

(19)
(12)

(KR)
(A)

(51) 。 Int. Cl.7
A61K 39/395

(11)
(43)

2003-0097604
2003 12 31

(21) 10-2002-7011377 ()
(22) 2002 08 30
(62) 1994-0700829
: 1994 03 12
2002 08 30

1998 07 13

(86) PCT/US1993/06599
(86) 1993 07 13

(87)
(87)

WO 1994/01131
1994 01 20

(30) 07/912,453 1992 07 13 (US)

(71) ,
01730

(72) -
02174 67

(74)

:

(54) ,

1

1

2 (1)

(2

2)

, (2)

C_H, V_H, C_L / V_L

3 HIV-1

-Tat

(, -) -Tat

4

(

-Tat
가

,

-gp120
-Tat

,

rsCD4 가 HLCD4-CAT
CAT

(75%)

CAT

1970

1

가

가

/

(effector)

가

가

가

(,)

, HIV

(life cycle)

가

가

가

가

()

[Connor and Huang(1985) *J. Cell Biol.* 101 : 582; Huang, L. (1985) *Biochemistry* 24 :29; Babbitt et al. (1984) *Biochemistry* 23 : 3920; Connor et al. (1984) *Proc. Natl. Acad. Sci. (U.S.A.)* 81 : 1715; Huang et al. (1983) *J. Biol. Chem.* 258 : 14034; Shen et al. (1982) *Biochim. Biophys. Acta* 689 : 31; Huang et al. (1982) *Biochim. Biophys. Acta* 716 : 140; Huang et al. (1981) *J. Immunol. Methods* 46 : 141; and Huang et al. (1980) *J. Biol. Chem.* 255 : 8015].

N-

가

, 가

가 ,
가 (EP 0 329 185),
(U.S .S.N. 07/693,872).
(
90%).

1
2 3

가 /

(WO 91/17242)
(WO 91/16024)

가

DNA , DNA가
가
(Behr et al., (1989) *Proc. Natl. Acad. Sci. USA* 86 : 6982), DNA

Rose et al., (1991) *BioTechniques* 10 : 520).

(

1
(trans cytosin)

(U.S. 4,902,505)

가

1

2 , 3 / 4

가 (,)

가 가
(, Fos, Jun, AP-1, OCT -1, NF - AT)
()

1 (,)

Arg, Lys, Cys, Asp, Glu (mimetic) (Fauchere, J.(1986) *Adv. Drug Res.* 15 : 29; Veber and Freidinger(1985) *TINS* p. 392; Evans et al. (1987) *J. Med. Chem* 30 : 1229,

H₂-CH₂-, -CH=CH-(), -COCH₂-, -CH(OH)CH₂-, -CH₂SO- (Spatola, A. F. in 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins,' B. Weinstein, eds., Marcel Dekker, New York, p. 267(1983); Spatola, A. F., Vega Date(March 1983), Vol. 1, Issue 3, 'Peptide Backbone Modifications' (general review); Morley, J. S., *Trends Pharm Sci* (1980) pp. 463-468(general review); Hudson, D. et al., *Int J Pept Prot Res* (1979) 14 : 177-185(-CH₂ NH-, -CH₂ CH₂ -); Spatola, A.F. et al., *Life Sci* (1986) 38 : 1243-1249(-CH₂ -S); Hann, M.M., *J Chem Soc Perkin Trans I* (1982) 307-314 (-CH-CH, cis and trans); Almquist, R. G. et al., *J Med Chem* (1980) 23 : 1392-1398(-COCH₂ -); Jennings-White, C. et al., *Tetrahedron Lett* (1982) 23 : 2533 (-COCH₂ -); Szelke, M. et al., European Appln. EP 45665(1982) CA: 97 :39 405(1982) (-CH(OH)CH₂ -); Holladay, M. W. et al., *Tetrahedron Lett* (1983) 24 : 4401-4404 (-C(OH)CH₂ -); and Hruby, V. J., *Life Sci* (1982) 31 :189-199(-CH₂ -S-);

-CH₂ NH- (), 가 () 1 가 () () ()

(HIV-1 Tat), (c-fos, c-src, c-myc, c-lck(p56), c-fyn(p59), c-abl), (IL-2, PDGF, EGF, NGF, GH, TNF)

G / G (GAPs), NF-AT, c-ras p21, c-her-2, c-raf, (radiocontrast agent)

(c-ras^H p21, rasGAP) HIV-1 Tat () ()

(c-src, c-abl)

가

unology - A Synthesis , 20 , E.S. Golub D.R. Gren, (Imm (1991),).

