse-phase HPLC on a Gilson system using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. MS (ESI) m/e 1171.6 $(M+H)^+$.

2.57.9 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.57.8 (0.040 g) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (10.77 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.027 mL), and the reaction was stirred for 3 hours. The reaction was diluted with a 1:1 mixture of N,N-dimethylformamide :water (2 mL) and purified by preparatory reverse-phase HPLC on a Gilson system using a gradient of 10% to 85% acetonitrile water over 35 minutes. The product-containing fractions were lyophilized to give the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 2H), 8.00 (dd, 1H), 7.84 (t, 1H), 7.76 (d, 1H), 7.58 (dd, 1H), 7.50 – 7.35 (m, 4H), 7.38 – 7.25 (m, 2H), 7.25 (s, 1H), 7.13 (t, 1H), 6.97 – 6.87 (m, 4H), 6.80 (d, 1H), 5.05 (s, 2H), 4.97 (d, 1H), 4.92 (s, 2H), 3.89 – 3.81 (m, 6H), 3.77 (s, 2H), 3.55 (t, 2H), 3.45 – 3.34 (m, 2H), 3.33 – 3.20 (m, 4H), 3.02 – 2.79 (m, 8H), 2.27 (t, 2H), 2.06 (s, 3H), 1.49 (h, 2H), 1.32 (t, 4H), 1.26 – 1.19 (m, 2H), 1.19 (s, 4H), 1.12 – 0.94 (m, 4H), 0.93 (s, 1H), 0.79 (d, 6H). MS (ESI) m/e 1344.4 $(M+Na)^+$.

2.58 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid (Synthon QS)

2.58.1 tert-butyl (2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)carbamate

To a stirred solution of tert-butyl (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)carbamate (0.854 g) in dichloromethane (20 mL) was added sodium hydroxide (0.5 g) and 3-bromoprop-1-yne (0.7 mL). The mixture was stirred at 50 °C overnight, filtered through Celite and concentrated under reduced pressure to give the title compound.

2.58.2 (9H-fluoren-9-yl)methyl (2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)carbamate

To a stirred solution of Example 2.58.1 (0.986 g) in dichloromethane (20 mL) was added hydrochloric acid (20 mL, 2M in ether). The mixture was stirred at room temperature for 2 hours and concentrated under reduced pressure. The residue was suspended in dichloromethane (20 mL). Triethylamine (3 mL) and 9-fluorenylmethyl chloroformate (1.5 g) were added, and the reaction stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure. Ethyl acetate was added, and the suspension was filtered. The eluent was concentrated under reduced pressure and purified by silica gel chromatography, eluting with a gradient of 5% to 40% heptanes/ethyl acetate, to give the title compound. MS (ESI) m/e 410.0 $(M+H)^+$.

2.58.3 (3R,4S,5S,6S)-2-(2-formyl-5-iodophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a stirred solution of (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.0 g) in acetonitrile (12 mL) was added 2-hydroxy-4-iodobenzaldehyde (0.999 g), I_2 (0.192 g) and silver oxide (2.001 g). The mixture was covered with aluminum foil and stirred at room temperature for 4 hours. The reaction was filtered through Celite and washed with ethyl acetate. The solvent was removed. The residue was purified by silica gel chromatography, eluting with 10%-25% petroleum ether/ethyl acetate, to give the title compound. ^{1}H-NMR ($CDCl_3$, 400 MHz):2.07 (s, 9H), 3.76 (s, 3H), 4.26-4.28 (m, 1H), 5.25-5.27 (m, 1H), 5.34-5.40 (m, 3H), 7.51-7.59 (m, 3H), 10.28 (s, 1H). MS (ESI) *m/z* 587 $(M+Na)^+$.

2.58.4 (2S,3R,4S,5S,6S)-2-(5-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadec-15-yn-16-yl)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.58.3 (0.280 g), Example 2.58.2 (0.264 g), bis(triphenylphosphine)palladium(II) dichloride (0.035 g) and copper(I) iodide (9.45 mg) were weighed into a flask and flushed with a stream of nitrogen. N,N-Diisopropylethylamine (0.173 mL) and N,N-dimethylformamide (3 mL) were added, and the reaction stirred at room temperature for 4 hours. The reaction was diluted with diethyl ether (100 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 10% to 75% ethyl acetate/heptanes, to give the title compound. MS (ESI) m/e 846.4 $(M+H)^+$.

2.58.5 (2S,3R,4S,5S,6S)-2-(5-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-yl)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.58.4 (0.225 g) and tetrahydrofuran (10 mL) were added to 10% Pd/C (45 mg, dry) in a 50 mL pressure bottle and shaken at room temperature for 1 hour under 30 psi H_2. The reaction was filtered and concentrated under reduced pressure to give the title compound. MS (ESI) m/e 850.4 $(M+H)^+$.

2.58.6 (2S,3R,4S,5S,6S)-2-(5-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-yl)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.58.5 (0.200 g) in tetrahydrofuran (0.75 mL) and methanol (0.75 mL) was cooled to 0 °C and sodium borohydride (4.45 mg) was added. After 30 minutes, the reaction was poured into a mixture of ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was separated, washed with brine (25 mL), dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 20% to 85% ethyl acetate/hexanes over 30 minutes, to give the title compound. MS (ESI) m/e 852.4 $(M+H)^+$.

2.58.7 (2S,3R,4S,5S,6S)-2-(5-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-yl)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.58.6 (0.158 g), bis(4-nitrophenyl) carbonate (0.113 g) and N,N-diisopropylethylamine (0.049 mL) was stirred in N,N-dimethylformamide (1.0 mL) at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and residue was purified by silica gel chromatography, eluting with a gradient of 20% to 80% ethyl acetate/hexanes, to give the title compound. MS (ESI) m/e 1017.2 $(M+H)^+$.

2.58.8 3-(1-(((1S,3r)-3-(2-((((4-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid, Trifluoroacetic Acid

To a solution of Example 1.1.17 (0.030 g) and Example 2.58.7 in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.030 mL), and the reaction was stirred for 3 hours. The reaction was concentrated under reduced pressure, dissolved in tetrahydrofuran (0.5 mL), methanol (0.5 mL) and treated with lithium hydroxide hydrate (0.022 g) as a solution in water (1 mL). After 1 hour, the reaction was quenched with trifluoroacetic acid (0.132 mL), diluted with N,N-dimethylformamide:water (1:1) (1 mL) and purified by preparatory reverse-phase HPLC on a Gilson PLC 2020 system using a gradient of 5% to 75% acetonitrile water over 30 minutes. Product-containing fractions were combined and lyophilized to give the title compound. MS (ESI) m/e 1275.7 $(M+H)^+$.

2.58.9 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid

To a solution of Example 2.58.8 (0.023 g) and 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (5.73 mg) in N,N-dimethylformamide (0.4 mL) was added N,N-diisopropylethylamine (0.014 mL), and the reaction was stirred at room temperature for 1 hour. The reaction was quenched with a mixture of water (1.5 mL), N,N-dimethylformamide (0.5 mL) and trifluoroacetic acid (0.064 mL) and purified via preparatory reverse-phase HPLC on a Gilson PLC 2020 system using a gradient of 5% to 75% acetonitrile/water over 30 minutes. Product-containing fractions were combined and lyophilized to give the title compound. ^{1}H NMR (501 MHz, DMSO-d_6) δ 8.01 (dd, 1H), 7.97 (t, 1H), 7.60 (d, 1H), 7.51 – 7.39 (m, 3H), 7.39 – 7.31 (m, 2H), 7.26 (s, 1H), 6.96 (s, 2H), 6.95 – 6.90 (m, 2H), 6.82 (d, 1H), 5.15 – 4.96 (m, 4H), 4.94 (s, 2H), 3.94 – 3.83 (m, 4H), 3.79 (d, 2H), 3.57 (dd, 12H), 3.41 – 3.23 (m, 10H), 3.12 (q, 2H), 2.99 (t, 2H), 2.86 (d, 4H), 2.55 (t, 2H), 2.33 – 2.26 (m, 2H), 2.07 (s, 3H), 1.74 (p, 2H), 1.45 – 0.87 (m, 12H), 0.81 (d, 6H). MS (ESI) m/e 1448.4 $(M+Na)^+$.

2.59 Synthesis of (6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid (Synthon SG)

2.59.1 2-iodo-4-nitrobenzoic acid

A 3L fully jacketed flask equipped with a mechanical stirrer, temperature probe and an addition funnel, under a nitrogen atmosphere, was charged with 2-amino-4-nitrobenzoic acid (69.1 g, Combi-Blocks) and sulfuric acid, 1.5 M aqueous (696 mL). The resulting orange suspension was cooled to 0 °C internal temperature, and a solution of sodium nitrite (28.8 g) in water (250 mL) was added dropwise over 43 minutes with the temperature kept below 1 °C. The reaction was stirred at ca. 0 °C for 1 hour. A solution of potassium iodide (107 g) in water (250 mL) was added dropwise over 44 minutes with the internal temperature kept below 1 °C. (Initially addition is exothermic and there is gas evolution). The reaction was stirred 1 hour at 0 °C. The temperature was raised to 20 °C and then stirred at ambient temperature overnight. The reaction mixture became an orange suspension. The reaction mixture was filtered, and the collected orange solid was washed with water. The wet orange solid (~ 108 g) was stirred in 10 % sodium sulfite (350 ml, with ~ 200 mL water used to wash in the solid) for 30 minutes. The orange suspension was acidified with concentrated hydrochloric acid (35 mL), and the solid was collected by filtration and washed with water. The solid was slurried in water (1L) and re-filtered, and the solid was left to dry in the funnel overnight. The solid was then dried in a vacuum oven for 2 hours at 60 °C. The resulting bright orange solid was triturated with dichloromethane (500 mL), and the suspension was filtered and washed with additional dichloromethane. The solid was air-dried to give the title product

2.59.2 (2-iodo-4-nitrophenyl)methanol

A flame-dried 3 L 3-necked flask was charged with Example 2.59.1 (51.9 g) and tetrahydrofuran (700 mL). The solution was cooled in an ice bath to 0.5 °C, and borane-tetrahydrofuran complex (443 mL, 1M in THF) was added dropwise (gas evolution) over 50 minutes, reaching a final internal temperature of 1.3 °C. The reaction mixture was stirred for 15 minutes, and the ice bath was removed. The reaction left to come to ambient temperature over 30 minutes. A heating mantle was installed, and the reaction was heated to an internal temperature of 65.5 °C for 3 hours, and then allowed to cool to room temperature while stirring overnight. The reaction mixture was cooled in an ice bath to 0 °C and quenched by dropwise addition of methanol (400 mL). After a brief incubation period, the temperature rose quickly to 2.5 °C with gas evolution. After the first 100 mL are added over ~ 30 minutes, the addition was no longer exothermic, and

the gas evolution ceased. The ice bath was removed, and the mixture was stirred at ambient temperature under nitrogen overnight. The mixture was concentrated to a solid, dissolved in dichloromethane/methanol and adsorbed on to silica gel (~ 150 g). The residue was loaded on a plug of silica gel (3000 mL) and eluted with dichloromethane to give the title product.

2.59.3 (4-amino-2-iodophenyl)methanol

A 5 L flask equipped with a mechanical stirrer, heating mantle controlled by a JKEM temperature probe and condenser was charged with Example 2.59.2 (98.83 g) and ethanol (2 L). The reaction was stirred rapidly, and iron (99 g) was added, followed by a solution of ammonium chloride (20.84 g) in water (500 mL). The reaction was heated over the course of 20 minutes to an internal temperature of 80.3 °C, where it began to reflux vigorously. The mantle was dropped until the reflux calmed. Thereafter, the mixture was heated to 80 °C for 1.5 hour. The reaction was filtered hot through a membrane filter, and the iron residue was washed with hot 50% ethyl acetate/methanol (800 mL). The eluent was passed through a Celite pad, and the clear yellow filtrate was concentrated. The residue was partitioned between 50% brine (1500 mL) and ethyl acetate (1500 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (400 mL x 3). The combined organic layers were dried over sodium sulfate, filtered and concentrated to give the title product, which was used without further purification.

2.59.4 4-(((tert-butyldimethylsilyl)oxy)methyl)-3-iodoaniline

A 5 L flask with a mechanical stirrer was charged with Example 2.59.3 (88 g) and dichloromethane (2 L). The suspension was cooled in an ice bath to an internal temperature of 2.5 °C, and tert-butylchlorodimethylsilane (53.3 g) was added portion-wise over 8 minutes. After 10 minutes, 1H-imidazole (33.7 g) was added portionwise to the cold reaction. The reaction was stirred 90 minutes while the internal temperature rose to 15 °C. The reaction mixture was diluted with water (3 L) and dichloromethane (1 L). The layers were separated, and the organic layer was dried over sodium sulfate, and concentrated to an oil. The residue was purified by silica gel chromatography (1600 g silica gel), eluting a gradient of 0 - 25% ethyl acetate in heptane, to give the title product as an oil.

2.59.5 (S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanoic acid

To a solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoic acid (6.5 g) in DME (40 mL) was added (S)-2-aminopropanoic acid (1.393 g) and sodium bicarbonate (1.314 g) in water (40 mL). Tetrahydrofuran (20 mL) was added to aid solubility. The resulting mixture was stirred at room temperature for 16 hours. Aqueous citric acid (15%, 75 mL) was added, and the mixture was extracted with 10% 2-propanol in ethyl acetate (2 x 100 mL). A precipitate formed in the organic layer. The combined organic layers were washed with water (2 x 150 mL). The organic layer was concentrated under reduced pressure and then triturated with

diethyl ether (80 mL). After brief sonication, the title compound was collected by filtration as a white solid. MS (ESI) m/e 411 $(M+H)^+$.

2.59.6 (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(((tert-butyldimethylsilyl)oxy)methyl)-3-iodophenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

A solution of Example 2.59.4 (5.44 g) and Example 2.59.5 (6.15 g) in a mixture of dichloromethane (70 mL) and methanol (35.0 mL) was added ethyl 2-ethoxyquinoline-1(2H)-carboxylate (4.08 g), and the reaction was stirred overnight. The reaction was concentrated and loaded onto silica gel, eluting with a gradient of 10% to 95% heptane in ethyl acetate followed by 5% methanol in dichloromethane. The product-containing fractions were concentrated, dissolved in 0.2% methanol in dichloromethane (50 mL), loaded onto silica gel and eluted with a gradient of 0.2% to 2% methanol in dichloromethane. The product containing fractions were collected to give the title compound. MS (ESI) m/e 756.0 $(M+H)^+$.

2.59.7 (2S,3S,4R,5S,6S)-2-((5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)ethynyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.55.9 (4.500 g), Example 2.59.6 (6.62 g), copper(I) iodide (0.083 g) and $PdCl_2(PPh_3)_2$ (0.308 g) were combined in vial and degassed. N,N-dimethylformamide (45 mL) and N-ethyl-N-isopropylpropan-2-amine (4.55 mL) were added, and the reaction vessel was flushed with nitrogen and stirred at room temperature overnight. The reaction was partitioned between water (100 mL) and ethyl acetate (250 mL). The layers were separated, and the organic was dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 5% to 95% ethyl acetate in heptane. The product containing fractions were collected, concentrated and purified by silica gel chromatography, eluting with a gradient of 0.25% to 2.5% methanol in dichloromethane to give the title compound. MS (ESI) m/e 970.4 $(M+H)^+$.

2.59.8 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((tert-butyldimethylsilyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Example 2.59.7 (4.7 g) and tetrahydrofuran (95 mL) were added to 5% Pt/C (2.42 g, wet) in a 50 mL pressure bottle and shaken for 90 minutes at room temperature under 50 psi of hydrogen. The reaction was filtered and concentrated to give the title compound. MS (ESI) m/e 974.6 $(M+H)^+$.

2.59.9 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

A solution of Example 2.59.8 (5.4 g) in tetrahydrofuran (7 mL), water (7 mL) and glacial acetic acid (21 mL) was stirred overnight at room temperature. The reaction was diluted with ethyl acetate (200 mL) and washed with water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL), brine (100 mL), dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 0.5% to 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 860.4 $(M+H)^+$.

2.59.10 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a solution of Example 2.59.9 (4.00 g) and bis(4-nitrophenyl) carbonate (2.83 g) in acetonitrile (80 mL) was added N-ethyl-N-isopropylpropan-2-amine (1.22 mL) at room temperature. After stirring overnight, the reaction was concentrated, dissolved in dichloromethane (250 mL) and washed with saturated aqueous $NaHCO_3$ solution (4 x 150 mL). The organic layer was dried over magnesium sulfate and concentrated. The resulting foam was purified by silica gel chromatography, eluting with a gradient of 5% to 75% ethyl acetate in hexanes to give the title compound. MS (ESI) m/e 1025.5 $(M+H)^+$.

2.59.11 3-(1-(((1r,3r)-3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

This example was prepared by substituting Example 1.1.17 for Example 1.3.7 and substituting Example 2.59.10 for Example 2.29.7 in Example 2.30.1. MS (ESI) m/e 1283.8 $(M+H)^+$.

2.59.12 (6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-

dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid

This example was prepared by substituting Example 2.59.11 for Example 2.30.1 and substituting 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate for 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate in Example 2.30.2. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 12.81 (s, 2H); 9.85 (s, 1H), 8.08 (d, 1H), 7.99 (dd, 1H), 7.81 – 7.72 (m, 2H), 7.58 (dd, 1H), 7.54 – 7.28 (m, 7H), 7.25 (s, 1H), 7.18 (d, 1H), 7.00 – 6.87 (m, 3H), 4.95 (d, 4H), 4.35 (p, 1H), 4.14 (dd, 1H), 3.90 – 3.71 (m, 4H), 3.53 (d, 1H), 3.22 (d, 2H), 3.10 (dt, 2H), 3.00 – 2.86 (m, 3H), 2.85 – 2.66 (m, 4H), 2.54 (d, 1H), 2.20 – 1.86 (m, 6H). MS (ESI-) m/e 1474.4 $(M-H)^-$.

2.60 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid (Synthon UF)

2.60.1 (3R,4S,5S,6S)-2-(2-formyl-5-iodophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a stirred solution of 2-hydroxy-4-iodobenzaldehyde (0.95 g) in acetonitrile (10 mL) was added (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (2.5 g) and silver oxide (2 g) . The mixture was protected from light and stirred at room temperature overnight. The reaction was filtered through diatomaceous earth, washed with ethyl acetate and concentrated. The residue was purified via silica gel chromatography eluting with 15-30% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 586.9 $(M+Na)^+$.

2.60.2 (3R,4S,5S,6S)-2-(5-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)prop-1-yn-1-yl)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

To a stirred solution of (9H-fluoren-9-yl)methyl prop-2-yn-1-ylcarbamate (332 mg), Example 2.60.1 (675 mg) and N,N-diisopropylethylamine (0.5 mL) in N,N-dimethylformamide (5 mL) was added bis(triphenylphosphine)palladium(II) dichloride (100 mg) and copper(I) iodide (23 mg). The mixture was stirred at room temperature overnight. The reaction was diluted with ethyl acetate and washed with water and brine. The aqueous layer was back extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified via silica gel chromatography eluting with 30-70% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 714.1 $(M+H)^+$.

2.60.3 (2S,3R,4S,5S,6S)-2-(5-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

Into a glass tube reactor was charged Example 2.60.2 (3.15 g), 10% Pd/C (3.2 g) and tetrahydrofuran (30 mL). Purged with H_2 and stirred at room temperature under 50 psig of H_2 for 22 hours. The catalyst was filtered off and washed with tetrahydrofuran. The solvent was removed by vacuum to afford title compound. MS (ESI) m/e 718.5 $(M+H)^+$.

2.60.4 (2S,3R,4S,5S,6S)-2-(5-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

This example was prepared by substituting Example 2.60.3 for Example 2.26.1 in Example 2.26.2. MS (ESI) m/e 742.2 $(M+Na)^+$.

2.60.5 (2S,3R,4S,5S,6S)-2-(5-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propyl)-2-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

This example was prepared by substituting Example 2.60.4 for Example 2.26.5 in Example 2.26.6. MS (ESI) m/e 885.2 $(M+Na)^+$.

2.60.6 3-(1-(((1r,3r)-3-(2-((((4-(3-aminopropyl)-2-(((3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

This example was prepared by substituting Example 1.1.17 for Example 1.3.7 and substituting Example 2.60.5 for Example 2.29.7 in Example 2.30.1. MS (ESI-) m/e 1141.4 $(M-H)^-$.

2.60.7 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid

This example was prepared by substituting 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate for 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate and substituting Example 2.60.6 for Example 2.30.1 in Example 2.30.2. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 12.84 (s, 2H); 8.12 (t, 1H), 8.00 (dd, 1H), 7.80 – 7.72

(m, 1H), 7.58 (dd, 1H), 7.50 – 7.37 (m, 3H), 7.36 – 7.29 (m, 2H), 7.25 (s, 1H), 7.18 – 7.11 (m, 1H), 7.03 (s, 2H), 6.97 – 6.88 (m, 2H), 6.82 (dd, 1H), 5.05 (s, 2H), 4.99 (d, 1H), 4.93 (s, 2H), 3.45 – 3.36 (m, 3H), 3.32 – 3.21 (m, 4H), 3.09 – 2.93 (m, 4H), 2.85 (d, 3H), 2.56 – 2.41 (m, 3H), 1.64 (p, 2H), 1.39 – 0.66 (m, 18H). MS (ESI-) m/e 1278.4 $(M-H)^-$.

2.61 Synthesis of 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{4-[({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)amino]butyl}phenyl beta-D-glucopyranosiduronic acid (Synthon VD)

2.61.1 (9H-fluoren-9-yl)methyl but-3-yn-1-ylcarbamate

A solution of but-3-yn-1-amine hydrochloride (9 g) and DIEA (44.7 mL) was stirred in dichloromethane (70 mL) and cooled to 0 °C. A solution of (9H-fluoren-9-yl)methyl carbonochloridate (22.06 g) in dichloromethane (35 mL) was added, and the reaction stirred for 2 hours. The reaction was concentrated, and the residue purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-25%) to give the title compound. MS (ESI) m/e 314 $(M+Na)^+$.

2.61.2 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)but-1-ynyl)-2-formylphenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

Example 2.58.3 (2.7 g), Example 2.61.1 (2.091 g), bis(triphenylphosphine)palladium(II) chloride (0.336 g) and copper(I) iodide (0.091 g) were weighed into a vial and flushed with a stream of nitrogen. Triethylamine (2.001 mL) and tetrahydrofuran (45 mL) were added, and the reaction stirred at room temperature. After stirring for 16 hours, the reaction was diluted with ethyl acetate (200 mL) and washed with water (100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-50%), to give the title compound. MS (ESI) m/e 750 $(M+Na)^+$.

2.61.3 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-formylphenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

Example 2.61.2 (1.5 g) and tetrahydrofuran (45 mL) were added to 10% Pd-C (0.483 g) in a 100 mL pressure bottle and stirred for 16 hours under 1 atm H_2 at room temperature. The reaction was filtered and concentrated to give the title compound. MS (ESI) m/e 754 $(M+Na)^+$.

2.61.4 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-(hydroxymethyl)phenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

A solution of Example 2.61.3 (2.0 g) in tetrahydrofuran (7.00 mL) and methanol (7 mL) was cooled to 0 °C and $NaBH_4$ (0.052 g) was added in one portion. After 30 minutes the reaction was diluted with ethyl acetate (150 mL) and water (100 mL). The organic layer was separated, washed with brine (100 mL), dried over magnesium suflate and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-40%), to give the title compound. MS (ESI) m/e 756 $(M+Na)^+$.

2.61.5 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-(((4-nitrophenoxy)carbonyloxy)methyl)phenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

To a solution of Example 2.61.4 (3.0 g) and bis(4-nitrophenyl) carbonate (2.488 g) in dry acetonitrile (70 mL) at 0 °C was added N,N-diisopropylethylamine (1.07 mL). After stirring at room temperature for 16 hours, the reaction was concentrated to give the residue, which was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-50%), to give the title compound. MS (ESI) m/e 921 $(M+Na)^+$.

2.61.6 (3R,7aS)-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

A solution of (S)-5-(hydroxymethyl)pyrrolidin-2-one (25g), benzaldehyde (25.5g) and para-toluensulfonic acid monohydrate (0.50 g) in toluene (300 mL) was heated to reflux using a Dean-Stark trap under a drying tube for 16 hours. The reaction was cooled to room temperature, and the solvent was decanted from the insoluble materials. The organic layer was washed with saturated aqueous sodium bicarbonate solution (2x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with 35/65 heptane/ethyl acetate, to give the title product. MS (DCI) m/e 204.0 (M+1).

