



(86) Date de dépôt PCT/PCT Filing Date: 1994/06/07
(87) Date publication PCT/PCT Publication Date: 1994/12/22
(45) Date de délivrance/Issue Date: 2012/10/09
(85) Entrée phase nationale/National Entry: 1995/12/07
(86) N° demande PCT/PCT Application No.: US 1994/005739
(87) N° publication PCT/PCT Publication No.: 1994/028920
(30) Priorité/Priority: 1993/06/07 (US073,028)

(51) Cl.Int./Int.Cl. *C12Q 1/70* (2006.01),
A61K 38/00 (2006.01), *A61K 39/155* (2006.01),
A61K 39/21 (2006.01), *A61K 39/42* (2006.01),
C07K 14/005 (2006.01), *C07K 14/02* (2006.01),
C07K 14/05 (2006.01), *C07K 14/11* (2006.01),
C07K 14/115 (2006.01), *C07K 14/12* (2006.01),
C07K 14/125 (2006.01), ...

(72) Inventeurs/Inventors:
BOLOGNESI, DANI P., US;
MATTHEWS, THOMAS J., US;
WILD, CARL T., US;
BARNEY, SHAWN O'LIN, US;
LAMBERT, DENNIS M., US; ...

(54) Titre : PEPTIDES SYNTHETIQUES, INHIBITEURS DE LA TRANSMISSION DU VIH
(54) Title: SYNTHETIC PEPTIDE INHIBITORS OF HIV TRANSMISSION

HIV1LAI (DP-178; SEQ ID:1) YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF

HIV1SF2 (DP-185; SEQ ID:3) YTNTIYNLLEESQNQQEKNEQELLELDKWASLWNWF

HIV1RF (SEQ ID:4) YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF

HIV1MN (SEQ ID:5) YTSLIYSLLEKSQTQQEKNEQELLELDKWASLWNWF

HIV2ROD (SEQ ID:6) LEANISKSLEQAQIQQEKMYELQKLNSWDIFGNWF

HIV2NIHZ (SEQ ID:7) LEANISQSLEQAQIQQEKMYELQKLNSWDVFTNWL

DP180 (SEQ ID:2) SSESFTLLEQWNNWKLQLAEQWLEQINEKHYLEDIS

DP118 (SEQ ID:10) QQLLDVVKRQQEMLRLTWGTKNLQARVTAIEKYLKDQ

DP125 (SEQ ID:8) CGGNLLRAIEAQQHLLQLTWGIKQLQARILAVERYLKDQ

DP116 (SEQ ID:9) LQARILAVERYLKDQQQ

(57) Abrégé/Abstract:

The present invention relates to peptides which exhibit potent anti-retroviral activity. The peptides of the invention comprise DP-178 (SEQ ID:1) peptide corresponding to amino acids 638 to 673 of the HIV-1LAI gp41 protein, and fragments, analogs and homologs of DP-178. The invention further relates to the uses of such peptides as inhibitory of human and non-human retroviral, especially HIV, transmission to uninfected cells.

(51) Cl.Int./Int.Cl. (suite/continued) *C07K 14/13* (2006.01), *C07K 14/135* (2006.01), *C07K 14/15* (2006.01), *C07K 14/155* (2006.01), *C07K 14/16* (2006.01), *C07K 14/21* (2006.01), *C07K 14/22* (2006.01), *C07K 14/285* (2006.01), *C07K 14/31* (2006.01), *C07K 5/08* (2006.01), *C07K 5/083* (2006.01), *C07K 5/087* (2006.01), *C07K 5/09* (2006.01), *C07K 5/093* (2006.01), *C07K 5/10* (2006.01), *C07K 5/103* (2006.01), *C07K 5/107* (2006.01), *C07K 5/113* (2006.01), *C07K 5/117* (2006.01), *C07K 7/06* (2006.01), *C07K 7/08* (2006.01), *A61K 39/00* (2006.01)