'GTATA'

'TATAC'

'TATAC'

70% 가

50%

25%

6

18

(object)

(sugar residues)

N- -Asn-X-Ser- -Asn-X-Thr- X -(Thr Ser) - X-X-Pro- -Ser-Gly-X-Gly- X 'N- 'O- 'N- 'O-

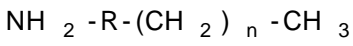
(Proteins, Structures and Molecular Principles , (1984) Creighton(ed.), W.H. Freeman and Company,).

가 , / O- 가 , 1 N- 1

(上科) 1 (The Immunoglobulin Gene Superfamily , A.F. Williams A.N. Barclay, in Immunoglobulin Genes, T. Honjo, F.W. Alt, T.H. Rabbitts, eds., (1989) Academic Press: , pp. 361 387,).

(IgG 1 , IgG 2 , IgG 3 , IgG 4), 가 (full-length)

(, -) 1
 (, ,) , ,
 , , ;
 (,) . , (,)
) , (,) (,)
 (, /) , ,
 1 , 1
 , (oligosaccharide)
 (, N- O-)
 , N- O- O- , 1
 N- () 3 (-N-)
 (NAG), , (6-), N- (), N-
 , N- , ,
 1 1 (, 1 2) 3 1
 . 1 1 : ,



R (), (-CH₂-); 1,4- ();
 1,4- (); -(CHR₁)-CO-NH- (, R₁);
 -O-PO₂-O- ; -CH₂
 n 1 50 , 5 30 , 10 25
 , 가 15 20 , n
 : 8 12 가 (, 10 KD), n 가
 , 가 , n (,), n 2 18
 , n

(,) , /

(,) , *Mol. Immunol.* 22 : 407 (1985).

(C) , IgG C_H2

297 IgG (, *J. Immunol.* 143 : 2595(1989)). IgM C_H3 402 (, *J. Immunol.* 142 : 695(1989)). IgA C_H1 C_H3 162 419 (, *Mol. Cell. Biol.* 8 : 4197(1988)).

가 (V) (*Proc. Natl. Acad. Sci. USA* 66 : 975(1970)) 20% 가 V Asn-Xaa-Ser/Thr 가 (V) 가

가 , 가

, 1 (,) (,) .

1 , Fab . 1 V_L CH₁ V_H DNA (Fab')₂ (,) .

(, V, J, D C) 가 (cDNA, RNA, 가 , cDNA (, 0239400 , *Nature*, 332, 323-327 (1988)).

/ DNA ((1976) *Eur. J. Immunol.* 6 : 511, (' ATCC ' , ATCC , ;) . DNA (, , *Molecular Cloning : A Laboratory Manual*, 2 (1989), *Methods in Enzymology*, Vol 152 , *Guide to Molecular Cloning Techniques* (1987), , ,) .

), DNA (, 가) , , DNA (, 가) . DNA G418- DNA (, , 4,704,362) .

(*E. coli*) DNA [, *Bacillus subtilis* ()] [, *Salmonella* ()], *Serratia* () *Pseudomonas* ()] [, (origin)] 가 (trp) , - , .

(,)
 (,).
 (*Saccharomyces*)
 3-
 ('From Genes to Clones', VCH (1987)).
 , HeLa , B- CHO , COS
 , 49-68(1986)], , RNA [, *Immunol. Rev.* , 89
 , SV40,
 DNA (,)
 (electroporation)
 (1982)). *Molecular Cloning : A Laboratory Manual*,
 ('Protein Purification', (1982)).
 가 가 90 95% , 98 99% ()
 가 가 (*Immunological Methods* ,
 Vols. I II, (1979, 1981)).
 Fab
 IgM IgG 가 . I
 gA, IgG, IgM, IgE, IgD , , , , ,
 가 , , , , , Fv ,
 (, Tc⁹⁹ , -)
 (, 2 50)
 가 (, N-) ,
 (, Asp , 1 Glu (,))
 Arg Lys Cys
 c, NF-AT, HMG CoA 가 , c-fos, c-myc, c-sr
 , , , 0.4% , 0.3%
 pH , , ,
 0.5 % , 1 % 15