2.61.7 (3R,6R,7aS)-6-bromo-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

To a cold (-77 °C) solution of Example 2.61.6 (44.6 g) in tetrahydrofuran (670 mL) was added lithium bis(trimethylsilyl)amide(1.0M in hexanes) (250 mL) dropwise over 40 minutes, keeping T_{rxn} < -73 °C. The reaction was stirred at -77 °C for 2 hours, and bromine (12.5 mL) was added dropwise over 20 minutes, keeping T_{rxn} < -64 °C. The reaction was stirred at -77 °C for 75 minutes and was quenched by the addition of 150 mL cold 10% aqueous sodium thiosulfate solution to the -77 °C reaction. The reaction was warmed to room temperature and partitioned

between half-saturated aqueous ammonium chloride solution and ethyl acetate. The layers were separated, and the organic was washed with water and brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography , eluting with a gradient of 80/20, 75/25, and 70/30 heptane/ethyl acetate to give the title product. MS (DCI) m/e 299.0 and 301.0 $(M+NH_3+H)^+$.

2.61.8 (3R,6S,7aS)-6-bromo-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

The title compound was isolated as a by-product from Example 2.61.7. MS (DCI) m/e 299.0 and 301.0 $(M+NH_3+H)^+$.

2.61.9 (3R,6S,7aS)-6-azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

To a solution of Example 2.61.7 (19.3 g) in N,N-dimethylformamide (100 mL) was added sodium azide (13.5 g). The reaction was heated to 60 °C for 2.5 hours. The reaction was cooled to room temperature and quenched by the addition of water (500 mL) and ethyl acetate (200 mL). The layers were separated, and the organic was washed brine. The combined aqueous layers were back-extracted with ethyl acetate (50 mL). The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 78/22 heptane/ethyl acetate, to give the title product. MS (DCI) m/e 262.0 $(M+NH_3+H)^+$.

2.61.10 (3R,6S,7aS)-6-amino-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

To a solution of Example 2.61.9 (13.5 g) in tetrahydrofuran (500 mL) and water (50 mL) was added polymer-supported triphenylphosphine (55 g). The reaction was mechanically stirred overnight at room temperature. The reaction was filtered through Celite, eluting with ethyl acetate and toluene. The solution was concentrated under reduced pressure, dissolved in dichloromethane (100 mL), dried with sodium sulfate, then filtered and concentrated to give the title compound, which was used in the subsequent step without further purification. MS (DCI) m/e 219.0 $(M+H)^+$.

2.61.11 (3R,6S,7aS)-6-(dibenzylamino)-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

To a solution of Example 2.61.10 (11.3 g) in N,N-dimethylformamide (100 mL) was added potassium carbonate (7.0 g), potassium iodide (4.2 g), and benzyl bromide (14.5 mL). The reaction was stirred at room temperature overnight and quenched by the addition of water and ethyl acetate. The layers were separated, and the organic was washed brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified

by silica gel chromatography, eluting with a gradient of 10 to 15% ethyl acetate in heptane to give a solid that was triturated with heptane to give the title product. MS (DCI) m/e 399.1 $(M+H)^+$.

2.61.12 (3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)pyrrolidin-2-one

To a solution of Example 2.61.11 (13 g) in tetrahydrofuran (130 mL) was added *para*-toluene sulfonic acid monohydrate (12.4 g) and water (50 mL), and the reaction was heated to 65 °C for 6 days. The reaction was cooled to room temperature and quenched by the addition of saturated aqueous sodium bicarbonate and ethyl acetate. The layers were separated, and the organic was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The waxy solids were triturated with heptane (150 mL) to give the title product. MS (DCI) m/e 311.1 $(M+H)^+$.

2.61.13 (3S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(dibenzylamino)pyrrolidin-2-one

To a solution of Example 2.61.12 (9.3 g) and 1H-imidazole (2.2 g) in N,N-dimethylformamide was added tert-butylchlorodimethylsilane (11.2 mL, 50 weight % in toluene), and the reaction was stirred overnight. The reaction was quenched by the addition of water and ethyl ether. The layers were separated, and the organic was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to give the title product. MS (DCI) m/e 425.1 $(M+H)^+$.

2.61.14 tert-butyl 2-((3S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl)acetate

To a cold (0 °C) solution of Example 2.61.13 (4.5 g) in tetrahydrofuran (45 mL) was added 95% sodium hydride (320 mg) in two portions. The cold solution was stirred for 40 minutes, and tert-butyl 2-bromoacetate (3.2 mL) was added. The reaction was warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the organic was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 5-12% ethyl acetate in heptane, to give the title product. MS (DCI) m/e 539.2 $(M+H)^+$.

2.61.15 tert-butyl 2-((3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)-2-oxopyrrolidin-1-yl)acetate

To a solution of Example 2.61.14 (5.3 g) in tetrahydrofuran (25 mL) was added tetrabutylammonium fluoride (11 mL, 1.0M in 95/5 tetrahydrofuran /water). The reaction was stirred at room temperature for one hour and then quenched by the addition of saturated aqueous ammonium chloride solution, water and ethyl acetate. The layers were separated, and the organic was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to give the title product. MS (DCI) m/e 425.1 $(M+H)^+$.

2.61.16 tert-butyl [(3S,5S)-3-(dibenzylamino)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxido-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradec-1-yl)pyrrolidin-1-yl]acetate

To a solution of Example 2.61.15 (4.7 g) in dimethyl sulfoxide (14 mL) was added a solution of 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (14.5 g) in dimethyl sulfoxide (14 mL). Then potassium carbonate (2.6 g) and water (28 µL) were added, and the reaction heated at 60 °C under nitrogen for one day. The reaction was then cooled to room temperature, and then quenched by the addition of brine solution, water and diethyl ether. The layers were separated, and the organic was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 15-25% ethyl acetate in heptane, to give the title product. MS (ESI+) m/e 871.2 $(M+H)^+$.

2.61.17 tert-butyl [(3S,5S)-3-amino-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxido-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradec-1-yl)pyrrolidin-1-yl]acetate

Example 2.61.16 (873 mg) was dissolved in ethyl acetate (5 mL) and methanol (15 mL), and palladium hydroxide on carbon, 20% by wt (180 mg) was added. The reaction was stirred under a hydrogen atmosphere (30 psi) at room temperature for 30 hours, then at 50 °C for one hour. The reaction was cooled to room temperature, filtered, and concentrated to give the desired product. MS (ESI+) m/e 691.0 $(M+H)^+$.

2.61.18 (2Z)-4-{[(3S,5S)-1-(2-tert-butoxy-2-oxoethyl)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxido-12,12-diphenyl-2,6,11-trioxa-5λ^6-thia-12-silatetradec-1-yl)pyrrolidin-3-yl]amino}-4-oxobut-2-enoic acid

Maleic anhydride (100 mg) was dissolved in dichloromethane (0.90 mL), and a solution of Example 2.61.17 (650 mg) in dichloromethane (0.90 mL) was added dropwise, then heated at 40

°C for 2 hours. The reaction was directly purified by silica gel chromatography, eluting with a gradient of 1.0-2.5% methanol in dichloromethane containing 0.2% acetic acid. After concentrating the product-bearing fractions, toluene (10 mL) was added and concentrated again to give the title product. MS (ESI-) m/e 787.3 $(M-H)^-$.

2.61.19 tert-butyl [(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-(8,8,13,13-tetramethyl-5,5-dioxido-12,12-diphenyl-2,6,11-trioxa-5λ⁶-thia-12-silatetradec-1-yl)pyrrolidin-1-yl]acetate

Example 2.61.18 (560 mg) was slurried in toluene (7 mL), and triethylamine (220 µL) and sodium sulfate (525 mg) were added. The reaction was heated at reflux under a nitrogen atmosphere for 6 hours, and the reaction stirred at room temperature overnight. The reaction was filtered, and the solids rinsed with ethyl acetate. The eluent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 45/55 heptane/ethyl acetate, ethyl acetate, and then 97.5/2.5/0.2 dichloromethane/methanol/acetic acid to give the title product.

2.61.20 2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetic acid

Example 2.61.19 (1.2 g) was dissolved in trifluoroacetic acid (15 mL) and heated to 65-70 °C under nitrogen overnight. The trifluoroacetic acid was removed under reduced pressure. The residue was dissolved in acetonitrile (2.5 mL) and purified by preparative reverse-phase liquid chromatography on a Luna C18(2) AXIA column (250 x 50 mm, 10µ particle size) using a gradient of 5-75% acetonitrile containing 0.1% trifluoroacetic acid in water over 30 min, to give the title compound. MS (ESI-) m/e 375.2 $(M-H)^-$.

2.61.21 3-(1-((3-(2-((((4-(4-aminobutyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.61.5 for Example 2.42.6 in Example 2.42.7. MS (ESI) m/e 1155.5 $(M-H)^-$.

2.61.22 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-4-(4-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)butyl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

Example 2.61.20 (35 mg) was dissolved in N,N-dimethylformamide (0.7 mL) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (41 mg) and N,N-diisopropylethylamine (37 μL) were added. The reaction was stirred for 3 minutes at room temperature, and a solution of Example 2.61.21 (120 mg) and N,N-diisopropylethylamine (78 μL) in N,N-dimethylformamide (0.7 mL) was added. The reaction was stirred at room temperature for 1 hour, then diluted with N,N-dimethylformamide /water 1/1 (1.5 mL) and purified by reverse phase chromatography (C18 column), eluting with 20-80% acetonitrile in 0.1% TFA water, to provide the title compound.. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 8.03 (d, 1H), 7.84 (br t, 1H), 7.79 (d, 1H), 7.61 (d, 1H), 7.51 (d, 1H), 7.46 (d, 1H), 7.44 (d, 1H), 7.36 (m, 2H), 7.29 (s, 1H), 7.16 (br d, 1H), 7.07 (s, 2H), 6.96 (m, 2H), 6.85 (br d, 1H), 5.08 (s, 2H), 5.03 (d, 1H), 4.96 (s, 2H), 4.70 (t, 1H), 4.05 (d, 1H), 3.93 (d, 1H), 3.87 (m, 2H), 3.82 (m, 3H), 3.74 (br m, 1H), 3.63 (t, 2H), 3.44 (m, 5H), 3.32 (m, 2H), 3.28 (m, 2H), 3.08 (m, 2H), 3.01 (br t, 2H), 2.90, 2.86 (both br s, total 3H), 2.74 (ddd, 2H), 2.54 (br t, 2H), 2.35 (br m, 1H), 2.09 (s, 3H), 1.81 (m, 1H), 1.55 (br m, 2H), 1.42 (m, 2H), 1.38 (br m, 2H), 1.25 (br m, 4H), 1.18-0.90 (m, 6H), 0.83 (br s, 6H); MS (ESI-) m/e 1513.5 $(M-H)^-$.

2.62 Synthesis of 3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium (Synthon VX)

2.62.1 3-((3-(4-((((2-(((1r,3S)-3-((4-(6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl)-3-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)propyl)amino)-N,N,N-trimethylpropan-1-aminium 2,2,2-trifluoroacetate

To an ice cooled stirred solution of Example 2.60.6 (30 mg) and N,N-diisopropylethylamine (20 µL) in N,N-dimethylformamide (1 mL) was added 3-bromo-N,N,N-trimethylpropan-1-aminium bromide (7 mg). The mixture was allowed to warm to room temperature and stirred for 5 hours. The reaction mixture was diluted with N,N-dimethylformamide/water (1mL, 1:1) and purified by Prep HPLC using a gradient of 20% to 100% acetonitrile/water. The product containing fractions were lyophilized to give the title compound. MS (ESI-) m/e 1240.6 $(M-H)^-$.

2.62.2 3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium

This example was prepared by substituting 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate for 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate and substituting Example 2.62.1 for Example 2.30.1 in Example 2.30.2. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 12.91 (s, 2H), 8.19 (t, 1H), 8.05 (dd, 1H), 7.81 (d, 1H), 7.63 (dd, 1H),7.55 (d, 1H), 7.51 – 7.43 (m, 2H), 7.41 – 7.35 (m, 2H), 7.32 (s, 1H), 7.18 (q, 1H), 7.08 (s, 2H), 7.03 –6.95 (m, 2H), 6.85 (d, 1H), 5.09 (s, 2H), 5.04 (d, 1H), 4.97 (s, 2H), 4.07 (t, 2H), 4.02 (s, 2H), 3.44 (dt,2H), 3.38 – 3.25 (m, 3H), 3.22 – 3.14 (m, 2H), 2.89 (d, 2H), 2.08 (s, 2H), 1.94 (d, 2H), 1.68 (p, 2H), 1.41– 0.72 (m, 17H). MS (ESI) m/e 1379.5 $(M+H)^+$.

2.63 Synthesis of (6S)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{[N-({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid (Synthon WD)

2.63.1 3-(1-((3-(2-((((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

The title compound was prepared by substituting Example 2.59.10 for Example 2.42.6 in Example 2.42.7. MS (ESI) m/e 1281.6 $(M-H)^-$.

2.63.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)-4-((S)-2-((S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

The title compound was prepared by substituting Example 2.63.1 for Example 2.61.21 in Example 2.61.22. ^{1}H NMR (500 MHz, dimethylsulfoxide-d_6) δ ppm 9.85 (br d, 1H), 8.18 (d, 1H), 8.05 (br s, 1H), 8.03 (d, 1H), 7.78 (d, 1H), 7.61 (d, 1H), 7.51 (d, 1H), 7.47 (m, 2H), 7.43 (m, 2H), 7.36 (m, 2H), 7.29 (s, 1H), 7.20 (d, 1H), 7.07 (s, 2H), 6.95 (d, 1H), 4.99 (s, 2H), 4.96 (s, 2H), 4.65 (t, 1H), 4.36 (m, 1H), 4.18 (m, 2H), 4.01 (d, 1H), 3.87 (br t, 2H), 3.81 (br d, 2H), 3.73 (br m, 1H), 3.63 (m, 2H), 3.53 (m, 2H), 3.44 (m, 2H), 3.32 (t, 2H), 3.24 (br m, 2H), 3.12 (m, 2H), 3.01 (m, 2H), 2.92 (t, 1H), 2.82 (m, 3H), 2.77 (m, 3H), 2.59 (v br s, 1H), 2.37 (m, 1H), 2.09 (s, 3H), 2.00 (m, 2H), 1.86 (m, 1H), 1.55 (br m, 1H), 1.36 (br m, 1H), 1.28 (br m, 6H), 1.10 (br m, 7H), 0.93 (br m, 1H), 0.88, 0.86, 0.81 (all d, total 12H); MS (ESI-) m/e 1639.6 $(M-H)^-$.

2.64 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfoethyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide (Synthon CZ)

Example 1.9.2 (100 mg) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate (purchased from Synchem, 114 mg) in N,N-dimethylformamide (7 mL) was cooled in an water-ice bath, and N,N-diisopropylethylamine (0.15 mL) was added. The mixture was stirred at 0 °C for 30 minutes and then at room temperature overnight. The reaction was purified by a reverse phase HPLC using a Gilson system, eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 12.85 (s, 1H), 9.99 (s, 1H), 8.04 (t, 2H), 7.75-7.82 (m, 2H), 7.40-7.63 (m, 6H), 7.32-7.39 (m, 2H), 7.24-7.29 (m, 3H), 6.99 (s, 2H), 6.95 (d, 1H), 6.01 (s, 1H), 4.83-5.08 (m, 4H), 4.29-4.48 (m, 1H), 4.19 (t, 1H), 3.84-3.94 (m, 2H), 3.80 (d, 2H), 3.14-3.29 (m, 2H), 2.87-3.06 (m, 4H),

2.57-2.69 (m, 2H), 2.03-2.24 (m, 5H), 1.89-2.02 (m, 1H), 1.53-1.78 (m, 2H), 1.26-1.53 (m, 8H), 0.89-1.27 (m, 12H), 0.75-0.88 (m, 12H). MS (ESI) m/e 1452.2 $(M+H)^+$.

2.65 Synthesis of (6*S*)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-carboxypyridin-3-yl}-5-methyl-1*H*-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfoethyl)carbamoyl}oxy)methyl]-5-{[*N*-({(3*S*,5*S*)-3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid (Synthon TX)

2.65.1 3-(1-(((1r,3s,5R,7S)-3-(2-((((4-((R)-2-((R)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

To a cold (0 °C) solution of Example 2.59.10 (70 mg) and Example 1.9.2 (58.1 mg) in N,N-dimethylformamide (4 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.026 mL). The reaction was slowly warmed to room temperature and stirred overnight. To the reaction mixture was added water (1 mL) and LiOH H_2O(20 mg). The mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title product. MS (ESI) m/e 1564.4 $(M-H)^-$.

2.65.2 (6*S*)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-carboxypyridin-3-yl}-5-methyl-1*H*-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfoethyl)carbamoyl}oxy)methyl]-5-{[*N*-({(3*S*,5*S*)-3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid

The title compound was prepared by substituting Example 2.65.1 for Example 2.61.21 in Example 2.61.22. ^{1}H NMR (400 MHz, dimethylsulfoxide-d_6) δ ppm 9.85 (s, 1H), 8.17 (br d, 1H), 8.01 (d, 2H), 7.77 (d, 1H), 7.59 (d, 1H), 7.53 (d, 1H), 7.43 (m, 4H), 7.34 (m, 3H), 7.19 (d, 1H), 7.06 (s, 2H), 6.96 (d, 1H), 4.99 (m, 2H), 4.95 (s, 2H), 4.63 (t, 1H), 4.36 (t, 1H), 4.19 (br m, 1H), 4.16 (d, 1H), 3.98 (d, 1H), 3.87 (br t, 2H), 3.81 (br d, 2H), 3.73 (brm, 1H), 3.63 (t, 2H), 3.53 (m, 2H), 3.44 (m, 4H), 3.31 (t, 2H), 3.21 (br m, 2H), 3.17 (m, 2H), 3.00 (m, 2H), 2.92 (br m, 1H),

2.75 (m, 3H), 2.65 (br m, 3H), 2.35 (br m, 1H), 2.07 (s, 3H), 1.98 (br m, 2H), 1.85 (m, 1H), 1.55 (br m, 1H), 1.34 (br m, 1H), 1.26 (br m, 6H), 1.09 (br m, 7H), 0.93 (br m, 1H), 0.87, 0.83, 0.79 (all d, total 12H). MS (ESI) m/e 1733.4 $(M-H)^-$.

Example 3. Synthesis of Exemplary Bcl-xL Inhibitory ADCs

Exemplary ADCs were synthesized using one of nine exemplary methods, described below. Table 6 correlates which method was used to synthesize each exemplary ADC.

Method A. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to a solution of antibody (10 mg/mL, 1 mL) preheated to 37 °C. The reaction mixture was kept at 37 °C for 1 hour. The solution of reduced antibody was added to a solution of synthon (3.3 mM, 0.160 mL in dimethyl sulfoxide (DMSO)) and gently mixed for 30 minutes. The reaction solution was loaded onto a desalting column (PD10, washed with DPBS 3x before use), followed by Dulbecco's phosphate-buffered saline (DPBS) (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

Method B. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) preheated to 37 °C. The reaction mixture was kept at 37 °C for 1 hour. The solution of reduced antibody was adjusted to pH=8 by adding boric buffer (0.05 mL, 0.5 M, pH8), added to a solution of synthon (3.3 mM, 0.160 mL in DMSO) and gently mixed for 4 hours. The reaction solution was loaded onto a desalting column (PD10, washed with DPBS 3x before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 °C.

Method C. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid handling system equipped with an I235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

A Pall Filter plate 5052 was prewet with 100 µL 1x DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8 well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8 tip Varispan arm, equipped with 1mL conductive tips, was used to aspirate the resin (250 µL) and transfer to a 96-well filter plate. A vacuum was applied for 2 cycles to remove most of the buffer. Using the MDT, 150 µL of 1xPBS was aspirated and dispensed to the 96-well filter plate holding the resin. A vacuum was applied,

removing the buffer from the resin. The rinse/vacuum cycle was repeated 3 times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 µL of 5x DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 µL) DPBS was prepared as described above for Conditions A and preloaded into a 96 well plate. The solutions of reduced antibody were transferred to the filter plate wells containing the resin, and the mixture was mixed with the MDT by repeated aspiration/dispensation of a 100 µL volume within the well for 45 seconds per cycle. The aspiration/dispensation cycle was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied to the filter plate for 2 cycles, thereby removing excess antibody. The MDT tips were rinsed with water for 5 cycles (200 µL, 1 mL total volume). The MDT aspirated and dispensed 150 µL of DPBS to the filter plate wells containing resin –bound antibody, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. After the last vacuum cycle, 100 µL of 1x DPBS was dispensed to the wells containing the resin-bound antibody. The MDT then collected 30 µL each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the filter plate containing resin-bound antibody in DPBS. The wells containing the conjugation mixture were mixed with the MDT by repeated aspiration/dispensation of a 100 µL volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon to waste. The MDT tips were rinsed with water for 5 cycles (200 µL, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 µL) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 µL of 10x DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 µL, 1 mL total volume). The MDT aspirated and dispensed 100 µL of IgG Elution Buffer 3.75 (Pierce) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 µL of 5x DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

Method D. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid handling system equipped with an I235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

A Pall Filter plate 5052 was prewet with 100 µL 1x DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8-well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8 tip Varispan arm, equipped with 1mL conductive tips, was used to aspirate the resin (250 µL) and transfer to a 96-well filter plate. A vacuum was applied to the filter plate for 2 cycles to remove most of the buffer. The MDT aspirated and dispensed 150 µL of DPBS to the filter plate wells containing the resin. The wash and vacuum sequence was repeated two more times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 µL of 5x DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 µL) DPBS was prepared as described above for Method A and dispensed into the 96-well plate. The MDT then collected 30 µL each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the plate loaded with reduced antibody in DPBS. The mixture was mixed with the MDT by twice repeated aspiration/dispensation of a 100 µL volume within the well. After five minutes, the conjugation reaction mixture (230 µL) was transferred to the 96-well filter plate containing the resin. The wells containing the conjugation mixture and resin were mixed with the MDT by repeated aspiration/dispensation of a 100 µL volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon and protein to waste. The MDT tips were rinsed with water for 5 cycles (200 µL, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 µL) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 µL of 10x DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 µL, 1 mL total volume). The MDT aspirated and dispensed 100 µL of IgG Elution Buffer 3.75 (P) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 µL of 5x DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

Method E. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of reduced antibody cooled to room temperature and was added to a solution of synthon (10 mM, 0.040 mL in DMSO) followed by addition of boric buffer (0.1 mL, 1M, pH 8). The reaction solution was

let to stand for 3 days at room temperature, loaded onto a desalting column (PD10, washed with DPBS 3x5mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

Method F. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system.

The solution of antibody (10 mg/mL) was preheated to 37 °C and aliquoted to a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37 C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37 °C for 75 minutes. The solution of reduced antibody was transferred to an unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL in DMSO) were added to the wells with reduced antibodies and treated for 15 minutes. The reaction solutions were loaded onto a platform (8 x 12) of desalting columns (NAP5, washed with DPBS 4x before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4 °C.

Method G. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system.

The solution of antibody (10 mg/mL) was preheated to 37 °C and aliquoted onto a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37 C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37 °C for 75 minutes. The solutions of reduced antibody were transferred to an unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL/well in DMSO) were added to the wells with reduced antibodies followed by addition of boric buffer (pH=8, 0.03 mL/well) and treated for 3 days. The reaction solutions were loaded onto a platform (8 x 12) of desalting columns (NAP5, washed with DPBS 4x before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4 °C.

Method H. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of synthon (10 mM, 0.40 mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2 – 2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in phase A (phase A: 1.5 M ammonium sulfate,

25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as "E2") and DAR4 (also referred to as "E4") eluted upon applying gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The purified ADC solutions were filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

Method I. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37 °C for 75 minutes. The solution of synthon (10 mM, 0.40 mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2 – 2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto a butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in Phase A (phase A: 1.5 M ammonium sulfate, 25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as "E2") and DAR 4 (also referred to as "E4") eluted upon applying a gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The ADC solutions were treated with boric buffer (0.1 mL, 1M, pH8). The reaction solution was let stand for 3 days at room temperature, then loaded onto a desalting column (PD10, washed with DPBS 3x5mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

Table 6, below, indicates which exemplary ADCs were synthesized via which exemplary method. The AbB, AbG, AbK, AbA1 are affinity matured variants of Ab1 described in Table 2. Monoclonal antibody to CMV glycoprotein H (MSL109) is an isotype matched non-targeting control.