(72) Inventeurs(suite)/Inventors(continued): PETTEWAY, STEPHEN R., JR., US

(73) Propriétaires(suite)/Owners(continued):DUKE UNIVERSITY, US

(74) Agent: OSLER, HOSKIN & HARCOURT LLP

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 37/02, 39/12, C12Q 1/70, G01N 33/53		A1	(11) International Publication Number: WO 94/28920 (43) International Publication Date: 22 December 1994 (22.12.94)
(21) International Application Number: PCT/US94/05739 (22) International Filing Date: 7 June 1994 (07.06.94) (30) Priority Data: 073,028 7 June 1993 (07.06.93) US (71) Applicant: DUKE UNIVERSITY [US/US]; Erwin Road, Durham, NC 27706 (US). (72) Inventors: BOLOGNESI, Dani, P.; 17 Harvey Place, Durham, NC 27705 (US). MATTHEWS, Thomas, J.; 5906 Newhall Road, Durham, NC 27713 (US). WILD, Carl, T.; 1702 B Vista Street, Durham, NC 27701 (US). BARNEY, Shaen, O'Lin; 106 Branchway Road, Cary, NC 27502 (US). LAMBERT, Dennis, M.; 101 Centerville Court, Cary, NC 27513 (US). PETTEWAY, Stephen, R., Jr.; 203 Le Gault Drive, Cary, NC 27513 (US). (74) Agents: CORUZZI, Laura, A. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).			(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, UA, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). 2164698 Published <i>With international search report.</i>
(54) Title: SYNTHETIC PEPTIDE INHIBITORS OF HIV TRANSMISSION			
(57) Abstract The present invention relates to peptides which exhibit potent anti-retroviral activity. The peptides of the invention comprise DP-178 (SEQ ID:1) peptide corresponding to amino acids 638 to 673 of the HIV-1 _{LAI} gp41 protein, and fragments, analogs and homologs of DP-178. The invention further relates to the uses of such peptides as inhibitory of human and non-human retroviral, especially HIV, transmission to uninfected cells.			

SYNTHETIC PEPTIDE INHIBITORS OF HIV TRANSMISSION1. INTRODUCTION

The present invention relates to DP-178 (SEQ ID:1), a peptide corresponding to amino acids 638 to 673 of the HIV-1_{LAI} transmembrane protein (TM) gp41, and portions, analogs, and homologs of DP-178 (SEQ ID:1), all of which exhibit anti-viral activity. Such anti-viral activity includes, but is not limited to, the inhibition of HIV transmission to uninfected CD-4⁺ cells. Further, the invention relates to the use of DP-178 (SEQ ID:1) and DP-178 fragments and/or analogs or homologs as inhibitors of human and non-human retroviral, especially HIV, transmission to uninfected cells. Still further, the invention relates to the use of DP-178 as a HIV subtype-specific diagnostic. The present invention also relates to antiviral peptides analogous to DP-107, a peptide corresponding to amino acids 558 to 595 of the HIV-1_{LAI} transmembrane protein (TM) gp41, that are present in other enveloped viruses. The present invention further relates to methods for identifying antiviral compounds that disrupt the interaction between DP-178 and DP-107, and/or between DP-107-like and DP-178-like peptides. The invention is demonstrated by way of a working example wherein DP-178 (SEQ ID:1), and a peptide whose sequence is homologous to DP-178 are each shown to be potent, non-cytotoxic inhibitors of HIV-1 transfer to uninfected CD-4⁺ cells. The invention is further demonstrated by working examples wherein peptides having antiviral and/or structural similarity to DP-107 and DP-178 are identified.