% 20 %

1ml

1 10mg

250ml

150mg

[Remington's Pharmaceutical Science (15

(1980)]

gM IgG

(,)

1 200mg

, 5 25mg

가

0.1 25mg

(2)

),

(, -CEA)

가

c-myc

, 12 Ser p21^{ras})

(,)
NF-AT

12, 13, 59

61

(

c-ras
T-

Fundamental

Immunology, 2 , W.E.

HIV-1 Tat

N. , (1990),

(,)).

(,

Virology , 2 , B.

Tris,

.001 %

5 %

1 99 %

가

2 가

2 . 2 ,
 , 2 .
 (, 가 ,), (, -
) / (, -
 ; , -) , / 가 (, , -
) ,) , 가 (, 10 /
) - 가 , 2
 . 2 FITC, , 2
 , - / , , 2
) 2 , 1 A 2 . 1(
) - , (, , G-) (,
 (, GTPase),
 () , Jun AP-1 (Fos/Jun
) Fos -Fos AP-1 AP-
 1 ras (, GTP/GDP- ras) ,
 ras , GAP ras) ,

[]
 1

IgG

[Behr et al. (1989) *Proc. Natl. Acad. Sci. (U.S.A)* 86 : 6982]

- -p- 1 1.1 CH₂Cl₂ 5 , H₂,
 CH₂Cl₂ /EtOH 10% Pd/C 가 1 .
 IgG () 2 mg 1.5 ml 300 mM NaHCO₃ 400 μl
 NaIO₄ (H₂O 42 mg/ml) 50 μl 가 90
 (1), 500 μl 10 mM Na₂CO₃ PD-10 ()
 IgG 1.6 mg 7 (3 ml 3.5 ml)
 DMSO (1 ml DMSO 5 mg
) 50 μl ()
) 350 μl 7() 가
 20 .
 , NaBH₄ (1 ml H₂O 10 mg) 100 μl 가 . 1 (1 ml H₂O 15
 μl) 40 μl 가 1 100 mM HEPES (pH 8.5) PD-
 10 IgG (3 3.5 ml)

14 C-

(10×10⁶ cpm/μl) ¹⁴C- IgG (500 μCi,) .5 μl ,
 IgG (100 mM HEPES 500 μl 10 2 가 .
 800 μg, pH 8.5) 500 μl .
 30 , 20 25 가 , PBS PD-10 ¹⁴C-
¹⁴C- IgG 500 μg 10×10⁶ cpm .
 (20 g) . PBS ¹⁴C- IgG ¹⁴C- I
 400,000 dpm) , () , 30 ¹⁴C- 3
 , EDTA- , () , , 500 μl
 pH 7.4 10 mM , 1 ml ()
 20 μl . 1 , , ¹⁴C
 1 μl 1 μl (μl/μg) .

[1]

	30		3	
	.20 ± .30	.32 ± .08	.94 ± .05	1.75 ± .09
	1.46 ± .31	9.50 ± 2.19	1.17 ± .04	3.54 ± .22
	1.26 ± .26	4.95 ± .82	.66 ± .04	7.59 ± .27
	1.08 ± .27	4.54 ± .76	1.13 ± .03	12.0 ± 2.15

30 3 4 6 IgG
 6
 ± s.e.m.
 2
 -Tat HIV-1
 HIV-1 Tat , PD-10 PBS
 1
 Sup T1 24- (2 ml RPMI 1640 1 ml 100,000)
 5 (1) 가 () , (2) -Tat (15 μg/ml) 가 ,
 (3) -Tat (11.7 μg/ml) 가 , .1 , 1
 -Tat - -Tat - HIV-1 IIIB 가
 HIV- 1 ml 500,000 HIV-1 IIIIB 가
 8 1 ml 1,000 ,
 0,000 -Tat -Tat HIV-1
 5
 2

Sup T1, HIV-1 III B, -Tat (1 µg/ml), -Ta
 t (1 µg/ml) 3가 1 7 가 2
 가 2 -Tat 가 HIV-1