Table 6

Ex. No.	ADC	Method
3.1	AbA-CZ	G
3.2	AbA-TX	G
3.3	AbA-TV	G
3.4	AbA-YY	G
3.5	AbA-AAA	G
3.6	AbA-AAD	G
3.7	AbB-CZ	G
3.8	AbB-TX	G

Ex. No.	Table 6 ADC	Method
3.9	AbB-TV	G
3.10	AbB-YY	G
3.11	AbB-AAD	G
3.12	AbG-CZ	G
3.13	AbG-TX	G
3.14	AbG-TV	G
3.15	AbG-YY	G
3.16	AbG-AAA	G
3.17	AbG-AAD	G
3.18	AbK-CZ	G
3.19	AbK-TX	G
3.20	AbK-TV	G
3.21	AbK-YY	G
3.22	AbK-AAA	G
3.23	AbK-AAD	G
3.24	MSL109-CZ	G
3.25	MSL109-TX	G
3.26	MSL109-TV	G
3.27	MSL109-YY	G
3.28	MSL109-AAA	G
3.29	MSL109-AAD	G
3.30	AbA-WD	E
3.31	AbA-LB	A
3.32	AbB-WD	E
3.33	AbB-LB	A
3.34	AbG-WD	E
3.35	AbG-LB	A
3.36	AbK-WD	E
3.37	AbK-LB	A
3.38	MSL109-WD	E
3.39	MSL109-LB	A
3.40	AbA-ZT	G
3.41	AbA-ZZ	G
3.42	AbA-XW	G
3.43	AbA-SE	A
3.44	AbA-SR	A
3.45	AbA-YG	E
3.46	AbA-KZ	A
3.47	AbB-ZT	G

Ex. No.	Table 6 ADC	Method
3.48	AbB-ZZ	G
3.49	AbB-XW	G
3.50	AbB-SE	A
3.51	AbB-SR	A
3.52	AbB-YG	E
3.53	AbB-KZ	A
3.54	AbG-ZT	G
3.55	AbG-ZZ	G
3.56	AbG-XW	G
3.57	AbG-SE	A
3.58	AbG-SR	A
3.59	AbG-YG	E
3.60	AbG-KZ	A
3.61	AbK-ZT	G
3.62	AbK-ZZ	G
3.63	AbK-XW	G
3.64	AbK-SE	A
3.65	AbK-SR	A
3.66	AbK-YG	E
3.67	AbK-KZ	A
3.68	MSL109-ZT	G
3.69	MSL109-ZZ	G
3.70	MSL109-XW	G
3.71	MSL109-SE	A
3.72	MSL109-SR	A
3.73	MSL109-YG	E
3.74	MSL109-KZ	A

Example 4. Drug to Antibody Ratio (DAR) and Aggregation of Exemplary ADCs

The DAR and percentage aggregation of exemplary ADCs synthesized as described in Example 3, above, were determined by LC-MS and size exclusion chromatography (SEC), respectively.

4.1 LC-MS General Methodology

The DAR and percentage aggregation of exemplary ADCs synthesized as described in Example 3, above, were determined by LC-MS and size exclusion chromatography (SEC), respectively.

4.1 LC-MS General Methodology

LC-MS analysis was performed using an Agilent 1100 HPLC system interfaced to an Agilent LC/MSD TOF 6220 ESI mass spectrometer. The ADC was reduced with 5 mM (final concentration) BOND BREAKER TCEP solution (Thermo Scientific, Rockford, IL), loaded onto a Protein Microtrap (Michrom Bioresorces, Auburn, CA) desalting cartridge, and eluted with a gradient of 10% B to 75% B in 0.2 minutes at ambient temperature. Mobile phase A was H_20 with 0.1% formic acid (FA), mobile phase B was acetonitrile with 0.1% FA, and the flow rate was 0.2 ml/min. Electrospray-ionization time-of-flight mass spectra of the co-eluting light and heavy chains were acquired using Agilent MassHunter(TM) acquisition software. The extracted intensity vs. m/z spectrum was deconvoluted using the Maximum Entropy feature of MassHunter software to determine the mass of each reduced antibody fragment. DAR was calculated from the deconvoluted spectrum by summing intensities of the naked and modified peaks for the light chain and heavy chain, normalized by multiplying intensity by the number of drugs attached. The summed, normalized intensities were divided by the sum of the intensities, and the summing results for two light chains and two heavy chains produced a final average DAR value for the full ADC.

Thiosuccinimide hydrolysis of a bioconjugate can be monitored by electrospray mass spectrometry, since the addition of water to the conjugate results in an increase of 18 Daltons to the observable molecular weight of the conjugate. When a conjugate is prepared by fully reducing the interchain disulfides of a human IgG1 antibody and conjugating the maleimide derivative to each of the resulting cysteines, each light chain of the antibody will contain a single maleimide modification and each heavy chain will contain three maleimide modifications, as described in Figure 4. Upon complete hydrolysis of the resulting thiosuccinimides, the mass of the light chain will therefore increase by 18 Daltons, while the mass of each heavy chain will increase by 54 Daltons. This is illustrated in Figure 5, with the conjugation and subsequent hydrolysis of an exemplary maleimide drug-linker (synthon TX, molecular weight 1736 Da) to the fully reduced AM1 antibody. The presence of the single N-linked glycosylation site on the heavy chain results in the heterogeneity of mass observed in the unconjugated antibody.

Figure 5 shows MS characterization of light chain and heavy chain of an exemplary antibody Aba 1) prior to conjugation, 2) after conjugation to a maleimide derivative to give a thiosuccinimide intermediate and 3) post pH8-mediated hydrolysis of the thiosuccinimide ring.

4.2 Size Exclusion Chromatography General Methodology

Size exclusion chromatography (SEC) was performed using a Shodex KW802.5 column in 0.2M potassium phosphate pH 6.2 with 0.25 mM potassium chloride and 15% isopropyl alcohol at a flow rate of 0.75 ml/min. The peak area absorbance at 280 nm was determined for

each of the high molecular weight and monomeric eluents by integration of the area under the curve. The % aggregate fraction of the conjugate sample was determined by dividing the peak area absorbance at 280 nM for the high molecular weight eluent by the sum of the peak area absorbances at 280 nM of the high molecular weight and monomeric eluents multiplied by 100%.

4.3. Results

The average DAR values were determined using the above LC-MS method. The % aggregate fraction for the ADCs was also determined using the SEC method described in Example 2.2. The DAR and % aggregation are both reported below in Table 7.

Table 7: ADC Analytical Characterization

Appln Ex. No.	ADC Code	DAR (by MS)	% Agg (by SEC)
3.1	AbA-CZ	3.2	4.7
3.2	AbA-TX	2.8	0.7
3.3	AbA-TV	3.7	2.4
3.4	AbA-YY	2.2	18.8
3.5	AbA-AAA	2	19
3.6	AbA-AAD	3.3	3.6
3.7	AbB-CZ	3.5	0
3.8	AbB-TX	2.2	0
3.9	AbB-TV	2.3	0.7
3.10	AbB-YY	2.2	0
3.11	AbB-AAD	2.7	0
3.12	AbG-CZ	3.4	4
3.13	AbG-TX	3.3	1.6
3.14	AbG-TV	3.7	1.4
3.15	AbG-YY	2.2	16.5
3.16	AbG-AAA	1.9	17.5
3.17	AbG-AAD	3.4	2.5
3.18	AbK-CZ	3.4	3.3
3.19	AbK-TX	2.2	1.6
3.20	AbK-TV	2.4	2.6
3.21	AbK-YY	1.7	20
3.22	AbK-AAA	1.6	20.4
3.23	AbK-AAD	2.8	3.6
3.24	MSL109-CZ	3.4	4.1
3.25	MSL109-TX	3.5	0.7
3.26	MSL109-TV	4.2	0.7
3.27	MSL109-YY	2.3	17.5
3.28	MSL109-AAA	2.2	17.7
3.29	MSL109-AAD	3.6	2.9
3.30	AbA-WD	1.8	0
3.31	AbA-LB	2.4	14.5
3.32	AbB-WD	1.6	0
3.33	AbB-LB	1.8	0

Appln Ex. No.	ADC Code	DAR (by MS)	% Agg (by SEC)
3.34	AbG-WD	3.4	3.2
3.35	AbG-LB	2.5	15.3
3.36	AbK-WD	1.7	4.9
3.37	AbK-LB	1.8	13.6
3.38	MSL109-WD	2.9	0
3.39	MSL109-LB	1.8	0
3.40	AbA-ZT	2	17.1
3.41	AbA-ZZ	1.3	19.2
3.42	AbA-XW	3.7	6.6
3.43	AbA-SE	2.8	0
3.44	AbA-SR	2.3	37.1
3.45	AbA-YG	1.9	0
3.46	AbA-KZ	2	4.4
3.47	AbB-ZT	1.4	0
3.48	AbB-ZZ	1.1	0
3.49	AbB-XW	3.2	0
3.50	AbB-SE	2.2	0
3.51	AbB-SR	2.1	0
3.52	AbB-YG	1.1	0
3.53	AbB-KZ	1.9	0
3.54	AbG-ZT	1.6	12.4
3.55	AbG-ZZ	1.4	16.8
3.56	AbG-XW	3.7	5.9
3.57	AbG-SE	3.8	2.1
3.58	AbG-SR	2.8	36.7
3.59	AbG-YG	3.7	2.4
3.60	AbG-KZ	2.7	11.6
3.61	AbK-ZT	1.3	13.4
3.62	AbK-ZZ	1.9	4.5
3.63	AbK-XW	2.8	6.2
3.64	AbK-SE	2.7	2.5
3.65	AbK-SR	2.3	30.1
3.66	AbK-YG	0.9	0
3.67	AbK-KZ	2.3	10.2
3.68	MSL109-ZT	2.3	7.5
3.69	MSL109-ZZ	1.4	15
3.70	MSL109-XW	3.3	3.7
3.71	MSL109-SE	3.6	33.4
3.72	MSL109-SR	1.8	2.3
3.73	MSL109-YG	3.1	13.2
3.74	MSL109-KZ	2.5	18

Example 5. EGFR-Targeted ADCs Inhibit the Growth of Cancer Cells *In Vitro*

The cytotoxicity of the anti-EGFR antibodies AbB, AbG, AbK, and AbL as Bcl-xL ADCs against the EGFR positive non-small cell lung cancer cells (NCI-H1650) was compared to non-targeting MSL109 isotype matched exemplary ADCs. To further evaluate the in vitro efficacy of these exemplary EGFR-targeted Bcl-xL-ADCs, human EGFR was over-expressed in *mcl-1*$^{-/-}$ mouse embryonic fibroblasts (MEFs). Mcl-1 refers to the gene myeloid cell leukemia 1. *Mcl-1*$^{-/-}$ MEFs are dependent upon Bcl-xL for survival (Lessene *et al.*, 2013, *Nature Chemical Biology* 9:390-397).

5.1 Methods

Retroviral supernatants were produced through transfection of the GP2-293 packaging cell line (Clontech) with the retroviral construct pLVC-IRES-Hygro (Clontech) containing huEGFR sequence or the empty vector utilizing FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany). After 48 hours of culture, virus-containing supernatant was harvested and applied to *mcl-1*$^{-/-}$ MEFs in 75 cm^2 culture flasks ($0.5x10^6$ per flask) for a further 48 hours in the presence of polybrene (8 µg/ml; Sigma). *Mcl-1*$^{-/-}$ MEFs were washed and selected after 3 days with 250 µg/mL hygromycin B (Invitrogen) in the full complement of media. The expression of huEGFR was confirmed by flow cytometry and compared to the parental cell line or those transfected with the empty vector.

Mcl-1$^{-/-}$ MEFs expressing huEGFR or the pLVX empty vector (Vct Ctrl) were treated with AB033-targeted Bcl-xL-ADCs, AB033 alone or MSL109-targeted Bcl-xL-ADCs for 96 hours in DMEM containing 10% FBS. For the assay, the cells were plated at 250 cells per well in 384-well tissue culture plates (Corning, Corning, NY) in a total volume of 25 µL of assay media (DMEM and 10% HI FBS). The plated cells were treated with a 4-fold serial dilution of the Antibody Drug Conjugates of interest from either 1 µM or 0.5 µM to either 1 50 pM or 25 pM, respectively . Each concentration was tested in at least three replicates for the *Mcl-1*$^{-/-}$ MEF huEGFR cell line and for the *Mcl-1*$^{-/-}$ MEF vector cell line. The fraction of viable cells following 96 hours of Antibody Drug Conjugate treatment at 37 °C and 5% CO_2 was determined using the CellTiter-Glo Luminescent Cell Viability Assay according to the manufacturer's recommendations (Promega Corp., Madison, WI). The plates were read in a Perkin Elmer Envision using a Luminescence protocol with 0.1 sec integration time. The replicate values for each dilution point were averaged and the EC_{50} values for the Antibody Drug Conjugates were generated by fitting the data with GraphPad Prism 5 (GraphPad Software, Inc.) to a sigmoidal curve model using linear regression, $Y=((Bottom-Top)/(1+((x/K)^n)))+Top$, where Y is the measured response, x is the compound concentration, n is the Hill Slope and K is the EC_{50} and Bottom and Top are the lower and higher asymptotes respectively. Visual inspection of curves

was used to verify curve fit results. *Mcl-1*$^{-/-}$ MEFs were obtained from David C. S. Huang of the Walter and Eliza Hall Institute of Medical Research.

NCI-H1650 cells stably overexpressing eGFP were maintained in RPMI media (Invitrogen cat#22400) containing 10% Fetal Bovine Serum (Invitrogen cat# 10082). The cells were removed from plates with Trypsin and plated at 300 cells/well in 25 µL of the same media in Corning 384 well spheroid plates (cat# 3830). The plates were centrifuged at 500 x g for 5 minutes and placed in an Essen INCUCYTE Zoom Live Cell Analysis System in a 37°C with 5% CO_2 and 95% humidity. The cells were allowed to form spheroids for 3 days before dosing with an equal volume of the antibody drug conjugates at 2X the indicated concentration. The spheroids were incubated for an additional 6 days while monitoring growth and GFP Fluorescence in the Incucyte Zoom prior to addition of 40 µL of Promega CELLTITER-GLO 3D (cat# G968B) and subsequent luminescent reading. IC_{50}s were determined from both the final GFP fluorescence monitored by the Incucyte Zoom (referred to as "H1650 GFP Flourescence EC_{50} (µg/mL)" in Table 8) and the chemiluminescent readings from the CellTiter-Glo reagent (referred to as "H1650 CTG-3D EC_{50} (µg/mL)" in Table 8).

5.2 Results

Cell viability assay results (EC_{50} in nanomolar or µg/mL) for representative ADCs are provided below in Table 8.

Table 8: *In Vitro* Cell Viability Efficacy of Exemplary EGFR-Targeted ADCs

ADC Code.	DAR	huEGFR$^+$ *mcl-1*$^{-/-}$ MEF EC_{50} (µM)	*mcl-1*$^{-/-}$ MEF Vector Control EC_{50} (µM)	H1650 GFP Flourescence EC_{50} (µg/mL)	H1650 CTG-3D EC_{50} (µg/mL)
AbA-CZ	3.2	0.0003	>0.5	1.24	0.88
AbA-TX	2.8	0.016	>0.5	13.88	>40
AbA-TV	3.7	0.003	0.364	0.69	0.34
AbA-YY	2.2	0.46	>0.5	7.85	>40
AbA-AAA	2	0.22	0.306	0.98	0.65
AbA-AAD	3.3	0.065	>0.5	0.36	0.79
AbB-CZ	3.5	0.0059	0.104	0.88	1.27
AbB-TX	2.2	0.011	0.491	3.72	2.39
AbB-TV	2.3	0.0024	0.31	0.74	0.86
AbB-YY	2.2	0.051	0.4	7.73	8.6
AbB-AAD	2.7	0.0046	>10	0.59	0.16

ADC Code.	DAR	huEGFR^{+} *mcl-1*$^{-/-}$ MEF EC_{50} (μM)	*mcl-1*$^{-/-}$ MEF Vector Control EC_{50} (μM)	H1650 GFP Flourescence EC_{50} (μg/mL)	H1650 CTG-3D EC_{50} (μg/mL)
AbG-CZ	3.4	0.0034	0.194	0.25	0.07
AbG-TX	3.3	0.0053	0.368	0.51	0.15
AbG-TV	3.7	0.0026	0.196	0.04	0.03
AbG-YY	2.2	>0.5	>0.5	1.1	0.4
AbG-AAA	1.9	0.22	>0.5	0.17	0.12
AbG-AAD	3.4	0.108	>0.5	0.03	0.02
AbK-CZ	3.4	0.0001	>0.5	0.21	0.08
AbK-TX	2.2	0.00079	>1.0	1.47	1.07
AbK-TV	2.4	0.00015	0.455	0.16	0.05
AbK-YY	1.7	0.047	>1.0	1.09	0.49
AbK-AAA	1.6	0.0034	>1.0	0.16	0.11
AbK-AAD	2.8	0.0004	>0.5	0.06	0.01
MSL109-CZ	3.4	0.087	0.154	>40	>40
MSL109-TX	3.5	0.191	>0.5	24	22
MSL109-TV	4.2	0.143	0.411	>40	>40
MSL109-YY	2.3	>0.5	>0.5	>40	>40
MSL109-AAA	2.2	>0.5	>0.5	>40	>40
MSL109-AAD	3.6	>0.5	>0.5	>40	>40
AbA-WD	1.8	0.304	NT	7.62	23.59
AbA-LB	2.4	0.027	NT	2.48	4.05
AbB-WD	1.6	0.023	NT	1.01	2.69
AbB-LB	1.8	0.031	NT	2.38	1.07
AbG-WD	3.4	0.0058	NT	0.36	2.03
AbG-LB	2.5	0.024	NT	1.62	1.68
AbK-WD	1.7	0.035	NT	2.57	4.36
AbK-LB	1.8	0.017	NT	1.33	1.23
MSL109-WD	2.9	0.22	NT	1.791	7.668
MSL109-LB	1.8	0.025	NT	0.5744	0.6426
AbA-ZT	2	0.104	>0.5	1.13	0.35

ADC Code.	DAR	huEGFR^{+} *mcl-1*$^{-/-}$ MEF EC_{50} (μM)	*mcl-1*$^{-/-}$ MEF Vector Control EC_{50} (μM)	H1650 GFP Flourescence EC_{50} (μg/mL)	H1650 CTG-3D EC_{50} (μg/mL)
AbA-ZZ	1.3	0.186	>0.5	1.18	1.86
AbA-XW	3.7	0.032	0.229	>40	>40
AbA-SE	2.8	0.105	NT	4.23	4.00
AbA-SR	2.3	0.0059	NT	2.22	2.12
AbA-YG	1.9	0.0064	NT	2.84	3.80
AbA-KZ	2	0.152	NT	29.31	>40
AbB-ZT	1.4	0.0089	0.404	0.64	0.26
AbB-ZZ	1.1	0.0074	0.311	0.61	0.52
AbB-XW	3.2	0.00065	>0.5	12.14	6.92
AbB-SE	2.2	0.039	NT	2.18	0.59
AbB-SR	2.1	0.007	NT	2.10	0.62
AbB-YG	1.1	0.0033	NT	1.55	3.28
AbB-KZ	1.9	0.055	NT	37.73	16.47
AbG-ZT	1.6	0.033	>0.5	0.08	0.1
AbG-ZZ	1.4	0.068	>0.5	0.44	0.47
AbG-XW	3.7	0.019	0.246	>40	>40
AbG-SE	3.8	0.024	NT	0.99	0.96
AbG-SR	2.8	0.007	NT	1.38	1.31
AbG-YG	3.7	0.001	NT	0.63	1.24
AbG-KZ	2.7	0.096	NT	38.19	38.34
AbK-ZT	1.3	0.0002	>0.5	0.12	0.05
AbK-ZZ	1.9	0.045	>0.5	0.16	0.03
AbK-XW	2.8	0.0006	>0.5	8.53	>40
AbK-SE	2.7	0.161	NT	1.68	2.67
AbK-SR	2.3	0.0089	NT	1.36	1.74
AbK-YG	0.9	0.037	NT	10.40	12.32
AbK-KZ	2.3	0.224	NT	>40	>40
MSL109-ZT	2.3	>0.5	>0.5	>40	>40
MSL109-ZZ	1.4	>0.5	>0.5	>40	>40
MSL109-XW	3.3	0.297	0.494	>40	>40
MSL109-SE	3.6	>0.5	NT	3.692	6.079

ADC Code.	DAR	huEGFR$^+$ *mcl-1*$^{-/-}$ MEF EC_{50} (μM)	*mcl-1*$^{-/-}$ MEF Vector Control EC_{50} (μM)	H1650 GFP Flourescence EC_{50} (μg/mL)	H1650 CTG-3D EC_{50} (μg/mL)
MSL109-SR	1.8	0.142	NT	2.046	9.138
MSL109-YG	3.1	0.057	NT	3.895	5.852
MSL109-KZ	2.5	0.255	NT	>40	>40

NT = not tested

As described above in Table 8, anti-EGFR ADCs comprising an anti-EGFR antibody and a Bcl-xL inhibitor, were effective at reducing cell viability of the human EGFR expressing *mcl1*$^{-/-}$ fibroblasts with a range of potencies. The anti-EGFR Bcl-xL ADCs also inhibited the growth of NSCLC spheroids (H1650 GFP) as measured by remaining cell fluorescence and reduced viability. In contrast, many of the non-targeting (MSL109) control Bcl-xL ADCs displayed reduced potency as BCL-xL ADCs consistent with targeted delivery of the warhead by the anti-EGFR antibodies. This conclusion is further supported by the reduced activity of anti-EGFR Bcl-xL ADCs against the isogenic *mcl-1*$^{-/-}$ MEFs lacking huEGFR expression (Table 8).

Example 6. *In Vivo* Efficacy of anti-EGFR-Bcl-xL ADCs

The *in vivo* anti-tumor activity of the anti-EGFR antibodies AbB, AbG, AbK, and AbA as Bcl-xL inhibiting ADCs was evaluated using a murine xenograft non-small cell lung cancer (NSCLC) model. Specifically, EGFR positive NSCLC NCI-H1650 cells (ATCC deposit no. CRL-5883) were grown as a flank xenograft in mice. The activity of ADCs was compared to non-targeting IgG isotype matched antibody (AB095) (a human IgGl antibody recognizing tetanus toxoid; *see* Larrick *et al.*, 1992, *Immunological Reviews* 69-85) was used as a negative control. The results are presented in Tables 9, 10 and 11 below.

6.1 Evaluation of Efficacy in Xenograft Models Methods

The cell line NCI-H1650 was obtained from the American Type Culture Collection (ATCC Deposit No. CRL-5883, Manassas, VA). The cells were cultured as monolayers in RPMI-1640 that was supplemented with 10% Fetal Bovine Serum (FBS, Hyclone, Logan, UT). To generate xenografts, $5x10^6$ viable cells were inoculated subcutaneously into the right flank of immune deficient female SCID/bg mice (Charles River Laboratories, Wilmington, MA) respectively. The injection volume was 0.2 ml and composed of a 1:1 mixture of S MEM and Matrigel (BD, Franklin Lakes, NJ). Tumors were size matched at approximately 200 mm^3.

The control antibody and ADCs were formulated in 0.9% sodium chloride for injection and injected intraperitoneally. Injection volume did not exceed 200 µl. Therapy began within 24 hours after size matching of the tumors. Mice weighed approximately 22 g at the onset of therapy. Anti-EGFR ADCs and AB095 were administered intraperitoneally (IP) for a single dose (QDx1) or weekly for a total of six doses (Q7Dx6).

Tumor volume was estimated two to three times weekly. Measurements of the length (L) and width (W) of the tumor were taken via electronic caliper and the volume was calculated according to the following equation: $V = L \times W^2/2$. Eight mice were housed per cage. Food and water were available ad libitum. Mice were acclimated to the animal facilities for a period of at least one week prior to commencement of experiments. Animals were tested in the light phase of a 12-hour light: 12-hour dark schedule (lights on at 06:00 hours). Mice were euthanized when tumor volume reached 3,000 mm^3 or skin ulcerations occurred.

To refer to efficacy of therapeutic agents, parameters of amplitude (TGI_{max}), durability (TGD) of therapeutic response were used. TGI_{max} is the maximum tumor growth inhibition during the experiment. Tumor growth inhibition was calculated by $100*(1-T_v/C_v)$ where T_v and C_v are the mean tumor volumes of the treated and control groups, respectively. TGD or tumor growth delay is the extended time of a treated tumor needed to reach a volume of 1 cm^3 relative to the control group (AB095). TGD is calculated by $100*(T_t/C_t-1)$ where T_t and C_t are the median time periods to reach 1 cm^3 of the treated and control groups, respectively.