2164698

2. BACKGROUND OF THE INVENTION

2.1. THE HUMAN IMMUNODEFICIENCY VIRUS

The human immunodeficiency virus (HIV) has been implicated as the primary cause of the slowly degenerative immune system disease termed acquired
5 immune deficiency syndrome (AIDS) (Barre-Sinoussi, F. et al., 1983, Science 220:868-870; Gallo, R. et al., 1984, Science 224:500-503). there are at least two distinct types of HIV: HIV-1 (Barre-Sinoussi, F. et al., 1983, Science 220:868-870; Gallo R. et al., 1984,
10 Science 224:500-503) and HIV-2 (Clavel, F. et al., 1986, Science 233:343-346; Guyader, M. et al., 1987, Nature 326:662-669). Further, a large amount of genetic heterogeneity exists within populations of each of these types. Infection of human CD-4⁺ T-
15 lymphocytes with an HIV virus leads to depletion of the cell type and eventually to opportunistic infections, neurological dysfunctions, neoplastic growth, and ultimately death.

HIV is a member of the lentivirus family of
20 retroviruses (Teich, N. et al., 1984, RNA Tumor Viruses, Weiss, R. et al., eds., CSH-Press, pp. 949-956). Retroviruses are small enveloped viruses that contain a diploid, single-stranded RNA genome, and replicate via a DNA intermediate produced by a
25 virally-encoded reverse transcriptase, an RNA-dependent DNA polymerase (Varmus, H., 1988, Science 240:1427-1439). Other retroviruses include, for example, oncogenic viruses such as human T-cell leukemia viruses (HTLV-I, -II, -III), and feline
30 leukemia virus.

The HIV viral particle consists of a viral core, composed of capsid proteins, that contains the viral RNA genome and those enzymes required for early
35 replicative events. Myristylated Gag protein forms an

outer viral shell around the viral core, which is, in turn, surrounded by a lipid membrane envelope derived from the infected cell membrane. The HIV envelope surface glycoproteins are synthesized as a single 160 Kd precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane protein and gp120 is an extracellular protein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammarskjold, M. and Rekosh, D., 1989, Biochem. Biophys. Acta 989:269-280).

HIV is targeted to CD-4⁺ cells because the CD-4 cell surface protein acts as the cellular receptor for the HIV-1 virus (Dalglish, A. et al., 1984, Nature 312:763-767; Klatzmann et al., 1984, Nature 312:767-768; Maddon et al., 1986, Cell 47:333-348). Viral entry into cells is dependent upon gp120 binding the cellular CD-4⁺ receptor molecules (McDougal, J.S. et al., 1986, Science 231:382-385; Maddon, P.J. et al., 1986, Cell 47:333-348) and thus explains HIV's tropism for CD-4⁺ cells, while gp41 anchors the envelope glycoprotein complex in the viral membrane.

2.2. HIV TREATMENT

HIV infection is pandemic and HIV associated diseases represent a major world health problem. Although considerable effort is being put into the successful design of effective therapeutics, currently no curative anti-retroviral drugs against AIDS exist. In attempts to develop such drugs, several stages of the HIV life cycle have been considered as targets for therapeutic intervention (Mitsuya, H. et al., 1991, FASEB J. 5:2369-2381). For example, virally encoded reverse transcriptase has been one focus of drug development. A number of reverse-transcriptase-

2164698

targeted drugs, including 2',3'-dideoxynucleoside
analogues such as AZT, ddI, ddC, and d4T have been
developed which have been shown to be active against
HIV (Mitsuya, H. et al., 1991, Science 249:1533-1544).
While beneficial, these nucleoside analogues are not
curative, probably due to the rapid appearance of drug
resistant HIV mutants (Lander, B. et al., 1989,
Science 243:1731-1734). In addition, the drugs often
exhibit toxic side effects such as bone marrow
suppression, vomiting, and liver function
abnormalities.

Attempts are also being made to develop drugs
which can inhibit viral entry into the cell, the
earliest stage of HIV infection. Here, the focus has
thus far been on CD4, the cell surface receptor for
HIV. Recombinant soluble CD4, for example, has been
shown to inhibit infection of CD-4⁺ T-cells by some
HIV-1 strains (Smith, D.H. et al., 1987, Science
238:1704-1707). Certain primary HIV-1 isolates,
however, are relatively less sensitive to inhibition
by recombinant CD-4 (Daar, E. et al., 1990, Proc.
Natl. Acad. Sci. USA 87:6574-6579). In addition,
recombinant soluble CD-4 clinical trials have produced
inconclusive results (Schooley, R. et al., 1990, Ann.
Int. Med. 112:247-253; Kahn, J.O. et al., 1990, Ann.
Int. Med. 112:254-261; Yarchoan, R. et al., 1989,
Proc. Vth Int. Conf. on AIDS, p. 564, MCP 137).