[2]

HIV-1 Sup T1 -Tat							
	(×10 ⁶)						
	1	2	3	5	6	7	8
,	1.15	1.28	1.52	1.63	1.69	1.76	1.72
,	1.12	1.2	1.18	1.12	0.92	0.64	0.51
-Tat,	1.12	1.2	1.21	1.15	0.96	0.67	0.51
-Tat,	1.15	1.26	1.3	1.36	1.17	0.99	0.75
	(cmp/0 ⁹)						
	1	2	3	5	6	7	8
,	2	2	1	2	2	1	1
,	3	2	4	175	264	337	367
-Tat,	2	2	3	151	237	259	331
-Tat,	3	2	2	57	135	179	184

HIV-1- Sup T1 HIV-1 가 1 10
 -Tat rsCD4(1 µg/ml)
 RT (RT) 2 . 10 -Tat 가
 RT 70% 가 , RT 가 가 3 /
 -Tat 3 , -Tat 가 RT
 -Tat -Tat 10 µg/ml 가 RT rsCD4

3

HIV-1 LTR Tat -Tat

HeLa, HIV-1, HIV-1 (LTD) : CAT)
 , CD4, HIV-1 LTR, HIV-1, Tat (HLCD4-CAT) Tat 가
 , HIV-1 LTR, HIV-1, Tat, CAT, Tat 가
 HeLa (10% DMEM 1 ml 3 x 10⁵) (1 µg/ml 10
 µg/ml) () 가 CD4 (rsCD4) 1 (Ho) (1984)
 HIV- (100 µl) 가 . 24 () ()
 CAT 가 CAT (75%), () -Tat
 -Tat 가 CAT () -Tat
 -gp120, rsCD4, CAT
 -Tat 가 , Tat , LTR/
 , HeLa 가
 4

[(1989) *Proc. Natl. Acad. Sci. (U.S.A.)* 86 : 6982]

10% Pd/C 가 1.1 CH₂Cl₂ 5 , H₂, CH₂Cl₂/EtOH -p-

- c-Myc Ig

c-myc ([Evan et al. (1985) *Mol. Cell. Biol.* 5 : 3610] 10% RPMI 164
 0 MYC CT9-B7.3 (ATCC CRL 1725), MYC CT 14-G4.3(ATCC CRL 1727), MYC
 1-9E 10.2 (ATCC CRL 1729) ,

NaIO₄ 2 mg (H₂O 42 mg/ml) 50 µl 가 1.5 ml 300 mM NaHCO₃ 400 µl
 90) , 500 µl . 10 mM Na₂CO₃ (1) PD-10 (50
 0 µg () .

DMSO (1 ml DMSO 5 mg
 ,) . 50 µl ,
 , IgG 350 µl 가 .
 20

NaBH₄ (H₂O 1ml 10 mg) 100 µl 가 . 1 , (H₂O 1 ml 15
 µl) 40 µl 가 가 1 , PBS PD-10 .
 -myc IgG (3 3.5 ml)

-HMG CoA Ig

HMG CoA [Goldstein et al. (1983) *J. Biol. Chem.* 258 : 8450] 4.5
 g/l, 5% 2.5% DMEM A9 (ATCC CRL 1811)

2mg 1.5 ml 300 mM NaHCO₃ 400 µl .

0 NaIO₄ (H₂O 1 ml 42 mg) 50 μl 가 , 9
) , 500 μl 10 mM Na₂CO₃ (1) PD-10 (IgG 50
 0 μg () .

DMSO (DMSO 1 ml 5 mg
). 50 μl ,
 20 IgG 350 μl 가 . ,

NaBH₄ (H₂O 1 ml 10 mg) 100 μl 가 . 1 , 40 μl(H₂O 1 ml
 15 μl) 가 . 가 1 , PBS PD-10 .
 -HMG CoA IgG (3 3.5 ml) .