Certain anti-Bcl-xL inhibiting synthons were conjugated to EGFR targeting antibodies AbA, AbB, AbG and AbK according to the synthetic methods noted in Tables 9, 10 and 11 (and described in the above Examples).

Table 9. *In vivo* efficacy of anti-EGFR-Bcl-xL ADCs in NCI-H1650 model of NSCLC

Drug	Synthetic Method	DAR	Dose (mg/kg/day)	Regimen/Route	N	TGI_{max} (%)	TGD (%)
AB095**	-	-	10	QDx1 / IP	8	0	0
AbA-CZ	A	3.7	3	QDx1 / IP	8	81	100
AbA-CZ	A	3.7	10	QDx1 / IP	8	99	144
** IgG1 mAb							

Table 10. *In vivo* efficacy of anti-EGFR-Bcl-xL ADCs in NCI-H1650 model of NSCLC

Drug	Conjugation Method	DAR	Dose (mg/kg/day)	Regimen/Route	N	TGI_{max} (%)
AB095**	-	-	10	QDx1 / IP	8	0
AbG-TX	E	3.4	10	QDx1 / IP	8	88
AbG-AAA	E	3.6	10	QDx1 / IP	8	76
AbG-XW	E	4.2	10	QDx1 / IP	8	76
AbK-CZ	A	3.5	10	QDx1 / IP	8	77
AbK-AAA	E	3.1	10	QDx1 / IP	8	84
AbB-CZ	A	3.5	10	QDx1 / IP	8	82
** IgG1 mAb						

The results provided in Tables 9 and 10 indicate that the anti-EGFR antibodies AbB, AbG, AbK, AbA as Bcl-xL inhibitor ADCs were similarly effective at tumor growth inhibition of the H1650 xenograft non-small cell lung cancer (NSCLC) model.

The *in vivo* anti-tumor activity of anti-EGFR antibodies AbA and AbG were compared as DAR2 (E2) and DAR4 (E4) Bcl-xL inhibitor conjugates against the EGFR positive non-small cell lung cancer model NCI-H1650 grown as a flank xenograft in mice. The activity of these ADCs was compared to non-targeting IgG isotype matched antibody (AB095) as control. The results are presented in Table 11. The results shown in Table 11 indicate that the anti-EGFR antibodies AbA and AbG as Bcl-xL ADCs were effective as either purified DAR2 or DAR4 conjugates against the H1650 xenograft model, with TGI and TGD proportional to the total amount of Bcl-xL warhead dosed. Moreover, a comparison of the efficacy of the conjugates listed in Table 11 revealed that the growth inhibition was proportional to the amount of Bcl-xL administered.

Table 11. *In vivo* efficacy of anti-EGFR-Bcl-xL ADCs in NCI-H1650 model of NSCLC

Drug	Conjugation Method	DAR	Dose (mg/kg/day)	Regimen/Route	N	TGI_{max} (%)	TGD (%)
AB095**	-	-	8	Q7Dx6 / IP	8	0	0
AbA-CZE2	H	2	2	Q7Dx6 / IP	8	67	93
AbA-CZE2	H	2	4	Q7Dx6 / IP	8	67	93
AbA-CZE2	H	2	8	Q7Dx6 / IP	8	83	193
AbA-CZE4	H	4	1	Q7Dx6 / IP	8	62	75
AbA-CZE4	H	4	2	Q7Dx6 / IP	8	73	100
AbA-CZE4	H	4	4	Q7Dx6 / IP	8	77	114
AbG-CZE2	H	2	2	Q7Dx6 / IP	8	64	93
AbG-CZE2	H	2	4	Q7Dx6 / IP	8	80	143
AbG-CZE2	H	2	8	Q7Dx6 / IP	8	75	143
AbG-CZE4	H	4	1	Q7Dx6 / IP	8	61	64
AbG-CZE4	H	4	2	Q7Dx6 / IP	8	80	114
AbG-CZE4	H	4	4	Q7Dx6 / IP	8	74	114

** IgG1 mAb

As a control, the *in vivo* anti-tumor activity of an ADC comprising the non-targeting antibody MSL109 (MSL109 is a monoclonal antibody to CMV glycoprotein H) conjugated to Bcl-xL inhibitors was evaluated against the EGFR positive non-small cell lung cancer model NCI-H1650 grown as a flank xenograft in mice. The activity of these ADCs was compared to non-targeting IgG isotype matched antibody (AB095) as control showing very modest tumor growth inhibition and low or no tumor growth delay. The results are presented in Table 12, and show only modest tumor growth inhibition and low or no tumor growth delay caused by of Bcl-xL ADCs that use a non-targeting antibody as a carrier. This low anti-tumor activity is contrasted with much greater TGI and TGD observed with the EGFR-targeting Bcl-xL ADCs (Tables 9 and 10), and reflected the antigen dependent delivery of these ADCs in EGFR expressing models.

Table 12. *In vivo* efficacy of non-targeting (MSL109) Bcl-xL inhibiting ADCs in NCI-H1650 model of NSCLC

		Growth Inhibition	
Treatment	**Dose[a]/route/regimen**	**TGI_{max} (%)**	**TGD (%)**
MSL109†-H	3/IP/Q4Dx6	18*	0
MSL109†-H	10/IP/Q4Dx6	43*	20*
MSL109†-H	10/IP/Q4Dx6	8	0
MSL109†-CZ	3/IP/Q4Dx6	29*	0
MSL109†-CZ	3/IP/Q7Dx6	18*	0
MSL109†-CZ	10/IP/Q4Dx6	32*	16
MSL109†-CZ	3/IP/Q4Dx6	32*	12

† Non-targeting antibody
[a] dose is given in mg/kg/day
* = $p < 0.05$ as compared to control treatment (AB095)
Q4Dx6 indicates one dose every 4 days for a total of 6 doses

While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure.

SEQUENCE TABLE

SEQ ID NO:	Description
1	Ab1 VH amino acid sequence
2	Ab1, AbC, AbD, and AbE VH CDR1 amino acid sequence
3	Ab1, AbC, AbD, AbE, AbF, AbJ, and AbN VH CDR2 amino acid sequence
4	Ab1, AbC, AbD, and AbE VH CDR3 amino acid sequence
5	Ab1 and AbA VL amino acid sequence
6	Ab1, AbA, AbB, AbC, and AbF VL CDR1 amino acid sequence
7	Ab1, AbA, AbB, and AbC, and AbF VL CDR2 amino acid sequence
8	Ab1, AbA, AbB, and AbF VL CDR3 amino acid sequence
9	AbA VH amino acid sequence
10	AbA, AbF, and AbK VH CDR1 amino acid sequence
11	AbA, AbH, AbK, AbL, AbM, AbO, and AbQ VH CDR2 amino acid sequence
12	AbA, AbF, AbM, AbN, and AbO VH CDR3 amino acid sequence
13	Ab1 and AbA light chain amino acid sequence
14	Ab1 heavy chain amino acid sequence
15	AbA heavy chain amino acid sequence
16	AbB and AbG VH CDR1 amino acid sequence
17	AbB and AbG VH CDR2 amino acid sequence
18	AbG, AbH, AbJ, and AbL VH CDR3 amino acid sequence
19	AbB and AbK VH CDR3 amino acid sequence
20	AbM and AbN VH CDR1 amino acid sequence
21	AbP VH CDR1 amino acid sequence
22	AbP and AbQ VH CDR3 amino acid sequence
23	AbG, AbH, and AbJ VL CDR1 amino acid sequence
24	AbG, AbH, and AbJ VL CDR2 amino acid sequence
25	AbG, AbH, and AbJ VL CDR3 amino acid sequence
26	AbK, AbL, AbM, AbN, and AbO VL CDR1 amino acid sequence
27	AbE, AbK, AbL, AbM, AbN, and AbO VL CDR2 amino acid sequence
28	AbK, AbL, AbM, AbN, and AbO VL CDR3amino acid sequence
29	AbP and AbQ VL CDR1 amino acid sequence
30	AbP and AbQ VL CDR2 amino acid sequence
31	AbD, AbP, and AbQ VL CDR3 amino acid sequence
32	Human EGFR amino acid sequence (with signal sequence)
33	Human Epidermal Growth Factor Receptor variant III (hEGFRvIII) amino acid
34	Human EGFR extracellular domain (ECD) amino acid sequence
35	VH CDR1 consensus sequence of AbA, AbG, AbK, AbM, and AbP
36	VH CDR2 consensus sequence of AbA, AbG, AbK, AbM, and AbP
37	VH CDR3 consensus sequence of AbA, AbG, AbK, AbM, and AbP
38	VL CDR1 consensus sequence of AbA, AbG, AbK, AbM, and AbP
39	VL CDR2 consensus sequence of AbA, AbG, AbK, AbM, and AbP
40	VL CDR3 consensus sequence of AbA, AbG, AbK, AbM, and AbP
41	Ig gamma-1 constant region
42	Ig gamma-1 constant region mutant
43	Ig kappa constant region

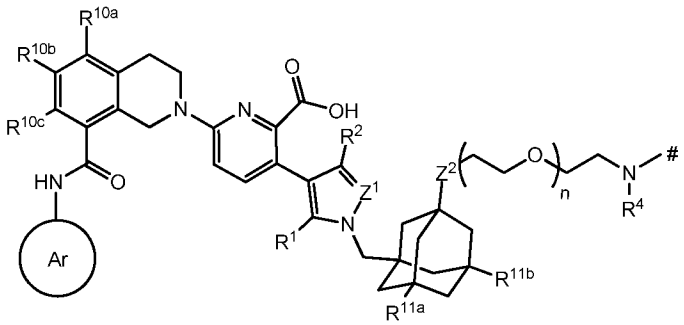
SEQ ID NO:	Description
44	Ig lambda constant region
45	Epitope of EGFR
46	ECD of EGFRvIII amino acid sequence
47	EGFR 1-525 amino acid sequence
48	Heavy chain amino acid sequence Ab2
49	Light chain amino acid sequence Ab2
50	VH amino acid sequence AbE
51	VL amino acid sequence AbE
52	VH amino acid sequence AbF
53	VL amino acid sequence AbF
54	VH amino acid sequence AbH
55	VL amino acid sequence AbH
56	VH amino acid sequence AbJ
57	VL amino acid sequence AbJ
58	VH amino acid sequence AbL
59	VL amino acid sequence AbL
60	VH amino acid sequence AbN
61	VL amino acid sequence AbN
62	VH amino acid sequence AbO
63	VL amino acid sequence AbO
64	VH amino acid sequence AbB
65	VL amino acid sequence AbB
66	VH amino acid sequence AbC
67	VL amino acid sequence AbC
68	VH amino acid sequence AbD
69	VL amino acid sequence AbD
70	VH amino acid sequence AbQ
71	VL amino acid sequence AbQ
72	VH amino acid sequence AbG
73	VL amino acid sequence AbG
74	VH amino acid sequence AbK
75	VL amino acid sequence AbK
76	VH amino acid sequence AbM
77	VL amino acid sequence AbM
78	VH amino acid sequence AbP
79	VL amino acid sequence AbP
80	AbH, AbJ, AbL, and AbO VH CDR1 amino acid sequence
81	AbQ VH CDR1 amino acid sequence
82	AbD and AbE VL CDR1 amino acid sequence
83	AbD VL CDR2 amino acid sequence
84	AbC VL CDR3 amino acid sequence
85	AbE VL CDR3 amino acid sequence
86	AbA heavy chain nucleic acid sequence
87	AbA light chain nucleic acid sequence
88	Heavy chain amino acid leader sequence
89	Light chain amino acid leader sequence

90	AbB heavy chain amino acid sequence
91	AbB heavy chain amino acid sequence, LALA mutation
92	AbB light chain amino acid sequence
93	AbG heavy chain amino acid sequence
94	AbG heavy chain amino acid sequence, LALA mutation
95	AbG light chain amino acid sequence
96	AbK heavy chain amino acid sequence
97	AbK heavy chain amino acid sequence, LALA mutation
98	AbK light chain amino acid sequence

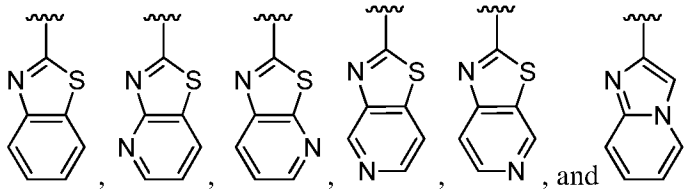
WHAT IS CLAIMED IS:

1. An anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents a point of attachment to a linker;

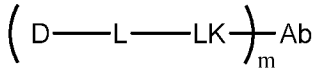
wherein the anti-hEGFR antibody has the following characteristics:

binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) or competes with a second anti-hEGFR antibody for binding to epidermal growth factor

receptor variant III (EGFRvIII) (SEQ ID NO: 33) in a competitive binding assay, wherein the second anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5; and

binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K_d) of about 1 x 10^{-6} M or less, as determined by surface plasmon resonance.

2. The ADC of claim 1, which is a compound according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

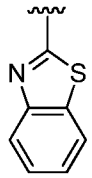
L is the linker;

Ab is the anti-hEGFR antibody;

LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and

m is an integer ranging from 1 to 20.

3. The ADC of claim 1 or 2, in which Ar is unsubstituted.

4. The ADC of claim 3, in which Ar is 

.

5. The ADC of claim 1 or 2, in which R^{10a}, R^{10b}, and R^{10c} are each hydrogen.

6. The ADC of claim 1 or 2, in which one of R^{10a}, R^{10b} and R^{10c} is halo and the others are hydrogen.

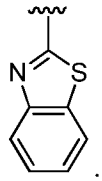
7. The ADC of claim 1 or 2, in which Z^1 is N.

8. The ADC of claim 1 or 2, in which R^1 is methyl or chloro.

9. The ADC of claim 1 or 2, in which R^2 is hydrogen or methyl.

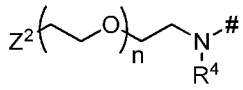
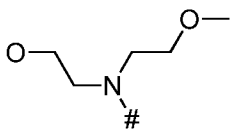
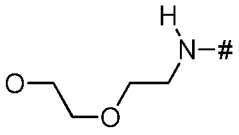
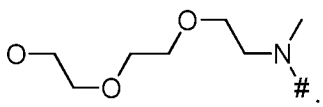
10. The ADC of claim 9, in which R^2 is hydrogen.

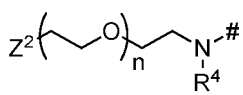
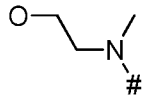
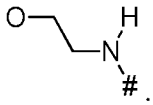
11. The ADC of claim 1 or 2, in which R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with $-OCH_3$.

12. The ADC of claim 1 or 2, in which Z^1 is N; R^1 is methyl; R^2 is hydrogen; R^4 is hydrogen or C_{1-4} alkanyl, wherein the C_{1-4} alkanyl is optionally substituted with $-OCH_3$; one of R^{10a}, R^{10b} and R^{10c} is hydrogen or halo, and the others are hydrogen; R^{11a} and R^{11b} are each methyl, and Ar is  .

13. The ADC of claim 1 or 2, in which Z^2 is CH_2 or O.

14. The ADC of claim 1 or 2, in which n is 0, 1 or 2.

15. The ADC of claim 1 or 2, in which the group  is  ,  , or  .

16. The ADC of claim 1 or 2, in which the group  is  or  .

17. The ADC of claim 1 or 2, wherein Z^2 oxygen, R^4 is hydrogen or C_{1-4} alkanyl optionally substituted with OCH_3, and n is 0, 1 or 2.

18. The ADC of claim 1 or 2, wherein the Bcl-xL inhibitor is selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.$1^{3,7}$]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.$1^{3,7}$]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.$1^{3,7}$]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

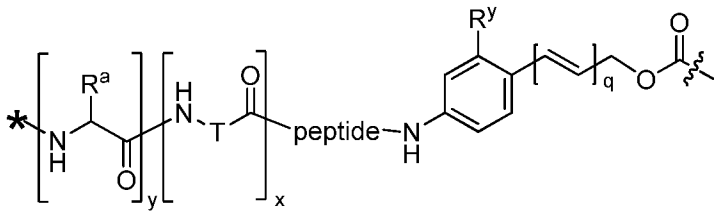
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

19. The ADC of any one of claims 1-18, in which the linker is cleavable by a lysosomal enzyme.

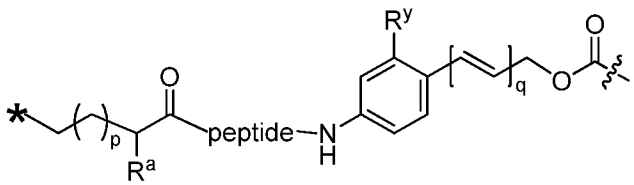
20. The ADC of claim 19, in which the lysosomal enzyme is Cathepsin B.

21. The ADC of any one of claims 1-18, in which the linker comprises a segment according to structural formula (IVa), (IVb), (IVc), or (IVd):

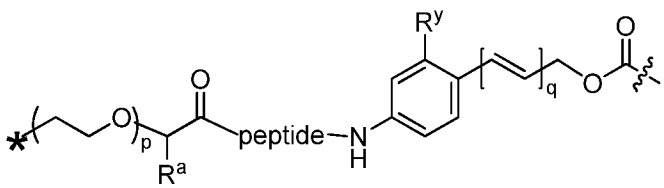
(IVa)

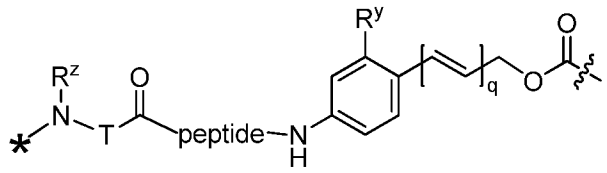


(IVb)



(IVc)



(IVd) 

wherein:

peptide represents a peptide (illustrated N→C, wherein peptide includes the amino and carboxy "termini") cleavable by a lysosomal enzyme;

T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof;

R^a is selected from hydrogen, C_{1-6} alkyl, SO_3H and CH_2SO_3H;

R^y is hydrogen or C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^1 or C_{1-4} alkyl-(N)-[(C_{1-4} alkylene)-G^1]$_2$;

R^z is C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^2;

G^1 is SO_3H, CO_2H, PEG 4-32, or sugar moiety;

G^2 is SO_3H, CO_2H, or PEG 4-32 moiety;

r is 0 or 1;

s is 0 or 1;

p is an integer ranging from 0 to 5;

q is 0 or 1;

x is 0 or 1;

y is 0 or 1;

$\sim\!\!\sim$ represents the point of attachment of the linker to the Bcl-xL inhibitor; and

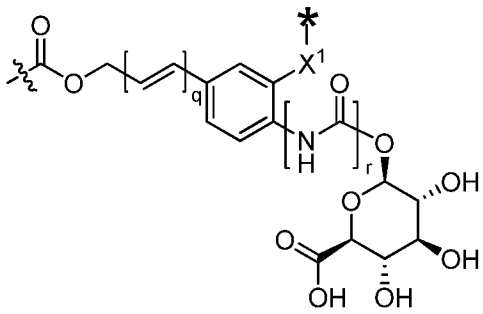
* represents the point of attachment to the remainder of the linker.

22. The ADC of claim 21, in which peptide is selected from the group consisting of 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.

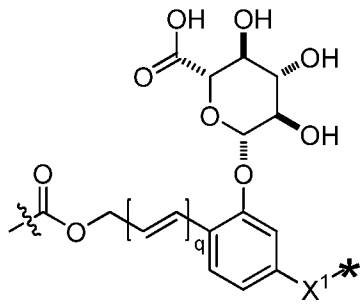
23. The ADC of claim 19, in which the lysosomal enzyme is β-glucuronidase or β-galactosidase.

24. The ADC of any one of claims 1-18, in which the linker comprises a segment according to structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

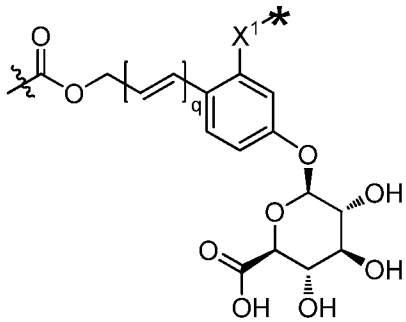
(Va)



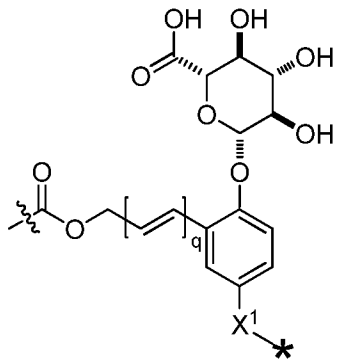
(Vb)



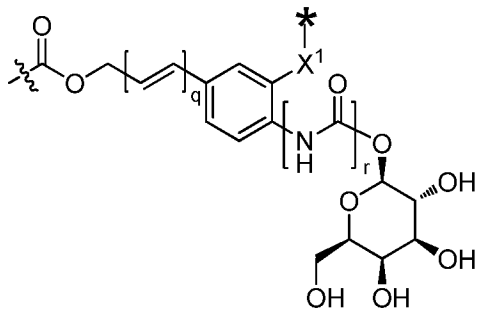
(Vc)



(Vd)



(Ve)



wherein:

q is 0 or 1;

r is 0 or 1;

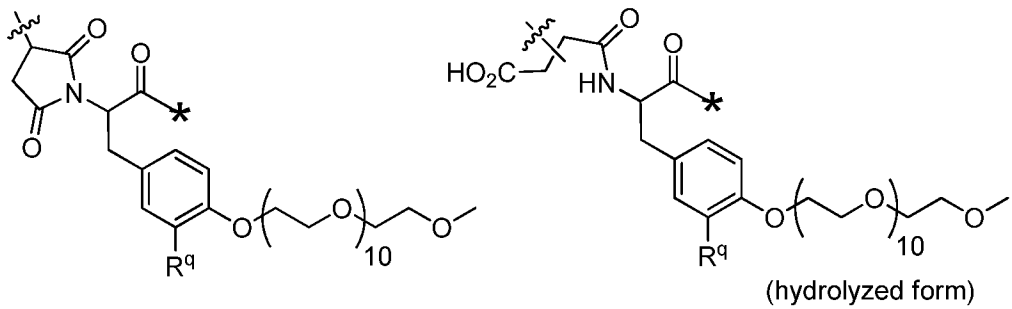
X^1 is CH_2, O or NH;

~ represents the point of attachment of the linker to the drug; and

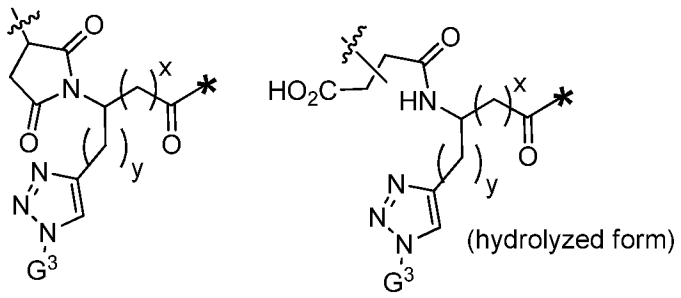
* represents the point of attachment to the remainder of the linker.

25. The ADC of any one of claims 1-18, in which the linker comprises a segment according to structural formula (VIIIa), (VIIIb), or (VIIIc):

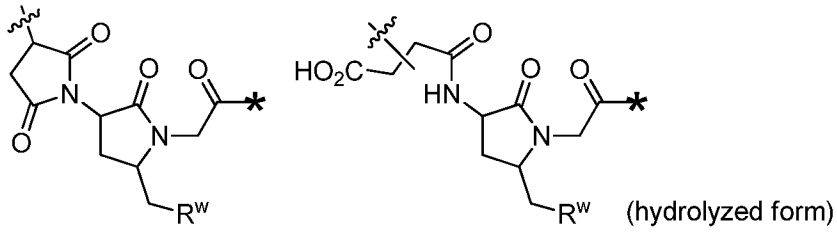
(VIIIa)



(VIIIb)



(VIIIc)



or a hydrolyzed derivative thereof, wherein:

R^q is H or $-O-(CH_2CH_2O)_{11}-CH_3$;

x is 0 or 1;

y is 0 or 1;

G^3 is $-CH_2CH_2CH_2SO_3H$ or $-CH_2CH_2O-(CH_2CH_2O)_{11}-CH_3$;

R^w is $-O-CH_2CH_2SO_3H$ or $-NH(CO)-CH_2CH_2O-(CH_2CH_2O)_{12}-CH_3$;

* represents the point of attachment to the remainder of the linker; and

 represents the point of attachment of the linker to the antibody.