The late stages of HIV replication, which involve
crucial virus-specific secondary processing of certain
viral proteins, have also been suggested as possible
anti-HIV drug targets. Late stage processing is
dependent on the activity of a viral protease, and
drugs are being developed which inhibit this protease
(Erickson, J., 1990, Science 249:527-533). The

clinical outcome of these candidate drugs is still in question.

Attention is also being given to the development of vaccines for the treatment of HIV infection. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for anti-HIV antibodies present in AIDS patients (Barin, et al., 1985, Science 228:1094-1096). Thus far, therefore, these proteins seem to be the most promising candidates to act as antigens for anti-HIV vaccine development. To this end, several groups have begun to use various portions of gp160, gp120, and/or gp41 as immunogenic targets for the host immune system. See for example, Ivanoff, L. et al., U.S. Pat. No. 5,141,867; Saith, G. et al., WO 92/22,654; Shafferman, A., WO 91/09,872; Formoso, C. et al., WO 90/07,119. Clinical results concerning these candidate vaccines, however, still remain far in the future.

Thus, although a great deal of effort is being directed to the design and testing of anti-retroviral drugs, a truly effective, non-toxic treatment is still needed.

3. SUMMARY OF THE INVENTION

The present invention relates to DP-178 (SEQ ID:1), a 36-amino acid synthetic peptide corresponding to amino acids 638 to 673 of the transmembrane protein (TM) gp41 from the HIV-1 isolate LAI, which exhibits potent anti-HIV-1 activity. As evidenced by the example presented below, in Section 6, the DP-178 (SEQ ID:1) anti-viral activity is so high that, on a weight basis, no other known anti-HIV agent is effective at concentrations as low as those at which DP-178 (SEQ ID:1) exhibits its inhibitory effects. The invention further relates to those portions, analogs, and

2164698

homologs of DP-178 which also show such antiviral activity. The antiviral activity of such DP-178 portions, analogs, and homologs, includes, but is not limited to the inhibition of HIV transmission to uninfected CD-4⁺ cells. The invention relates to the use of DP-178 (SEQ ID:1) and DP-178 fragments and/or analogs or homologs. Such uses may include, but are not limited to, the use of the peptides as inhibitors of human and non-human retroviral, especially HIV, transmission to uninfected cells, and as type and/or subtype-specific diagnostic tools.

An embodiment of the invention is demonstrated below wherein an extremely low concentration of DP-178 (SEQ ID:1), and very low concentrations of a DP-178 homolog (SEQ ID:3) are shown to be potent inhibitors of HIV-1 mediated CD-4⁺ cell-cell fusion (i.e., syncytial formation) and infection of CD-4⁺ cells by cell-free virus. Further, it is shown that DP-178 (SEQ ID:1) is not toxic to cells, even at concentrations 3 logs higher than the inhibitory DP-178 (SEQ ID:1) concentration.

The invention also relates to analogous DP178 peptides in other enveloped viruses that demonstrate similar antiviral properties.

The invention further relates to peptides analogous to DP-107, a peptide corresponding to amino acids 558-595 of the HIV-1_{LAI} transmembrane protein (TM) of gp41, that are present in other enveloped viruses, and demonstrate antiviral properties. The present invention is based, in part, on the surprising discovery that the DP-107 and DP-108 domains of the gp41 protein non-covalently complex with each other, and that their interaction is necessary for the normal activity of the virus. The invention, therefore, further relates to methods for identifying antiviral

2164698

compounds that disrupt the interaction between DP-107 and DP-178, and/or between DP-107-like and DP-178-like peptides.

Embodiments of the invention are demonstrated, below, wherein peptides having structural and/or
5 similarity to DP-107 and DP-178 are identified.