5

[Proc. Natl. Acad. Sci.(U.S.A.) 86:6982(Behr (1989))]()

- -p- 1 1.1 5 . CH₂Cl₂
 d/C, H₂ 가 1 . , CH₂Cl₂/EtOH 10% P

-Ras Ig

4.5g/l, L- 2mM, 1mM, , 1xBME , 0.1
 mM, 0.032mM, 0.05mg/ml, 10%가 , DMEM
 142-24E5(ATCC HB 8679; 5,015,571 5,030,565 ,) , 1%
 L- HT 10% Iscove DMEM MX(ATCC HB 91
 58) (4,820,631 ,) , ras

90 NaIO₄ (H₂O 2mg 1.5ml 300mM NaHCO₃ 400 μl
 500 μg IgG 500 μl () . Na₂CO₃ 10mM (1) PD-10 ()

DMSO (DMSO 1ml 5mg,) .
 20 IgG 350 μl 가 50 μl , ,

NaBH₄ (H₂O 10mg/ml) 100 μl 가 . 1 , (H₂O 1ml 15
 μl) 40 μl 가 . 가 1 , PBS PD-10
 -ras IgG(3 3.5ml) .

(American Type Culture Collection, Rock ville, MD; ATCC Cell Lines and Hybridomas(1992)7th Ed,)

6

- (GGT:EC 2.3.2.2) - 가
 . GGT 2

. GGT N-

(Barouki (1984) *J. Biol. Chem.* 259 : 7970; Curthoys Hughey(1979) *Enzyme* 24 : 383; Matsuda (1983) *J. Biochem.* 93 :1427; Taniguch (1985) *J. Natl. Cancer Inst.* 75 : 841; Tate Meiste r(1985) *Methods Enzymol.* 113 : 400; Toya (1983) *Ann. N. Y. Acad. Sci.* 417 : 86,

) GGT
 GGT 1mg 1.5ml 300mM NaHCO₃ 400 μl
 NaIO₄ (H₂O 42mg/ml) 50 μl 가
 60 10mM Na₂CO₃ (1) PD-10 (GGT
) 500 μl
 100 μg ()
 DMSO (DMSO 1ml 5mg,).
 GGT 350 μl 가 50 μl 20
 , NaBH₄ (H₂O 10mg/ml) 100 μl 가 .1 , (H₂O 1ml 15
 μl) 40 μl 가 가 1 , PBS PD-10
 . GGT(3 3.5ml)
 (Tate Meister(1983) ,) GGT GGT GGT
 - , , GGT GGT GGT
 GGT GGT GGT T 125 I
 GGT 50 μg .24 ,
 GGT

7

- 가 , (Swiss) 3T3 , - ()
 A- -
 . 0.2 1.0mg/ml 300mM NaHCO₃ 0.8ml . NaIO₄
 (42mg/ml) 50 μl 가 PD-10 (, Piscataway, NJ) , 1
 10mM Na₂CO₃ 10mg/ml 50 μl
 0mM Na₂CO₃ (, A₂₈₀) 가
 20 , (H₂O 1ml NaBH₄ (10mg/ml) 100 μl 가 1
 , 15 μl) 50 μl 가 가 1 가 1
 , PD-10

ELISA

ELISA () - -Tat - -Tat(()
) -Tat ()