26. The ADC of any one of claims 1-18, in which the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

27. The ADC of any one of claims 2-18, in which m is 2, 3 or 4.

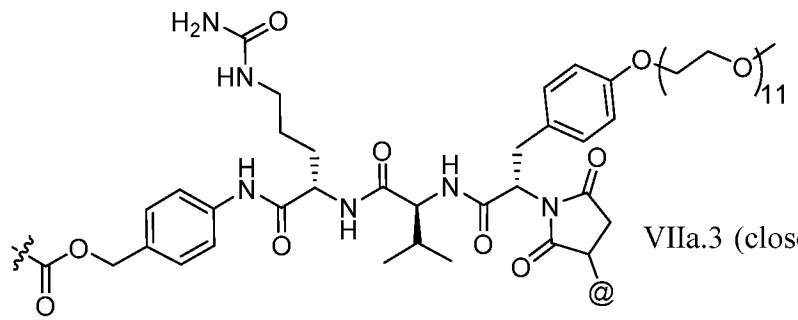
28. The ADC of any one of claims 1-18, in which the linker L is selected from IVa or IVb.

29. The ADC of any one of claims 1-18, in which the linker L is selected from the group consisting of IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-V1c.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 in the closed or open form.

30. The ADC of any one of claims 1-18, in which the linker L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

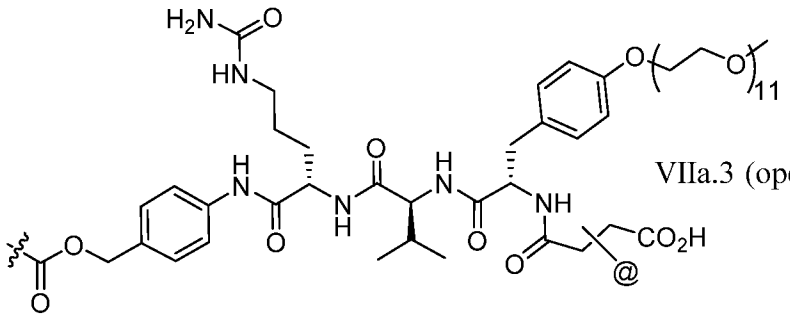
31. The ADC of any one of claims 1-18, in which the linker L is selected from the group consisting of IVc.5, IVc.6, IVd.4, VIIa.1, VIIa.3, VIIc.1, VIIc.3, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody, Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

32. The ADC of any one of claims 1-18, in which the linker L is selected from the group consisting of VIIa.3, IVc.6, VIIc.1, and VIIc.5, wherein  is the attachment point to drug D and @ is the attachment point to the LK, wherein when the linker is in the open form as shown below, @ can be either at the α-position or β-position of the carboxylic acid next to it:



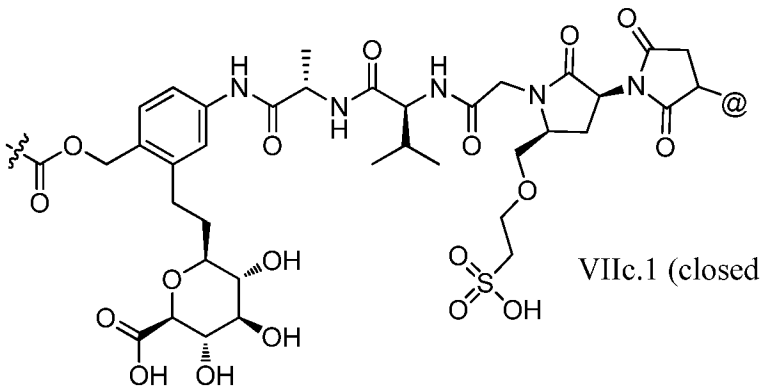
VIIa.3 (closed form)

,



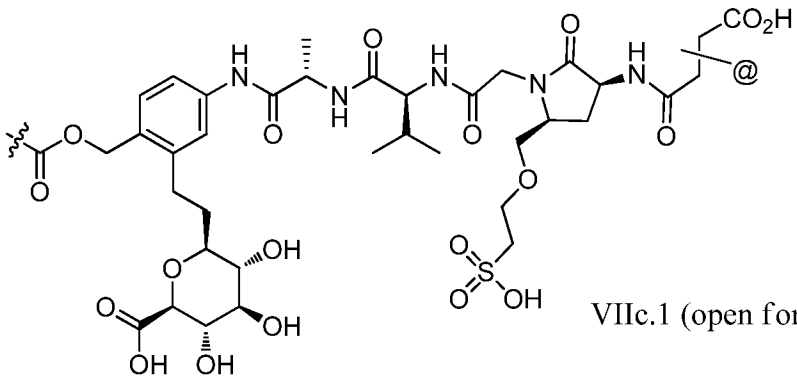
VIIa.3 (open form)

,



VIIc.1 (closed form)

,

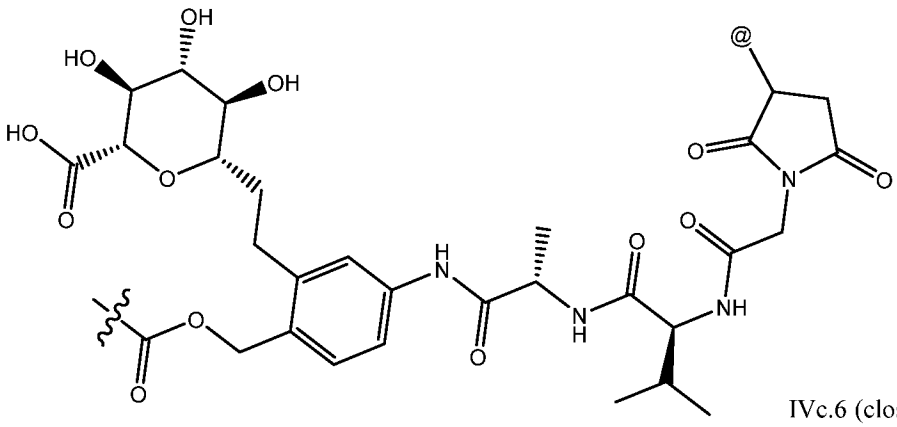


VIIc.1 (open form)

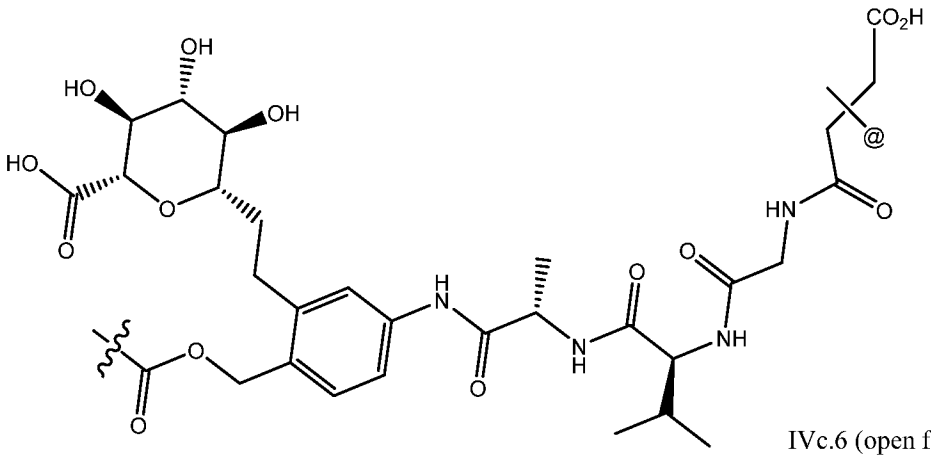
,

,

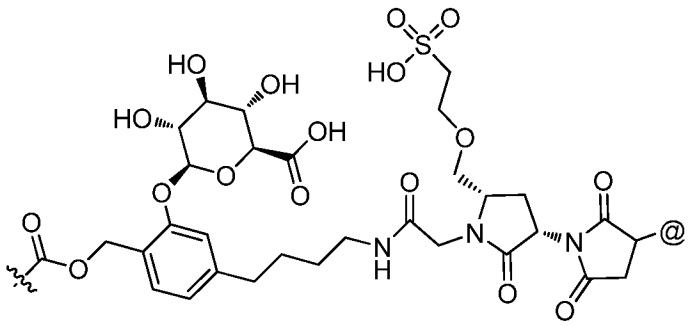
5



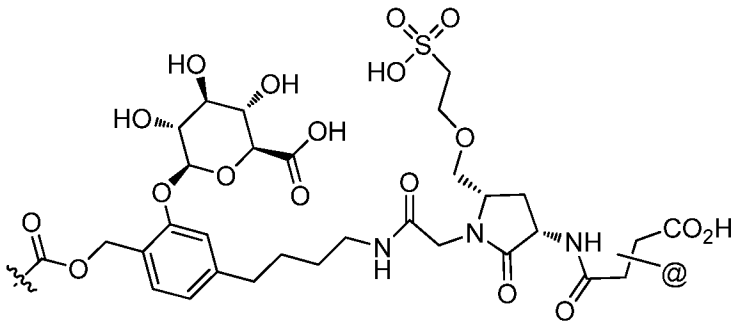
IVc.6 (closed form),



IVc.6 (open form),



VIIc.5 (closed form), and



VIIc.5 (open form)..

33. The ADC of any one of claims 2-18, in which LK is a linkage formed with an amino group on the anti-hEGFR antibody Ab.

34. The ADC of claim 32, in which LK is an amide or a thiourea.

35. The ADC of any one of claims 2-18, in which LK is a linkage formed with a sulfhydryl group on the anti-hEGFR antibody Ab.

36. The ADC of claim 35, in which LK is a thioether.

37. The ADC of any one of claims 2-18, in which:

LK is selected from the group consisting of amide, thiourea and thioether; and

m is an integer ranging from 1 to 8.

38. The ADC of claim 2 in which:

D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-V1c.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 wherein each linker has reacted with the anti-hEGFR antibody, Ab, forming a covalent attachment;

LK is thioether; and

m is an integer ranging from 1 to 8.

39. The ADC of claim 2 in which:

D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; and

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

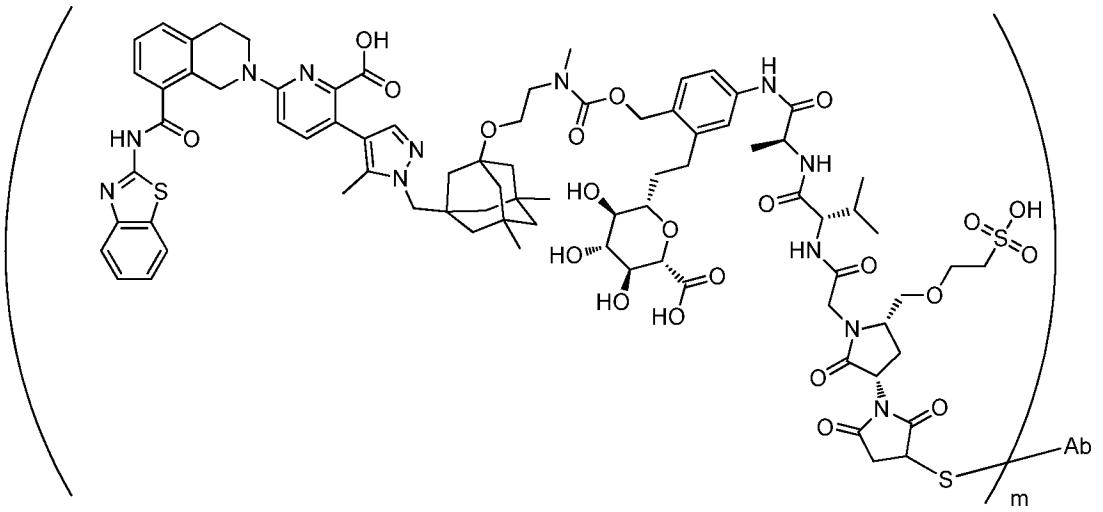
L is selected from the group consisting of linkers Vc.5, IVc.6, IVd.4, VIIa.1, VIIc.1, VIIc.3, VIIc.4, and VIIc.5 in either closed or open form;

LK is thioether; and

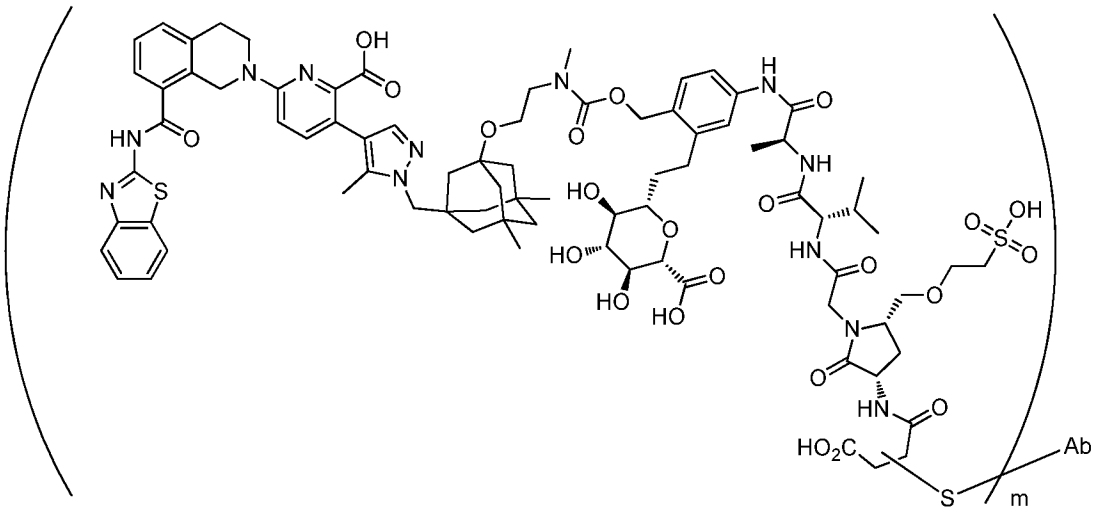
m is an integer ranging from 2 to 4.

40. The ADC of claim 2, selected from the group consisting of AbA-WD, AbA-LB, AbA-VD, AbB-WD, AbB-LB, AbB-VD, AbG-WD, AbG-LB, AbG-VD, AbK-WD, AbK-LB, and AbK-VD, wherein WD, LB, and VD are synthons disclosed in Table 5, and where in the synthons are either in open or closed form.

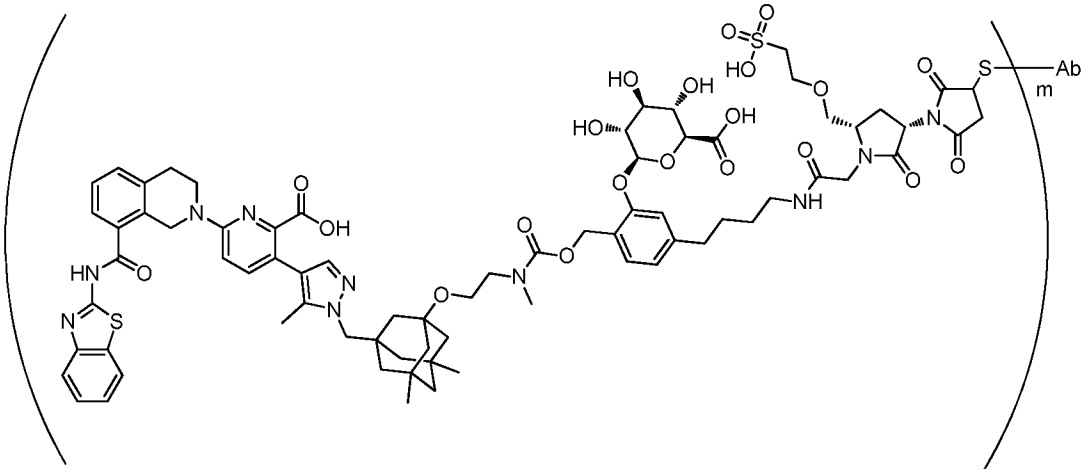
41. The ADC of claim 2, selected from the group consisting of formulas i-vi:



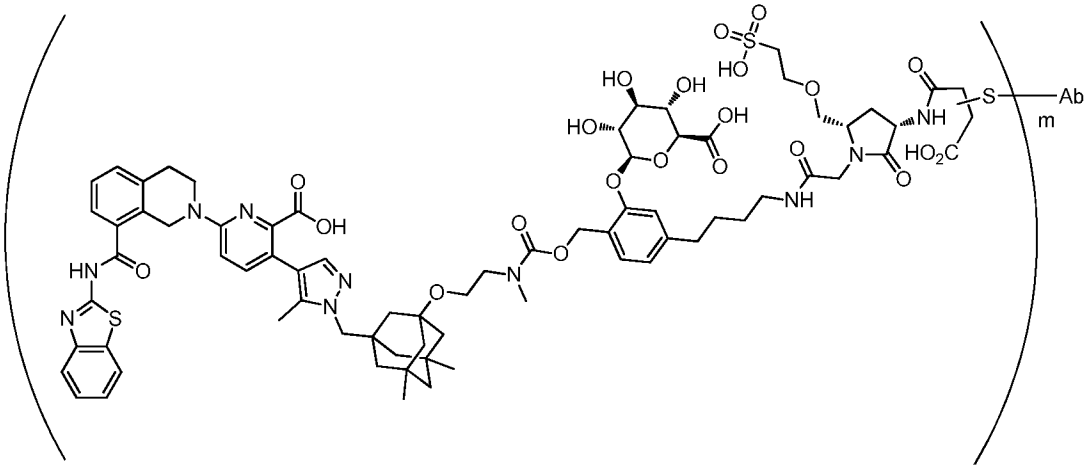
(i),



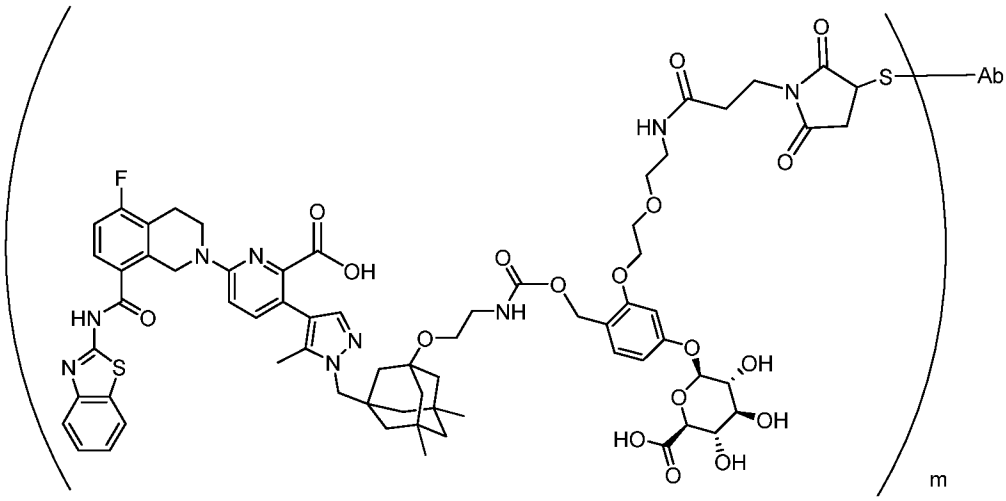
(ii),



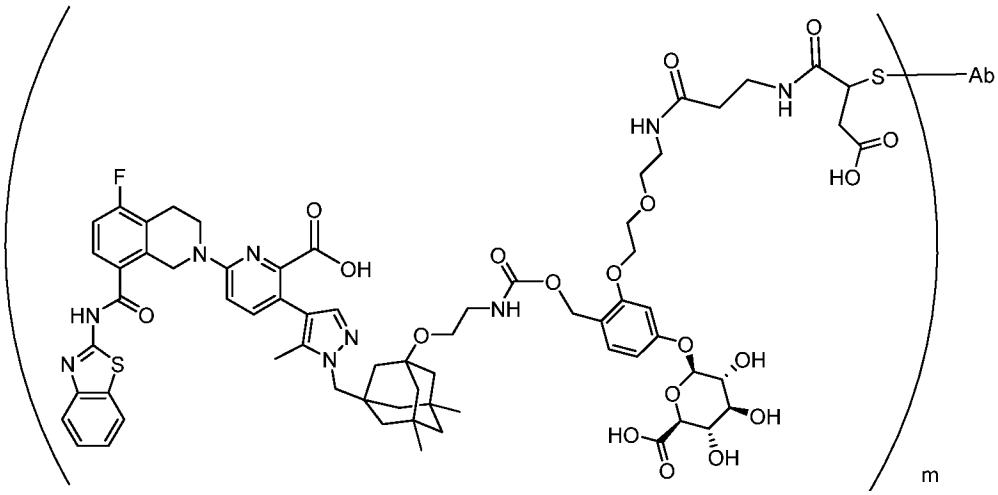
(iii),



(iv),



(v), and



(vi),

wherein m is an integer from 1 to 6.

42. The ADC of claim 41, wherein m is an integer from 2 to 6.

43. The ADC of any one of claims 1-42, wherein the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-10} M, as determined by surface plasmon resonance.

44. The ADC of any one of claims 1-42, wherein the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_d of between about 1 x 10^{-6} M and about 1 x 10^{-7} M, as determined by surface plasmon resonance.

45. The ADC of any one of claims 1-42, wherein the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of about 8.2 x 10^{-9} M or less, as determined by surface plasmon resonance.

46. The ADC of any one of claims 1-42, wherein the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of between about 8.2 x 10^{-9} M and about 6.3 x 10^{-10} M, as determined by surface plasmon resonance.

47. The ADC of any one of claims 1-42, wherein the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_d of between about 8.2 x 10^{-9} M and about 2.0 x 10^{-9} M, as determined by surface plasmon resonance.

48. The ADC of any one of claims 1-42, wherein the anti-hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6.

49. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

50. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

51. The ADC of any one of claims 1-42, wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

52. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

53. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and

a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85,

wherein the antibody does not comprise both the heavy chain CDR set of SEQ ID NOs: 2, 3, and 4, and the light chain CDR set of SEQ ID NOs: 6, 7, and 8.

54. The ADC of any one of claims 1-42, wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

55. The ADC of any one of claims 1-42, wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

56. The ADC of any one of claims 1-42, wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

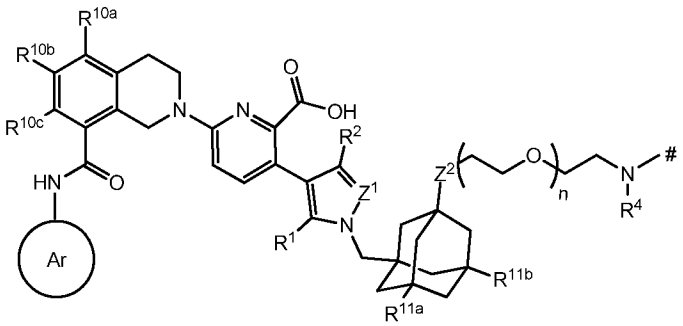
57. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 64, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65.

58. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

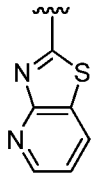
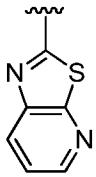
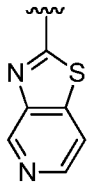
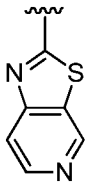
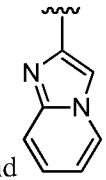
59. The ADC of any one of claims 1-42, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

60. An anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  ,  ,  ,  , and  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents a point of attachment to a linker; and

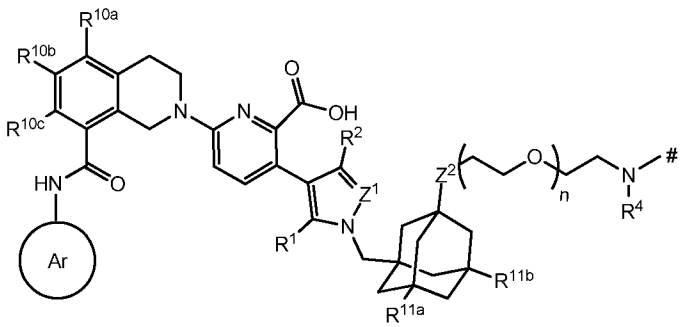
wherein the anti-hEGFR antibody is a monoclonal IgG antibody and comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light

chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6.