3.1. DEFINITIONS

Peptides are defined herein as organic compounds comprising two or more amino acids covalently joined
10 by peptide bonds. Peptides may be referred to with respect to the number of constituent amino acids, i.e., a dipeptide contains two amino acid residues, a tripeptide contains three, etc. Peptides containing
15 ten or fewer amino acids may be referred to as oligopeptides, while those with more than ten amino acid residues are polypeptides.

Peptide sequences defined herein are represented by one-letter symbols for amino acid residues as follows:

20 A (alanine)
R (arginine)
N (asparagine)
D (aspartic acid)
C (cysteine)
25 Q (glutamine)
E (glutamic acid)
G (glycine)
H (histidine)
I (isoleucine)
30 L (leucine)
K (lysine)
M (methionine)
F (phenylalanine)
P (proline)
35

S (serine)
T (threonine)
W (tryptophan)
Y (tyrosine)
V (valine)

5

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Amino acid sequence of DP-178 (SEQ ID:1) derived from HIV_{LAI}; DP-178 homologs derived from HIV-1_{SF2} (DP-185; SEQ ID:3), HIV-1_{RF} (SEQ ID:4), and
10 HIV-1_{MN} (SEQ ID:5); DP-178 homologs derived from amino acid sequences of two prototypic HIV-2 isolates, namely, HIV-2_{rod} (SEQ ID:6) and HIV-2_{NIH2} (SEQ ID:7); control peptides: DP-180 (SEQ ID:2), a peptide
15 incorporating the amino acid residues of DP-178 in a scrambled sequence; DP-118 (SEQ ID:10) unrelated to DP-178, which inhibits HIV-1 cell free virus infection; DP-125 (SEQ ID:8), unrelated to DP-178, was also previously shown to inhibit HIV-1 cell free virus
20 infection (Wild et al., 1992, Proc. Natl. Acad. Sci USA 89:10,537-10,541); DP-116 (SEQ ID:9), unrelated to DP-178 had previously been shown to be negative for inhibition of HIV-1 infection using the cell-free virus infection assay (Wild, et al., 1992, Proc. Natl. Acad. Sci USA 89:10,537-10,541). Throughout the
25 figures, the one letter amino acid code is used.

FIG. 2. Inhibition of HIV-1 cell-free virus infection by synthetic peptides. IC50 refers to the concentration of peptide that inhibits RT production from infected cells by 50% compared to the untreated
30 control. Control: the level of RT produced by untreated cell cultures infected with the same level of virus as treated cultures.

FIG. 3. Inhibition of HIV-1 and HIV-2 cell-free virus infection by the synthetic peptide DP-178 (SEQ
35

2164698

ID:1). IC50: concentration of peptide that inhibits RT production by 50% compared to the untreated control. Control: Level of RT produced by untreated cell cultures infected with the same level of virus as treated cultures.

5 FIG. 4A. Fusion Inhibition Assay. DP-178 (SEQ ID:1) inhibition of HIV-1 prototypic isolate-mediated syncytia formation. Data represents the number of virus-induced syncytia per cell.

10 FIG. 4B. Fusion Inhibition Assay. DP-180 (SEQ ID:2): scrambled control peptide. DP-185 (SEQ ID:3): DP-178 homolog derived from HIV-1_{SF2} isolate. Control: number of syncytia produced in the absence of peptide.

15 FIG. 5. Fusion inhibition assay: HIV-1 vs. HIV-2. Data represents the number of virus-induced syncytia per well. ND: not done.

 FIG. 6. Cytotoxicity study of DP-178 (SEQ ID:1) and DP-116 (SEQ ID:9) on CEM cells. Cell proliferation data is shown.

20 FIG. 7. Schematic representation of HIV-gp41 and maltose binding protein (MBP)-gp41 fusion proteins. DP107 and DP178 are synthetic peptides based on the two putative helices of gp41. The letter P in the DP107 boxes denotes an Ile to Pro mutation at amino acid number 578. Amino acid residues are
25 numbered according to Meyers et al., Human Retroviruses and AIDS, 1991, Theoret. Biol. and Biophys. Group, Los Alamos Natl. Lab., Los Alamos, NM.