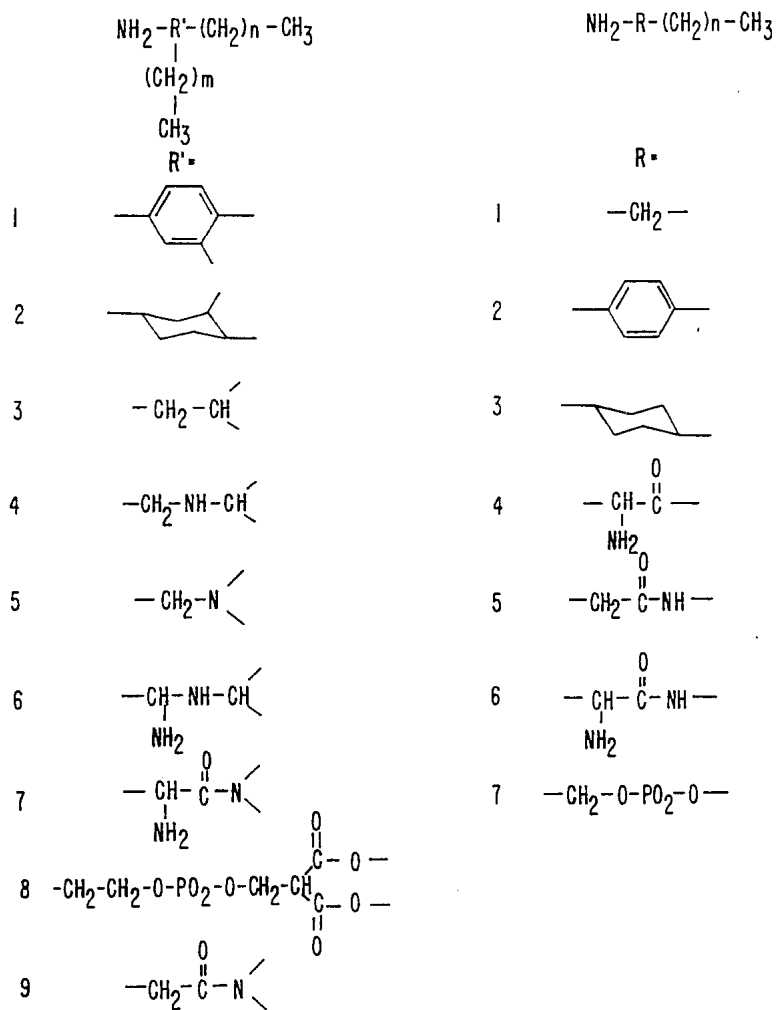
가 (Swiss) 3T3 1 2
 () 가 ()
 가 가 2
 가

1 2, 3 / 4
 가 ()

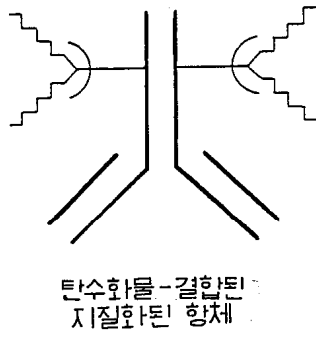
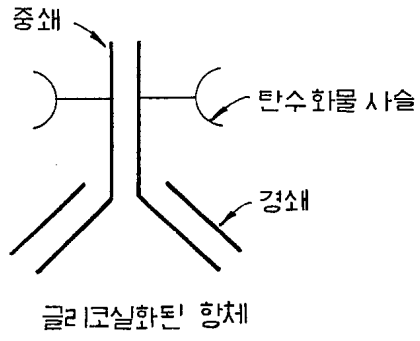
(57)

1. 1
2. 1
3. 1

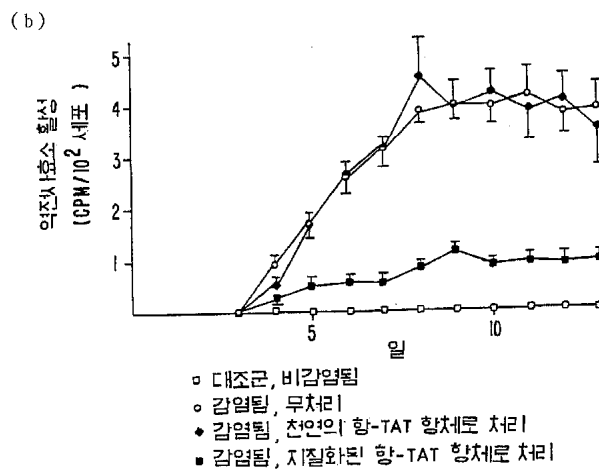
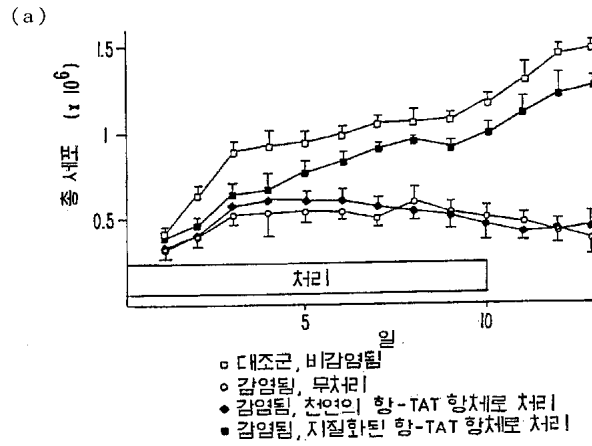
1



2



3



4