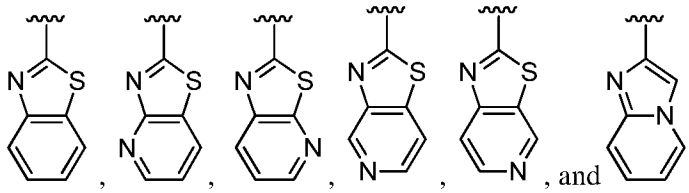
61. The ADC of claim 60, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

62. An anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

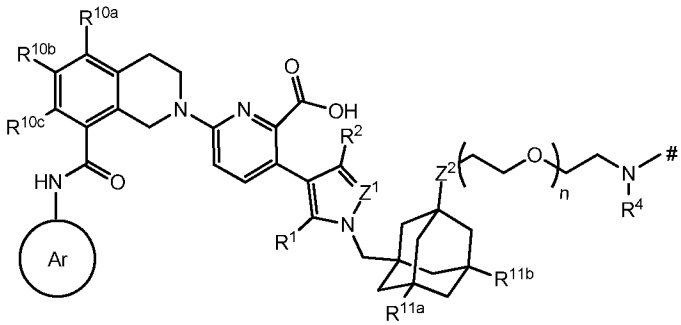
represents a point of attachment to a linker; and

wherein the antibody is a monoclonal IgG antibody and comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

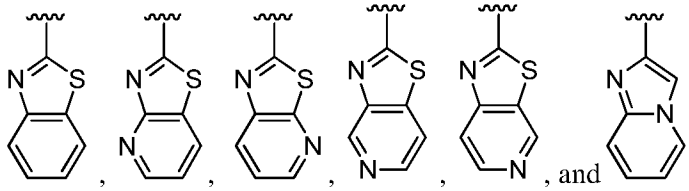
63. The ADC of claim 62, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

64. An anti-hEGFR ADC comprising a drug linked to an anti-hEGFR antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

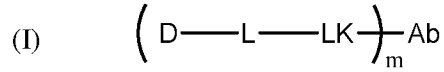
n is 0, 1, 2 or 3; and

represents a point of attachment to a linker; and

wherein the antibody is a monoclonal IgG antibody and comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

65. The ADC of claim 64, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

66. The ADC of any one of claims 60-65, which is a compound according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

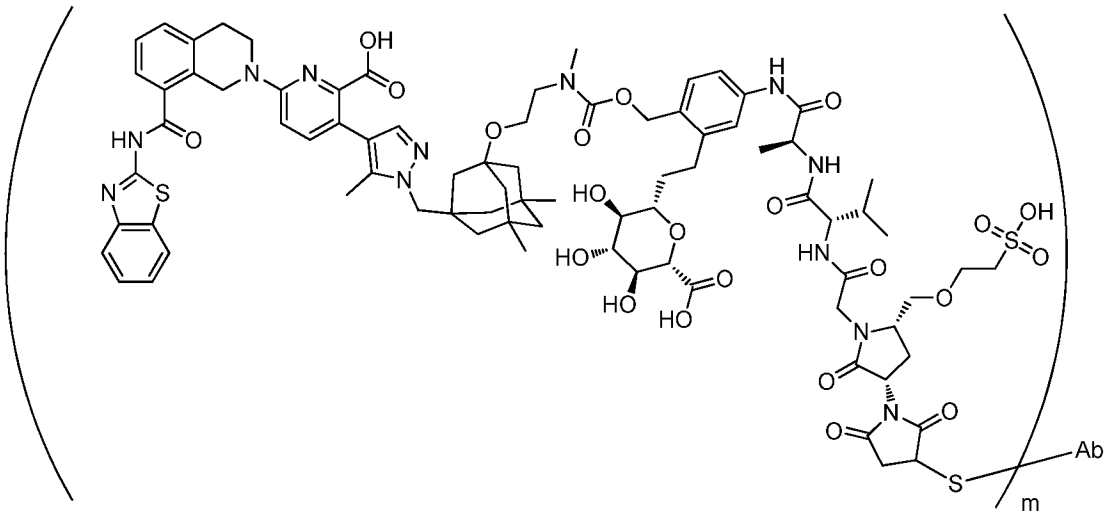
L is the linker;

Ab is the anti-hEGFR antibody;

LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and

m is an integer ranging from 1 to 20.

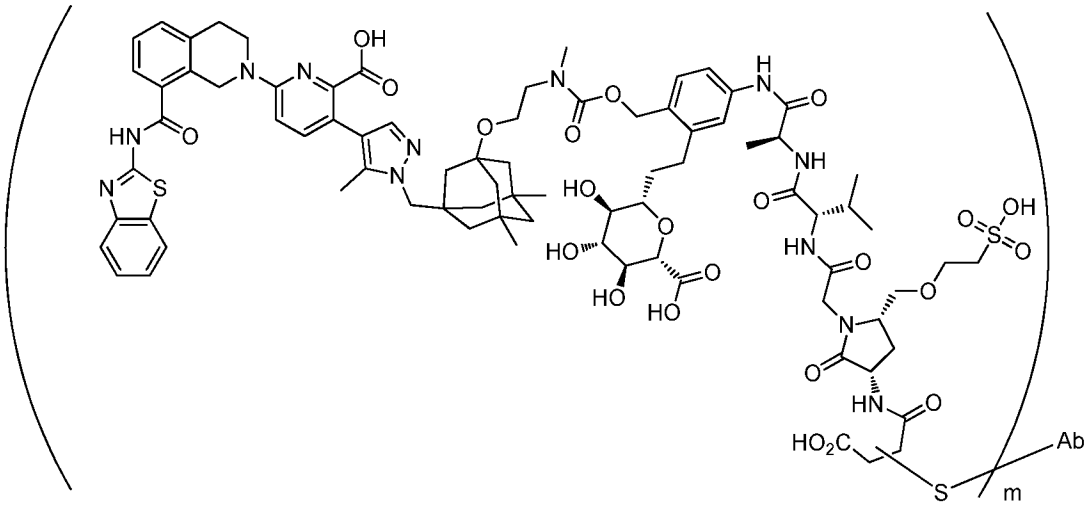
67. The ADC of claim 66, which is a compound according to structural formula (i)



(i),

wherein m is an integer from 1 to 6.

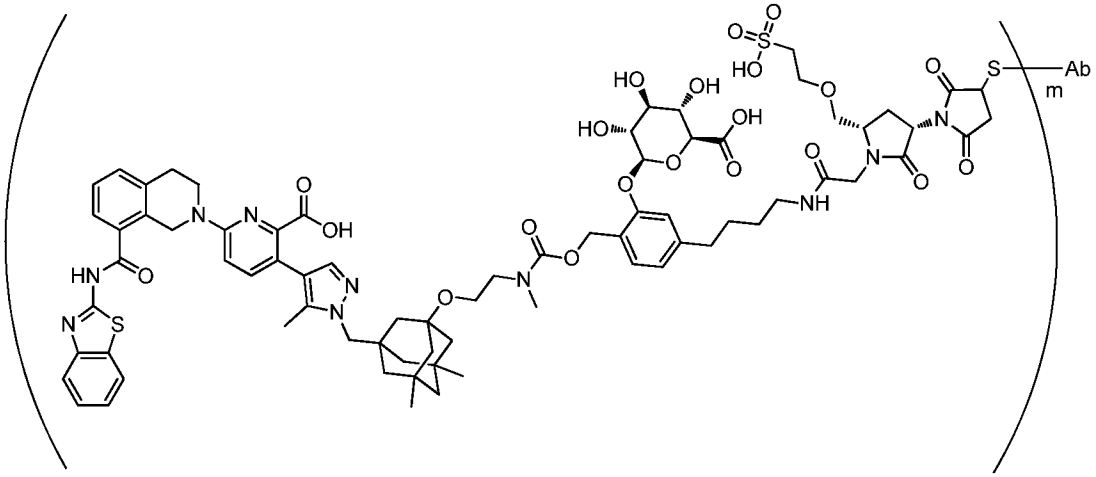
68. The ADC of claim 66, which is a compound according to structural formula (ii)



(ii),

wherein m is an integer from 1 to 6.

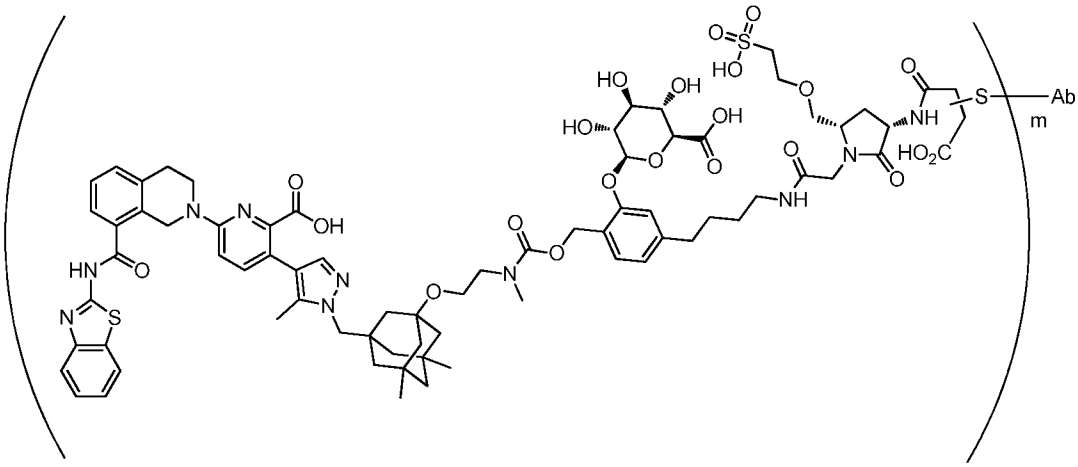
69. The ADC of claim 66, which is a compound according to structural formula (iii)



(iii)

wherein m is an integer from 1 to 6.

70. The ADC of claim 66, which is a compound according to structural formula (iv)



(iv),

wherein m is an integer from 1 to 6.

71. A pharmaceutical composition comprising an effective amount of an ADC according to any one of claims 1-70 or 103, and a pharmaceutically acceptable carrier.

72. A pharmaceutical composition comprising an ADC mixture comprising a plurality of the ADC of any one of claims 1-70 or 103, and a pharmaceutically acceptable carrier.

73. The pharmaceutical composition of claim 72, wherein the ADC mixture has an average drug to antibody ratio (DAR) of 2 to 4.

74. The pharmaceutical composition of claim 72, wherein the ADC mixture comprises ADCs each having a DAR of 2 to 8.

75. A method for treating cancer, comprising administering a therapeutically effective amount of an ADC of any one claims 1-70 or 103 to a subject in need thereof.

76. The method of claim 75, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, a glioblastoma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, and kidney cancer.

77. The method of claim 75, wherein the cancer is a squamous cell carcinoma.

78. The method of claim 77, wherein the squamous cell carcinoma is squamous lung cancer or squamous head and neck cancer.

79. The method of claim 75, wherein the cancer is triple negative breast cancer.

80. The method of claim 75, wherein the cancer is non-small cell lung cancer.

81. The method of any one of claims 76-80, wherein the cancer is characterized as having EGFR overexpression.

82. The method of any one of claims 76-80, wherein the cancer is characterized as having an activating EGFR mutation.

83. The method of claim 82, wherein the activating EGFR mutation is selected from the group consisting of an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

84. A method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering the ADC of any one of claims 1-70 or 103 to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased.

85. The method of claim 84, wherein the solid tumor is a non-small cell lung carcinoma or a glioblastoma.

86. The method of claim 84, wherein the solid tumor is a squamous cell carcinoma.

87. The method of any one of claims 84-86, wherein the solid tumor is an EGFRvIII positive solid tumor or is an EGFR-expressing solid tumor.

88. The method of any one of claims 84-86, wherein the solid tumor overexpresses EGFR.

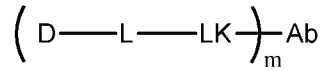
89. The method of any one of claims 75-88, wherein the ADC is administered in combination with an additional agent or an additional therapy.

90. The method of claim 89, wherein the additional agent is selected from the group consisting of an anti-PD1 antibody (e.g. pembrolizumab), an anti-PD-L1 antibody (atezolizumab), an anti-CTLA-4 antibody (e.g. ipilimumab), a MEK inhibitor (e.g. trametinib), an ERK inhibitor, a BRAF inhibitor (e.g. dabrafenib), osimertinib, erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g. dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-2 inhibitor (e.g. venetoclax), a Bcl-xL inhibitor, ibrutinib, a mTOR inhibitor (e.g. everolimus), a PI3K inhibitor (e.g. buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g. lapatinib), a taxane (e.g. docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g. rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g. TDM1), a TRAIL agonist, a proteasome inhibitor (e.g. bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor.

91. The method of claim 89, wherein the additional therapy is radiation.

92. The method of claim 89, wherein the additional agent is a chemotherapeutic agent.

93. A process for the preparation of an ADC according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

L is the linker;

Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA, AbB, AbG, and AbK;

LK represents a covalent linkage linking linker L to antibody Ab; and

m is an integer ranging from 1 to 20.

the process comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.63 (Table 5);

adjusting the pH of the solution to a pH of 7.5 to 8.5; and

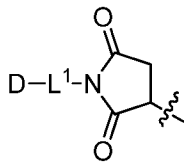
allowing the reaction to run for 48 to 80 hours to form the ADC;

wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

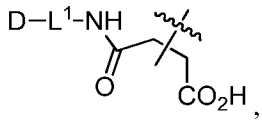
wherein the ADC is optionally purified by hydrophobic interaction chromatography.

94. The process of claim 93, wherein m is 2.

95. An ADC of any one of claims 1-70 or 103, formed by contacting an antibody that binds a hEGFR cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the synthon covalently links to the antibody through a maleimide moiety as shown in formulae (IId) and (IIe),



(IId)



(IIe),

wherein D is the Bcl-xL inhibitor drug of formula (IIa); and L^1 is the portion of the linker not formed from the maleimide upon attachment of the synthon to the antibody; and wherein the drug-linker synthon is selected from the list below:

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl) carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-

dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy) methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({(1s,3s)-3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[(2S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^{5}-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid;

1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,20-dioxo-7,10,13,16-tetraoxa-3,19-diazadocos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-methyl-10,26-dioxo-3,6,13,16,19,22-hexaoxa-9,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl](methyl)amino}ethoxy)ethoxy]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetratriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,26-dioxo-7,10,13,16,19,22-hexaoxa-3,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-

1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide;

(6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid;

(6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-

1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{4-[({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)amino]butyl}phenyl beta-D-glucopyranosiduronic acid;

3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium; and

(6S)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{[N-({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid.

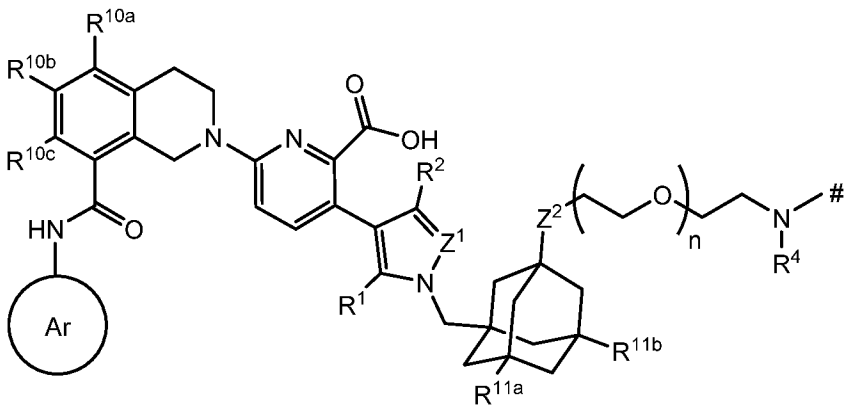
96. The ADC of claim 95 in which the contacting step is carried out under conditions such that the ADC has a DAR of 2, 3 or 4.

97. A synthon according to structural formula D-L^2-R^x, wherein:

D is the Bcl-xL inhibitor drug according to structural formula (IIa);

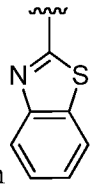
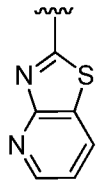
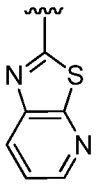
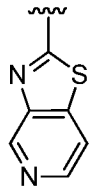
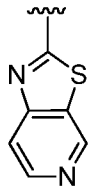
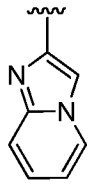
L^2 is the linker selected from the group consisting of IVa.8, IVb.16-IVb.19, IVc.3-IVc.6, IVd.1-IVd.4, Vb.5-Vb.10, Vc.11, Vd.3-Vd.6, VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8 and VIIc.1-VIIc.6; and

R^x is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,



(IIa)

wherein:

Ar is selected from  ,  ,  ,  ,  , and  , which is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents the point of attachment to linker L^2.

98. The synthon of claim 97, in which R^x comprises a maleimide, an acetyl halide, or a vinyl sulfone.

99. The synthon of claim 97, in which D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.$1^{3,7}$]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.$1^{3,7}$]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

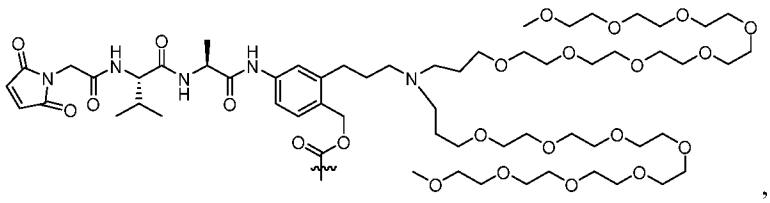
6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

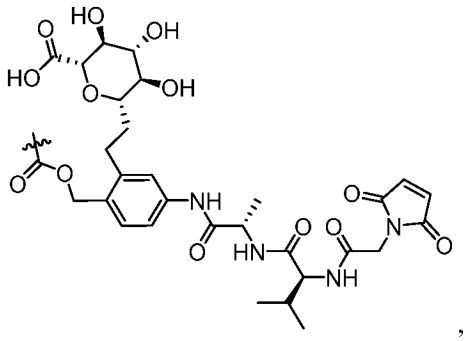
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

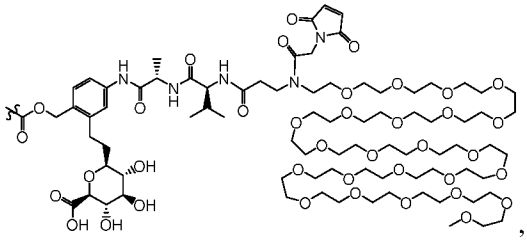
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

100. The synthon of claim 97, in which linker L^2 is selected from the group consisting of:

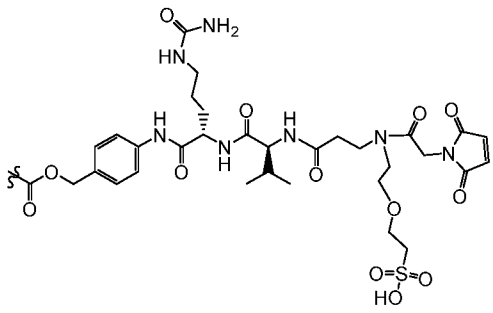
(IVc.5)  ,

(IVc.6)  ,

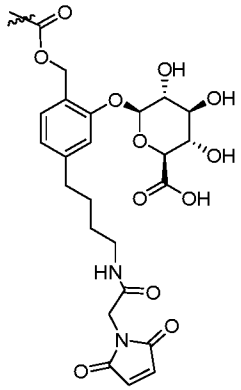
(IVd.3)



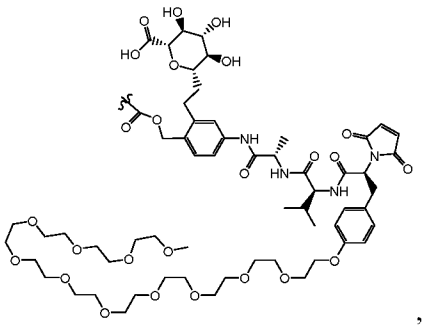
(IVd.4)



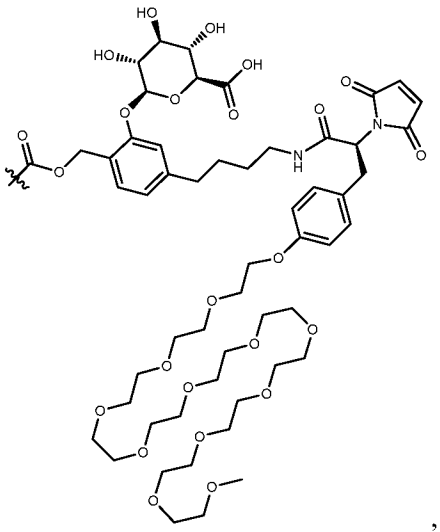
(Vb.9)



(VIIa.1)

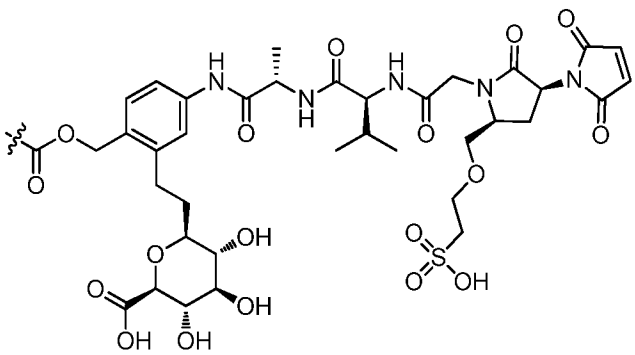


(VIIa.2)



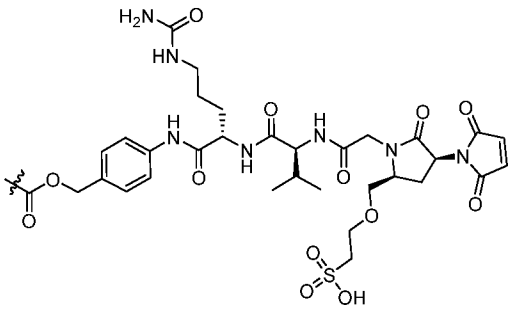
,

(VIIc.1)



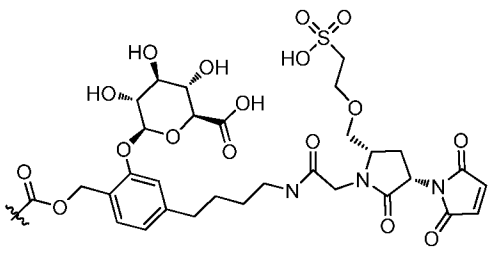
,

(VIIc.4)



, and

(VIIc.5)

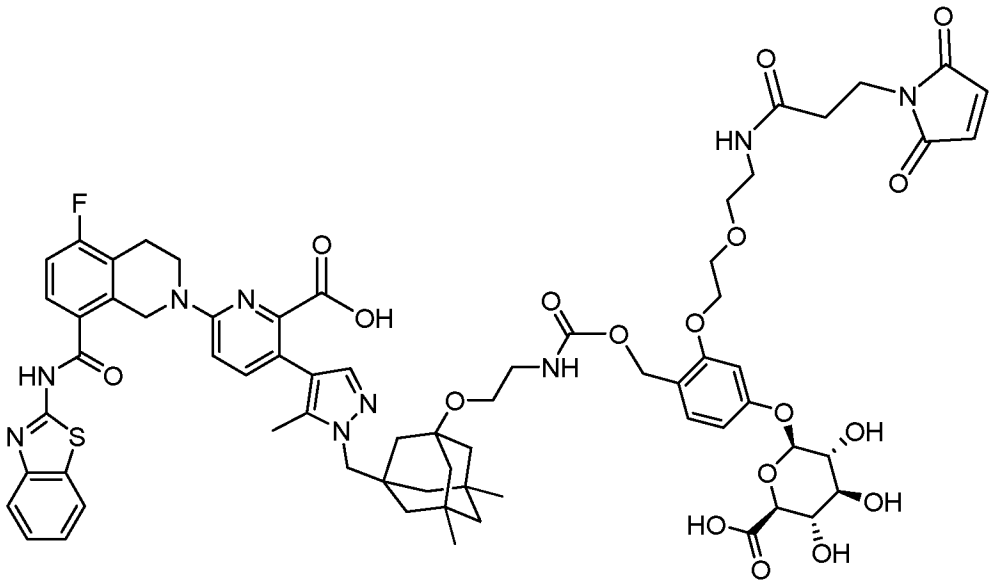


;

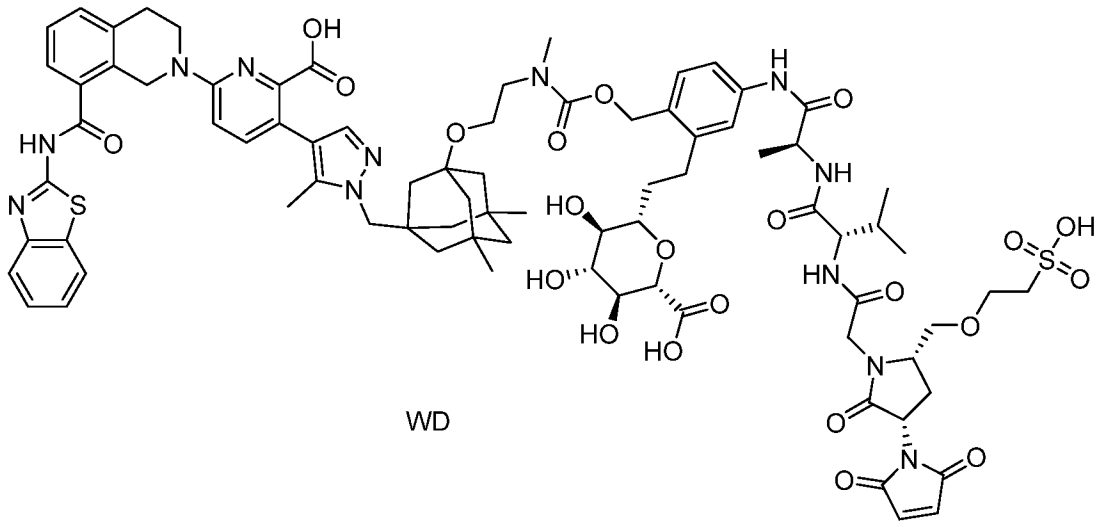
wherein, ~ represents the point of attachment of the linker L^2 to the Bcl-xL inhibitor.