 FIG. 8. A point mutation alters the conformation and anti-HIV activity of M41.

30 FIG. 9. Abrogation of DP178 anti-HIV activity. Cell fusion assays were carried out in the presence of 10 nM DP178 and various concentrations of M41Δ178 or M41PΔ178.

35

2164698

FIG. 10. Binding of DP178 to leucine zipper of gp41 analyzed by ELISA.

FIG. 11A-B. Models for a structural transition in the HIV-1 TM protein. Two models are proposed which indicate a structural transition from a native oligomer to a fusogenic state following a trigger event (possibly gp120 binding to CD4). Common features of both models include (1) the native state is held together by noncovalent protein-protein interactions to form the heterodimer of gp120/41 and other interactions, principally through gp41 interactive sites, to form homo-oligomers on the virus surface of the gp120/41 complexes; (2) shielding of the hydrophobic fusogenic peptide at the N-terminus (F) in the native state; and (3) the leucine zipper domain (DP107) exists as a homo-oligomer coiled coil only in the fusogenic state. The major differences in the two models include the structural state (native or fusogenic) in which the DP107 and DP178 domains are complexed to each other. In the first model (A; FIG. 11A) this interaction occurs in the native state and in B during the fusogenic state. When triggered, the fusion complex in the model depicted in (A) is generated through formation of coiled-coil interactions in homologous DP107 domains resulting in an extended α -helix. This conformational change positions the fusion peptide for interaction with the cell membrane. In the second model (B; FIG. 11B), the fusogenic complex is stabilized by the association of the DP178 domain with the DP107 coiled-coil.

FIG. 12. Motif design using heptad repeat positioning of amino acids of known coiled-coils.

FIG. 13. Motif design using proposed heptad repeat positioning of amino acids of DP-107 and DP-178.

2164698

FIG. 14. Hybrid motif design crossing GCN4 and DP-107.

FIG. 15. Hybrid motif design crossing GCN4 and DP-178.

5 FIG. 16. Hybrid motif design 107x178x4, crossing DP-107 and DP-178. This motif was found to be the most consistent at identifying relevant DP-107-like and DP-178-like peptide regions.

10 FIG. 17. Hybrid motif design ALLMOTI5, crossing GCN4, DP-107, and DP-178.

FIG. 18. Hybrid motif design crossing GCN4, DP-107, DP-178, c-Fos c-Jun, c-Myc, and Flu Loop 36.

FIG. 19. Motifs designed to identify N-terminal proline-leucine zipper motifs.

15 FIG. 20. Search results for HIV-1 (BRU isolate) envelope protein gp41. Sequence search motif designations: Spades (♠): 107x178x4; Hearts (♥) ALLMOTI5; Clubs (♣): PLZIP; Diamonds (♦): transmembrane region (the putative transmembrane domains were identified using a PC/Gene program
20 designed to search for such peptide regions). Asterisk (*): Lupas method. The amino acid sequences identified by each motif are bracketed by the respective characters. Representative sequences chosen based on all searches are underlined and in
25 bold. DP-107 and DP-178 sequences are marked, and additionally double-underlined and italicized.

30 FIG. 21. Search results for human respiratory syncytial virus (RSV) strain A2 fusion glycoprotein F1. Sequence search motif designations are as in FIG. 20.

35 FIG. 22. Search results for simian immunodeficiency virus (SIV) envelope protein gp41 (AGM3 isolate). Sequence search motif designations are as in FIG. 20.

2164698

FIG. 23. Search results for canine distemper virus (strain Onderstepoort) fusion glycoprotein 1. Sequence search motif designations are as in FIG. 20.

5 FIG. 24. Search results for newcastle disease virus (strain Australia-Victoria/32) fusion glycoprotein F1. Sequence search motif designations are as in FIG. 20.