101. The synthon of claim 97, selected from the group consisting of synthon examples 2.41 (LB), 2.54 (LX), 2.55 (MJ), 2.56 (NH), 2.57 (OV), 2.58 (QS), 2.59 (SG), 2.60 (UF), 2.61 (VD), 2.62 (VX), 2.63 (WD).

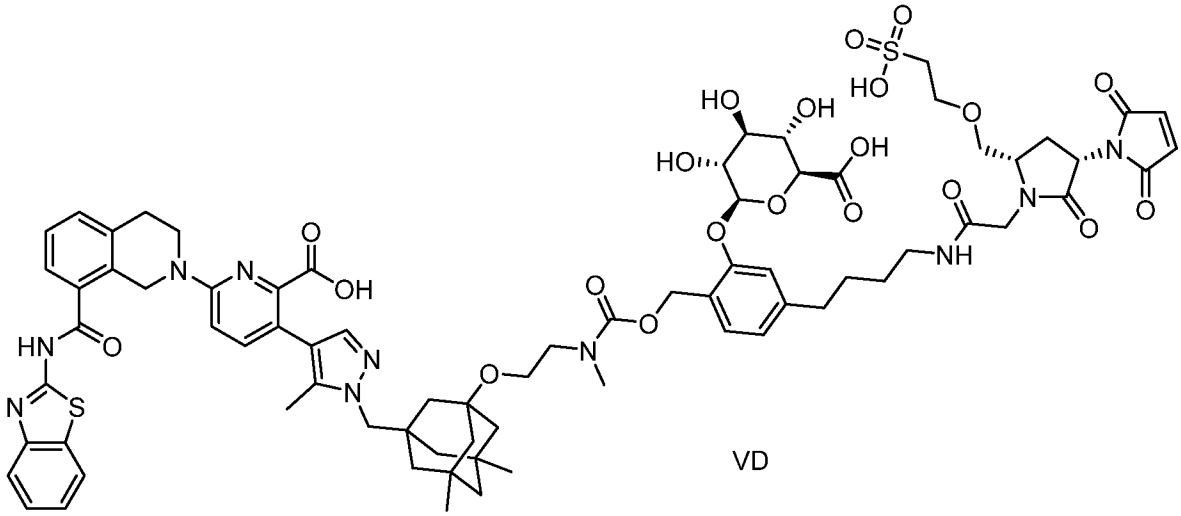
102. The synthon of claim 97, selected from the group consisting of synthon examples 2.42 (LB), 2.61 (VD) and 2.63 (WD).



LB



WD

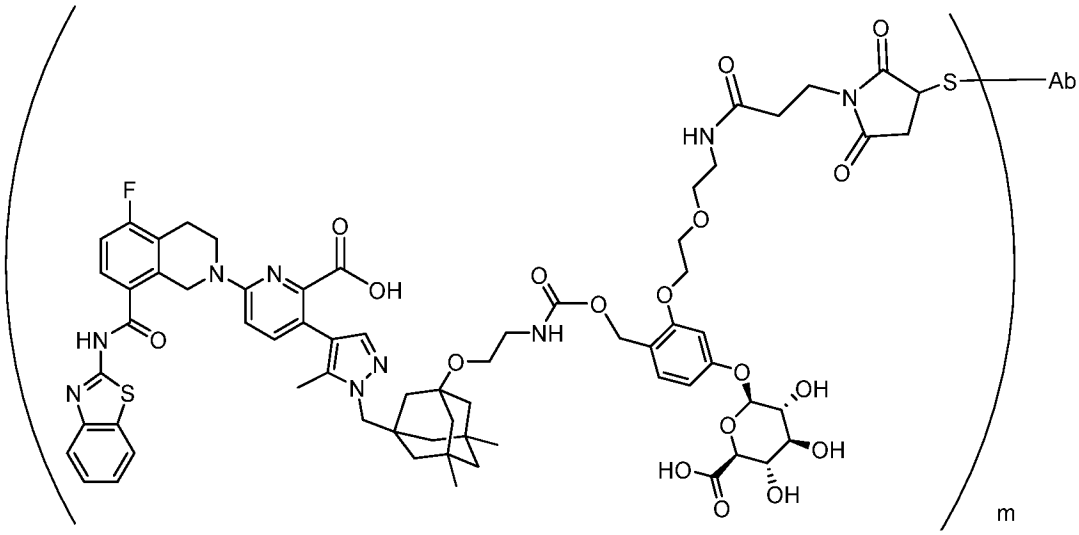


VD

103. The ADC of any one of claims 1-70, wherein the antibody is an IgG1 antibody having four polypeptide chains which are two heavy chains and two light chains.

104. An ADC prepared by the process of claim 93 or 94.

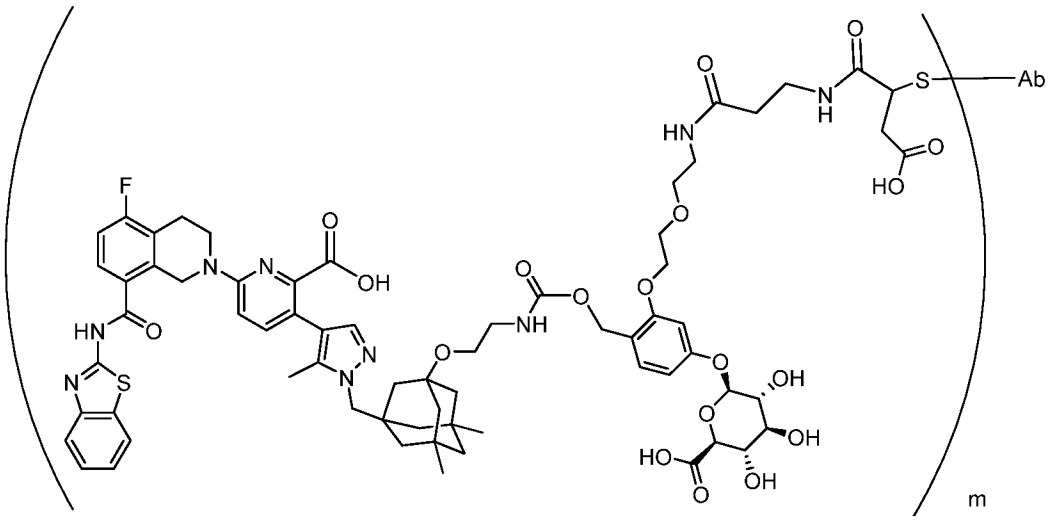
105. The ADC of claim 66, which is a compound according to structural formula (v)



(v)

wherein m is an integer from 1 to 6.

106. The ADC of claim 66, which is a compound according to structural formula (vi)



(vi)

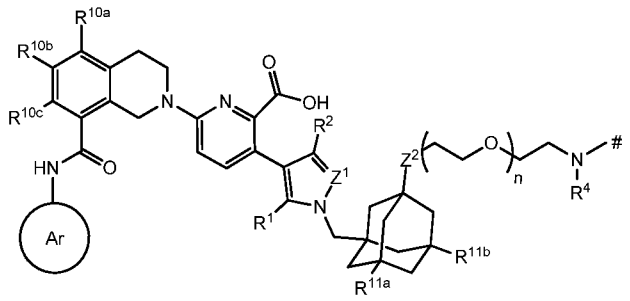
wherein m is an integer from 1 to 6.

AMENDED CLAIMS
received by the International Bureau on 6 November 2017 (06.11.2017)

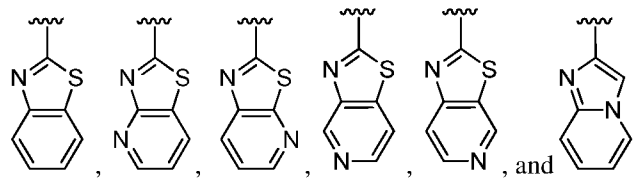
WHAT IS CLAIMED IS:

1. An anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising a drug linked to an anti-hEGFR antibody via a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa):

(IIa)



wherein:

Ar is selected from  , and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

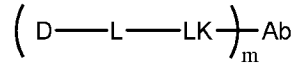
represents a point of attachment to a linker;

wherein the anti-hEGFR antibody has the following characteristics:

binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) or competes with a second anti-hEGFR antibody for binding to epidermal growth factor receptor variant III (EGFRvIII) (SEQ ID NO: 33) in a competitive binding assay, wherein the second anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5; and

binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K_d) of about 1 x 10^{-6} M or less, as determined by surface plasmon resonance.

2. The ADC of claim 1, which is a compound according to structural formula (I):

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

L is the linker;

Ab is the anti-hEGFR antibody;

LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and

m is an integer ranging from 1 to 20.

3. The ADC of claim 1 or 2, wherein the Bcl-xL inhibitor is selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

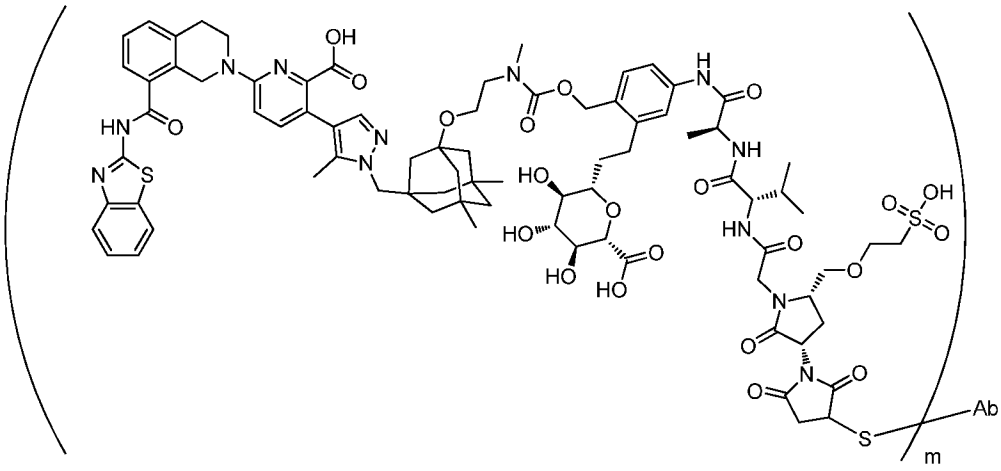
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

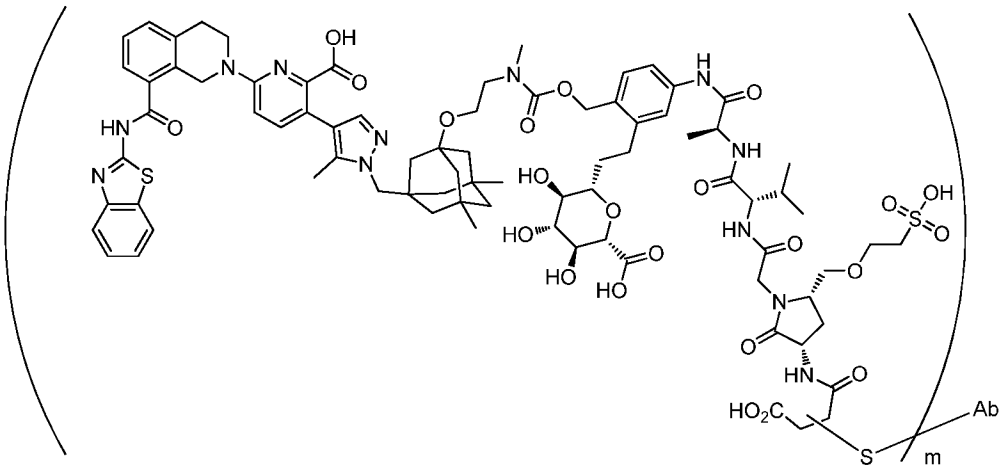
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

4. The ADC of claim 2, selected from the group consisting of AbA-WD, AbA-LB, AbA-VD, AbB-WD, AbB-LB, AbB-VD, AbG-WD, AbG-LB, AbG-VD, AbK-WD, AbK-LB, and AbK-VD, wherein WD, LB, and VD are synthons disclosed in Table 5, and where in the synthons are either in open or closed form.

5. The ADC of claim 2, selected from the group consisting of formulas i-vi:

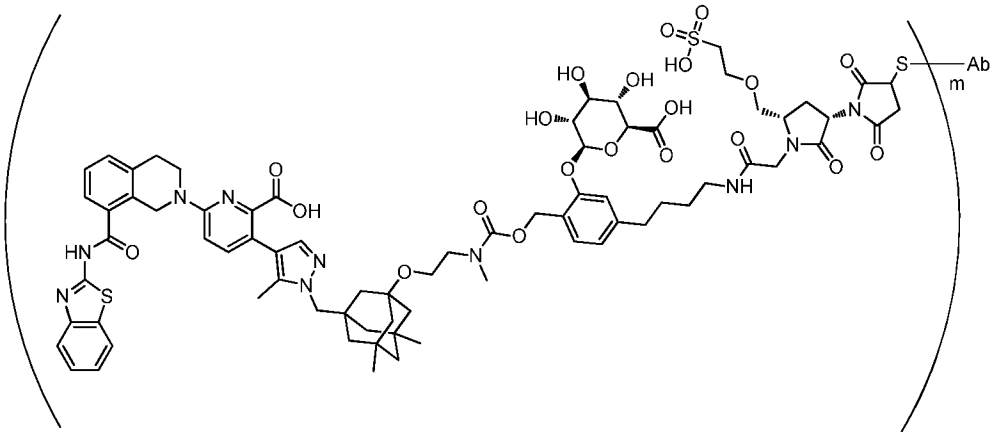


(i),

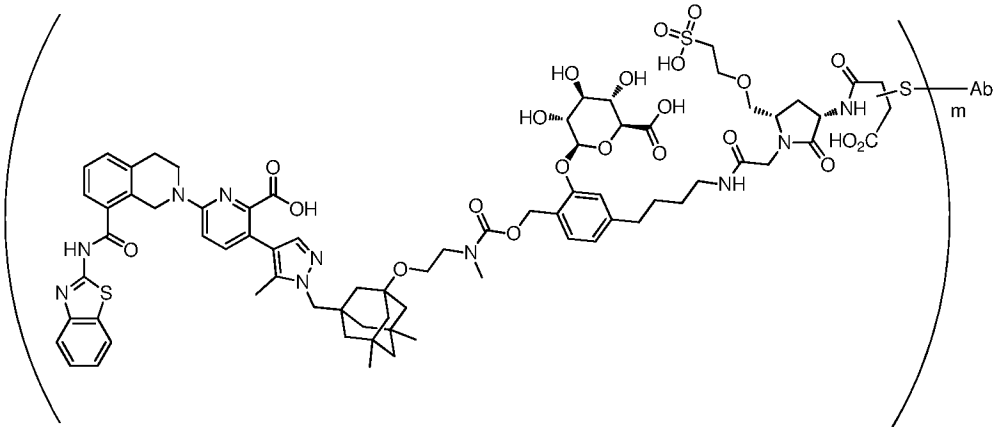


(ii),

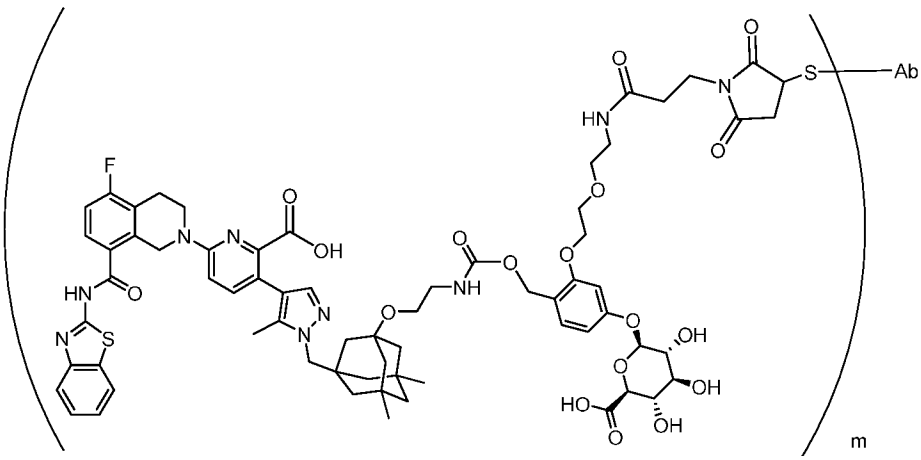
AMENDED SHEET (ARTICLE 19)



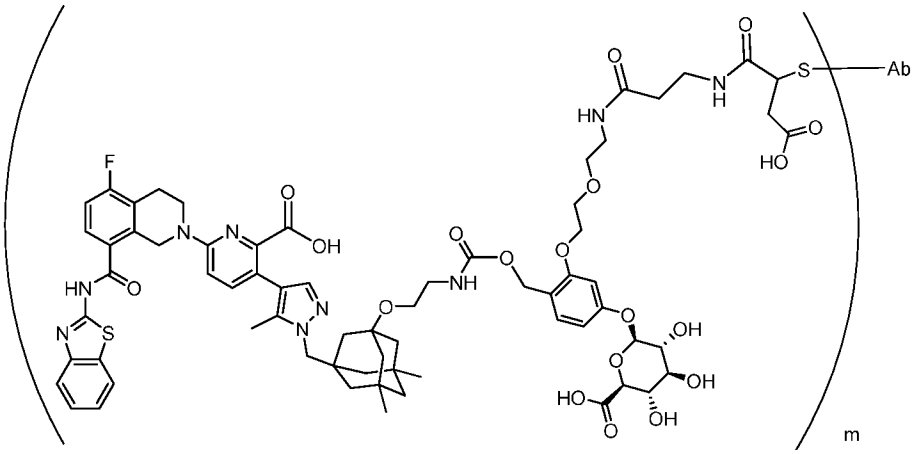
(iii),



(iv),



(v), and



(vi),

wherein m is an integer from 1 to 6.

5. The ADC of any one of claims 1-42, wherein the anti-hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; or

a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35; or

a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; or

a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; or

a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

7. The ADC of any one of claims 1-6, wherein the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

8. The ADC of any one of claims 1-6, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 9, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 5, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

9. The ADC of any one of claims 1-6, wherein the antibody comprises a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and

a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85,

wherein the antibody does not comprise both the heavy chain CDR set of SEQ ID NOs: 2, 3, and 4, and the light chain CDR set of SEQ ID NOs: 6, 7, and 8.

10. The anti-hEGFR ADC of claim 1, wherein the antibody is selected from the group consisting of

a monoclonal IgG antibody a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10;

a monoclonal IgG antibody comprising a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in

SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and

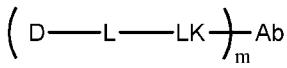
a monoclonal IgG antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6.

11. A pharmaceutical composition comprising an effective amount of an ADC according to any one of claims 1-10, or an ADC mixture comprising a plurality of the ADC of any one of claims 1-10, and a pharmaceutically acceptable carrier.

12. A method for treating cancer, comprising administering a therapeutically effective amount of an ADC of any one claims 1-10 to a subject in need thereof.

13. The method of claim 12, wherein the ADC is administered in combination with an additional agent or an additional therapy.

14. A process for the preparation of an ADC according to structural formula (I) according to claim 2:

(I) 

wherein:

D is the Bcl-xL inhibitor drug of formula (IIa);

L is the linker;

Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA, AbB, AbG, and AbK;

LK represents a covalent linkage linking linker L to antibody Ab; and

m is an integer ranging from 1 to 20.

the process comprising:

treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40 °C for at least 15 minutes, and then cooling the antibody solution to 20-27 °C;

adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.63 (Table 5);

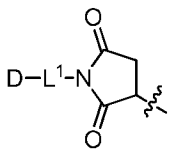
adjusting the pH of the solution to a pH of 7.5 to 8.5; and

allowing the reaction to run for 48 to 80 hours to form the ADC;

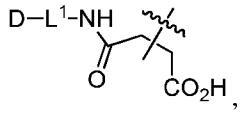
wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

15. An ADC of any one of claims 1-10, formed by contacting an antibody that binds a hEGFR cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the synthon covalently links to the antibody through a maleimide moiety as shown in formulae (IId) and (IIe),



(IId)



(IIe),

wherein D is the Bcl-xL inhibitor drug of formula (IIa); and L^1 is the portion of the linker not formed from the maleimide upon attachment of the synthon to the antibody; and wherein the drug-linker synthon is selected from the list below:

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl) carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy) methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({(1s,3s)-3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-

carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-L-valyl-N-{4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-alanyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

N-[(2R)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[(2S)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^5-carbamoyl-L-ornithinamide;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-{(1E)-3-[({2-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethoxy]ethyl}carbamoyl)oxy]prop-1-en-1-yl}-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[(1E)-14-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-6-methyl-5-oxo-4,9,12-trioxa-6-azatetradec-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[({[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)-4-(beta-D-galactopyranosyloxy)benzyl]oxy}carbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid;

1-O-({4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl}carbamoyl)-beta-D-glucopyranuronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[({3-[(N-{[2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-3-

sulfo-D-alanyl}amino)ethoxy]acetyl}-beta-alanyl)amino]-4-(beta-D-galactopyranosyloxy)benzyl}oxy)carbonyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[12-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)-4-methyl-3-oxo-2,7,10-trioxa-4-azadodec-1-yl]-2-{[N-({2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}acetyl)-beta-alanyl]amino}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-[(N-{6-[(ethenylsulfonyl)amino]hexanoyl}-beta-alanyl)amino]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[6-(ethenylsulfonyl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-

yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,20-dioxo-7,10,13,16-tetraoxa-3,19-diazadocos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-methyl-10,26-dioxo-3,6,13,16,19,22-hexaoxa-9,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl](methyl)amino}ethoxy)ethoxy]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-sulfobutanoyl](methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetratriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{[28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,26-dioxo-7,10,13,16,19,22-hexaoxa-3,25-diazaoctacos-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid;

N^2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N^6-(37-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-yl)-L-lysyl-L-alanyl-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid;

4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)prop-1-yn-1-yl]phenyl}-L-alaninamide;

(6S)-2,6-anhydro-6-({2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethynyl)-L-gulonic acid;

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-(3-sulfopropoxy)propyl]phenyl}-L-alaninamide;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(5-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}pentyl)phenyl beta-D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[16-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azahexadec-1-yl]phenyl beta-D-glucopyranosiduronic acid;

(6S)-2,6-anhydro-6-(2-{2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}propyl)phenyl D-glucopyranosiduronic acid;

2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{4-[({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)amino]butyl}phenyl beta-D-glucopyranosiduronic acid;

3-{(3-{4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(beta-D-glucopyranuronosyloxy)phenyl}propyl)[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-N,N,N-trimethylpropan-1-aminium; and

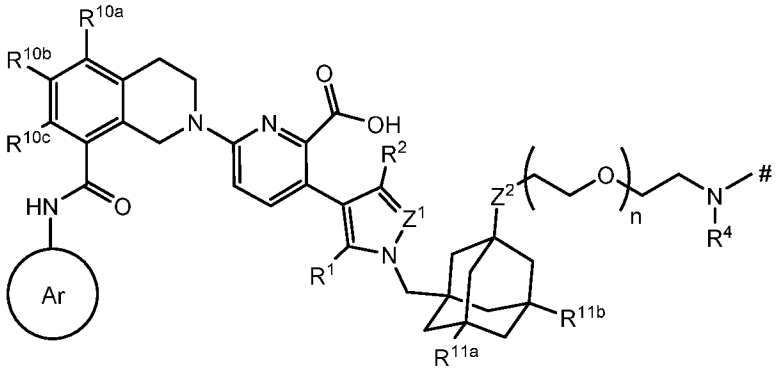
(6S)-2,6-anhydro-6-[2-(2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{[N-({(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid.

16. A synthon according to structural formula D-L^2-R^x, wherein:

D is the Bcl-xL inhibitor drug according to structural formula (IIa);

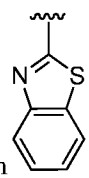
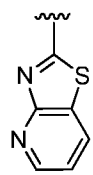
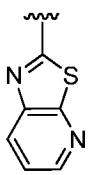
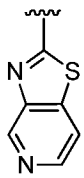
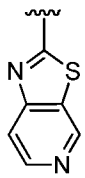
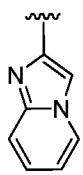
L^2 is the linker selected from the group consisting of IVa.8, IVb.16-IVb.19, IVc.3-IVc.6, IVd.1-IVd.4, Vb.5-Vb.10, Vc.11, Vd.3-Vd.6, VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8 and VIIc.1-VIIc.6; and

R^x is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,



(IIa)

wherein:

Ar is selected from  ,  ,  ,  ,  , and  , which is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl;

Z^1 is selected from N, CH and C-CN;

Z^2 is selected from NH, CH_2, O, S, S(O), and $S(O)_2$;

R^1 is selected from methyl, chloro, and cyano;

R^2 is selected from hydrogen, methyl, chloro, and cyano;

R^4 is hydrogen, C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl or C_{1-4} hydroxyalkyl, wherein the R^4 C_{1-4} alkanyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} haloalkyl and C_{1-4} hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH_3, $OCH_2CH_2OCH_3$, and $OCH_2CH_2NHCH_3$;

R^{10a}, R^{10b}, and R^{10c} are each, independently of one another, selected from hydrogen, halo, C_{1-6} alkanyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, methyl, ethyl, halomethyl, hydroxyl, methoxy, halo, CN and SCH_3;

n is 0, 1, 2 or 3; and

represents the point of attachment to linker L^2.

17. The synthon of claim 16, in which D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the # position of structural formula (IIa) is not present forming a monoradical:

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid;

6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[(1r,3R,5S,7s)-3,5-dimethyl-7-(2-{2-[2-(methylamino)ethoxy]ethoxy}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid;

3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

3-[1-({3-[2-(2-aminoethoxy)ethoxy]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

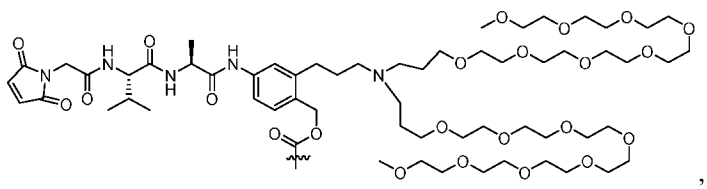
6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid;

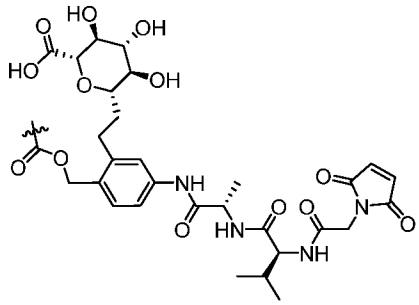
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid;

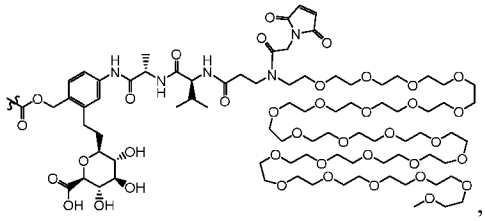
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and

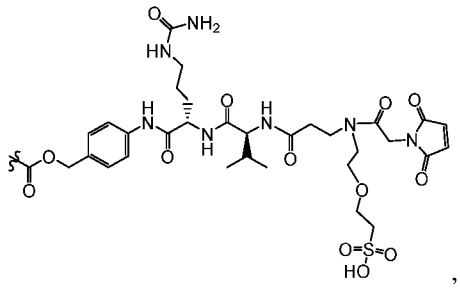
3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid.

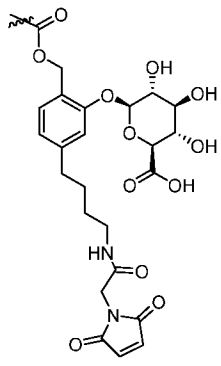
18. The synthon of claim 16, in which linker L^2 is selected from the group consisting of:

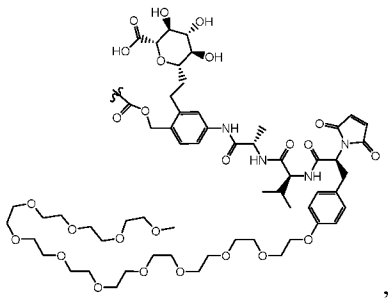
(IVc.5)  ,

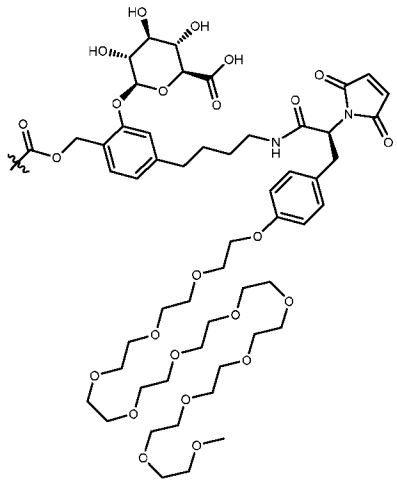
(IVc.6)  ,

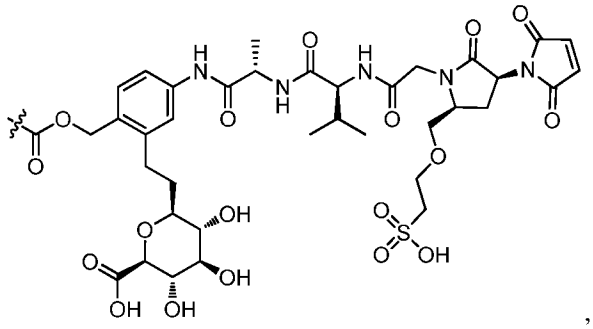
(IVd.3)  ,

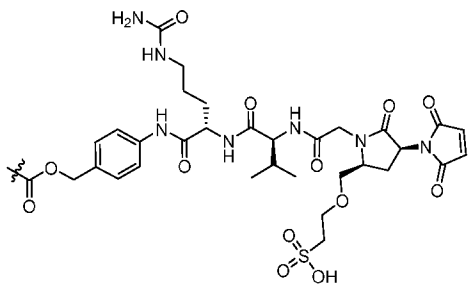
(IVd.4)  ,

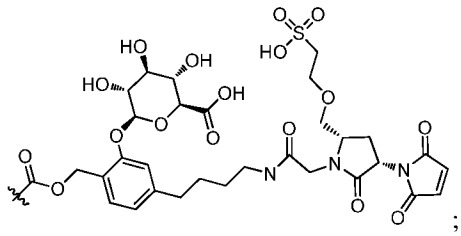
(Vb.9)  ,

(VIIa.1)  ,

(VIIa.2)  ,

(VIIc.1)  ,

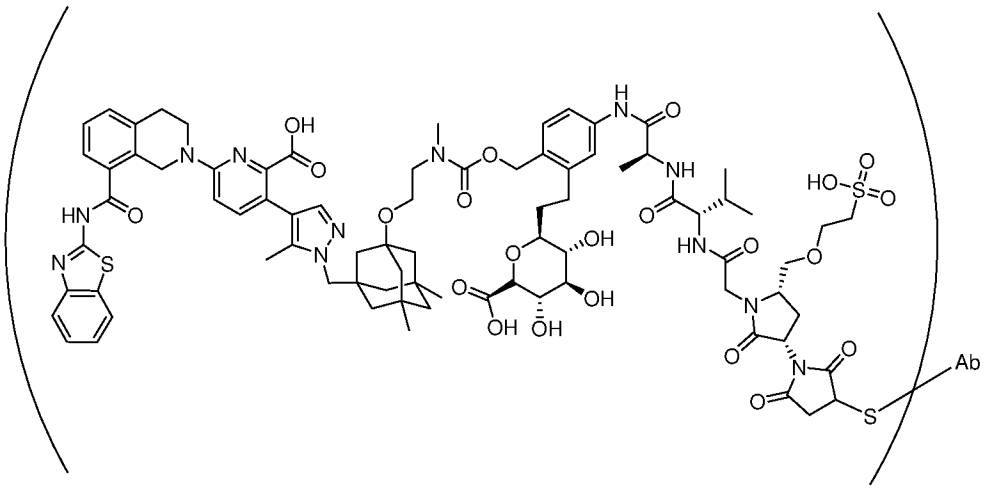
(VIIc.4)  , and

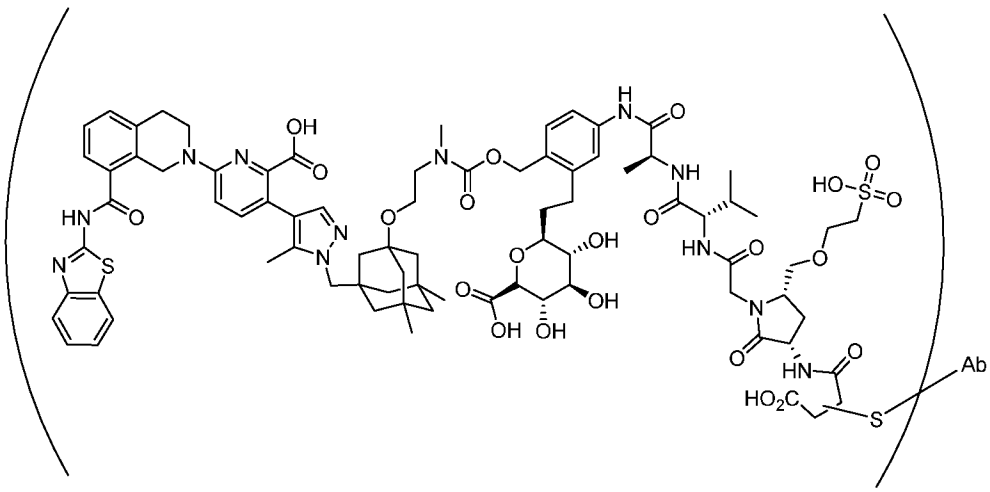
(VIIc.5)  ;

wherein, ~ represents the point of attachment of the linker L^2 to the Bcl-xL inhibitor.

19. An ADC prepared by the process of claim 14.

20. An anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC), wherein the ADC is selected from the group consisting of formulae (i) or (ii):


m (i), and


m (ii),

wherein m is an integer from 1 to 6, optionally from 2 to 6; and

wherein Ab is either

an anti-hEGFR antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a comprising light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; or

an anti-hEGFR antibody comprising a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

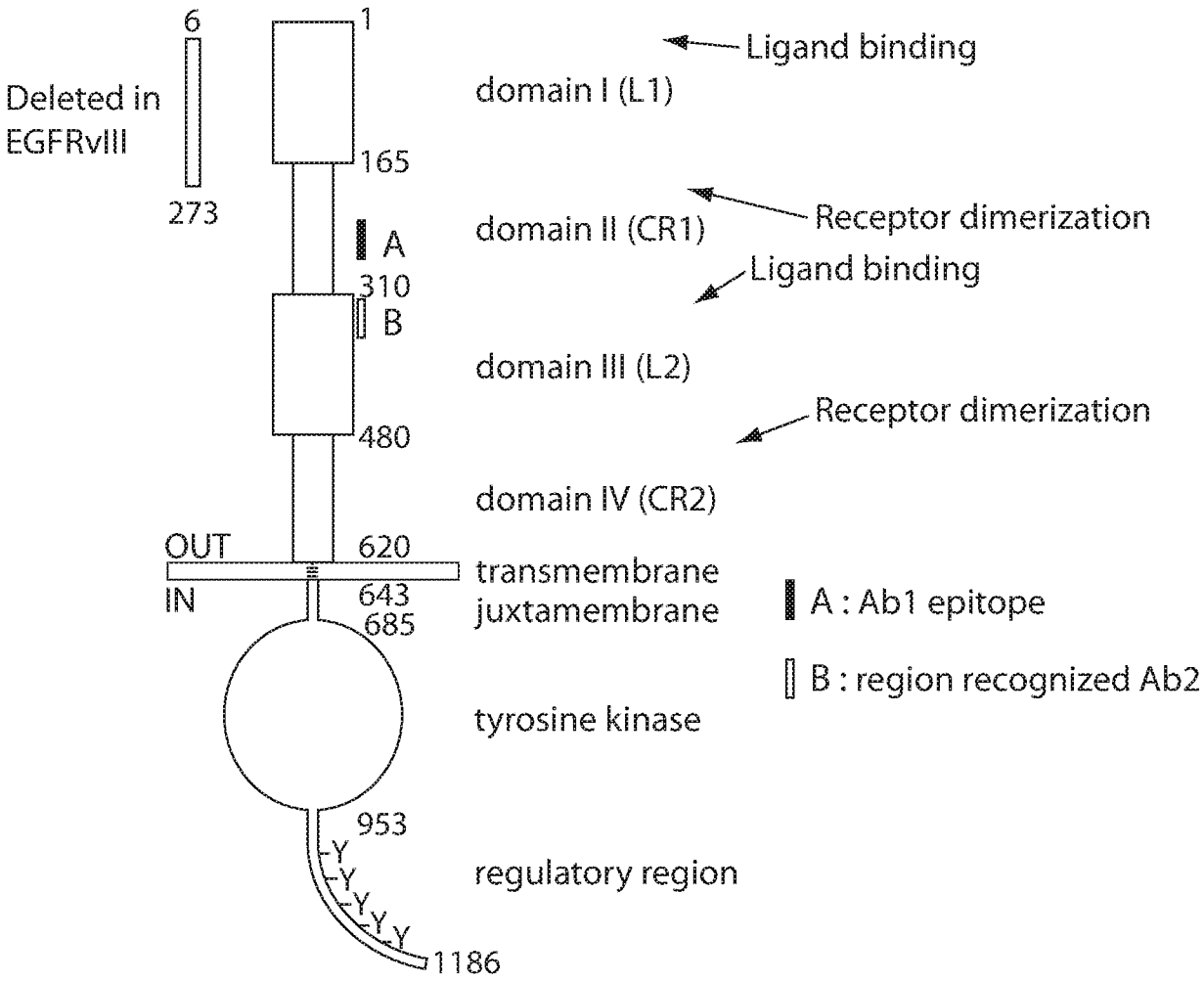

6
1
Deleted in
EGFRvIII
Ligand binding
domain I (L1)
165
273
domain II (CR1)
Receptor dimerization
A
Ligand binding
310
B
domain III (L2)
Receptor dimerization
480
domain IV (CR2)
OUT
620
transmembrane
IN
643
juxtamembrane
685
A : Ab1 epitope
B : region recognized Ab2
tyrosine kinase
953
Y
Y
Y
Y
Y
regulatory region
1186

Figure 1

VH domains:

Ab1 QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTPAGRGFPYWGQGTLVTVSS

AbA EVQLQESGPGLVKPSQTLSLTCTVSGYSISRDFAWNWIRQPPGKGLEWMGYISYNGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTPASRGFPYWGQGTLVTVSS

CDR1 CDR2 CDR3

VL domains: (Ab1 and AbA have the same VL sequence)

Ab1 DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLEIK

AbA DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLEIK

CDR1 CDR2 CDR3

Figure 2

LC:
the same

DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPSRFSGSGSGT
DYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC SEQ ID NO: 13

HC:
6 AA change

Ab1 QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYISYSGNTRYQPSLKSRITI
AbA EVQLQESGPGLVKPSQTLSLTCTVSGYSISRDFAWNWIRQPPGKGLEWMGYISYNGNTRYQPSLKSRITI

Ab1 SRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
AbA SRDTSKNQFFLKLNSVTAADTATYYCVTASRGFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA

Ab1 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
AbA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK

Ab1 VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
AbA VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD

Ab1 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
AbA GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT

Ab1 LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
AbA LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ

Ab1 GNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 14
AbA GNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 15

Figure 3

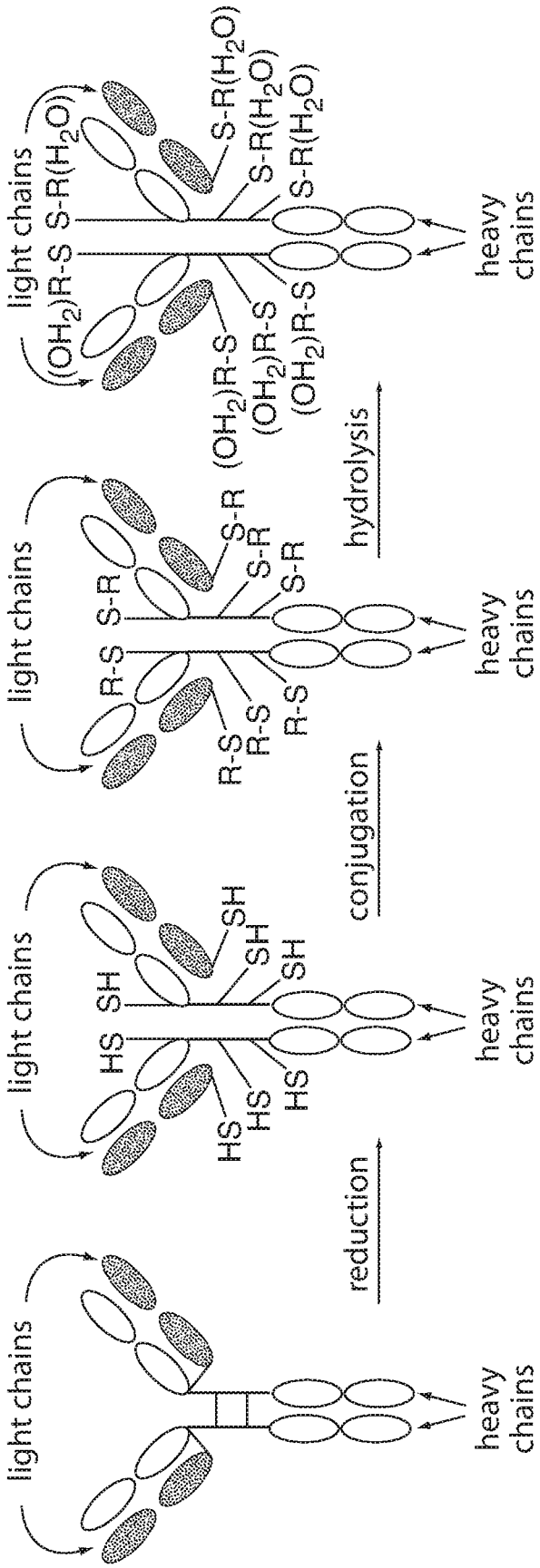

light chains
heavy chains
reduction
HS
SH
conjugation
R-S
S-R
hydrolysis
(OH2)R-S
S-R(H2O)

Figure 4

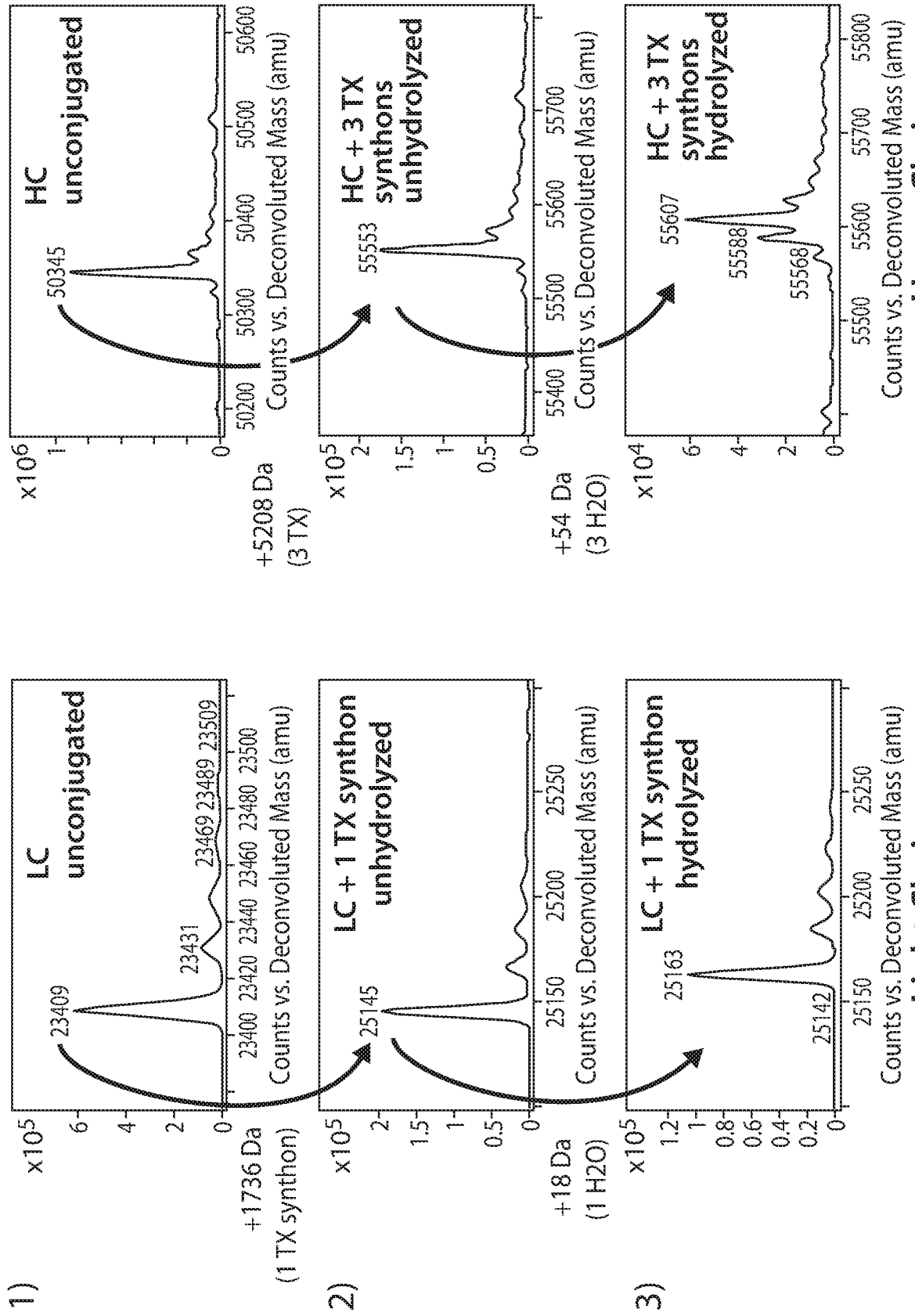

1)
LC
unconjugated
23409
23431
23469 23489 23509
23400 23420 23440 23460 23480 23500
Counts vs. Deconvoluted Mass (amu)
+1736 Da
(1 TX synthon)
2)
LC + 1 TX synthon
unhydrolyzed
25145
25150 25200 25250
Counts vs. Deconvoluted Mass (amu)
+18 Da
(1 H2O)
3)
LC + 1 TX synthon
hydrolyzed
25163
25142
25150 25200 25250
Counts vs. Deconvoluted Mass (amu)
Light Chain
HC
unconjugated
50345
50200 50300 50400 50500 50600
Counts vs. Deconvoluted Mass (amu)
+5208 Da
(3 TX)
HC + 3 TX
synthons
unhydrolyzed
55553
55400 55500 55600 55700
Counts vs. Deconvoluted Mass (amu)
+54 Da
(3 H2O)
HC + 3 TX
synthons
hydrolyzed
55607
55588
55568
55500 55600 55700 55800
Counts vs. Deconvoluted Mass (amu)
Heavy Chain

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/036288

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/68 A61P35/00
ADD. C07K16/28

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 C07K

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, BIOSIS, EMBASE, WPI Data, CHEM ABS 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 2013/055897 A1 (ABBVIE INC [US]) 18 April 2013 (2013-04-18) claims 1-16; table 2	1-106
A	WO 2015/143382 A1 (ABBVIE INC [US]) 24 September 2015 (2015-09-24) claims 25-49,57-89; figures 1,2; examples 7,9,11	1-106
X,P	WO 2016/094505 A1 (ABBVIE INC [US]) 16 June 2016 (2016-06-16) claims 1-40; examples 7,9,10	1-106
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2017	07/09/2017

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	Bliem, Barbara

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/036288

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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