10 FIG. 25. Search results for human parainfluenza 3 virus (strain NIH 47885) fusion glycoprotein F1. Sequence search motif designations are as in FIG. 20.

15 FIG. 26. Search results for influenza A virus (strain A/AICHI/2/68) hemagglutinin precursor HA2. Sequence search designations are as in FIG. 20.

20 FIG. 27. Coiled-coil structural similarity and anti-RSV antiviral activity of 35-mer peptides synthesized utilizing the sequence of a 48-amino acid RSV F2 peptide which spans sequences identified utilizing the computer-assisted searches described herein. For the exact location and motifs utilized, see FIG. 21. "+" symbols are relative indicators of either structural similarity or antiviral activity, with a greater number of "+" symbols indicating a higher relative similarity or antiviral activity.

25 FIG. 28. Coiled-coil structural similarity and anti-RSV antiviral activity of 35-mer peptides synthesized utilizing the sequence of a 53-amino acid RSV F1 peptide which spans sequences identified utilizing the computer-assisted searches described herein. See FIG. 21 for the exact location and motifs used. "+" symbols are as described for FIG. 27.

30 FIG. 29. Coiled-coil structural similarity and anti-human parainfluenza 3 virus (HPF3) antiviral activity of 35-mer peptides synthesized utilizing the

35

2164698

sequence of a 56-amino acid HPF3 peptide which spans sequences identified utilizing computer-assisted searches described herein. For the exact location and motifs utilized, see FIG. 25. "+" symbols are as described in FIG. 27.

5 FIG. 30. Coiled-coil structural similarity and anti-HPF3 antiviral activity of 35-mer peptides synthesized utilizing the sequence of a 70-amino acid HPF3 peptide which spans sequences identified
10 utilizing the computer-assisted searches described herein. For the exact location and motifs utilized, see FIG. 25. "+" symbols are as described in FIG. 27.

5. DETAILED DESCRIPTION OF THE INVENTION

15 Described herein are peptides that exhibit potent antiviral activity. These peptides include DP-178 (SEQ ID:1), a gp41-derived 36 amino acid peptide, fragments and/or analogs of DP-178, and peptides which are homologous to DP-178. In addition, these peptides may include peptides exhibiting anti-viral activity
20 which are analogous to DP-107, a 38 amino acid peptide corresponding to residues 558 to 595 of the HIV-1_{LAI} transmembrane (TM) gp41 protein, and which are present in other enveloped viral proteins. Also described
25 here are assays for testing the antiviral activities of such peptides. The present invention is based, in part, of the surprising discovery that the DP-107 and DP-178 domains of the gp41 protein complex with each other via non-covalent protein-protein interactions which are necessary for normal activity of the virus.
30 As such, methods are described for the identification of antiviral compounds that disrupt the interaction between DP-107 and DP-178 peptides, and between DP-107-like and DP-178-like peptides. Finally, the use
35 of the peptides of the invention as inhibitors of non-

2164698

human and human viral and retroviral, especially HIV, transmission are detailed, as is the use of the peptides as diagnostic indicators of the presence of specific, viruses, especially retroviruses.

5 While not limited to any theory of operation, the following model is proposed to explain the potent anti-HIV activity of DP178, based, in part, on the experiments described in the working examples, infra. In the viral protein, gp41, DP178 corresponds to a putative α -helix region located in the C-terminal end
10 of the gp41 ectodomain, and appears to associate with a distal site on gp41 whose interactive structure is influenced by the leucine zipper motif, a coiled-coil structure, referred to as DP107. The association of these two domains may reflect a molecular linkage or
15 "molecular clasp" intimately involved in the fusion process. It is of interest that mutations in the C-terminal α -helix motif of gp41 (i.e., the D178 domain) tend to enhance the fusion ability of gp41, whereas mutations in the leucine zipper region (i.e.,
20 the DP107 domain) decrease or abolish the fusion ability of the viral protein. It may be that the leucine zipper motif is involved in membrane fusion while the C-terminal α -helix motif serves as a molecular safety to regulate the availability of the
25 leucine zipper during virus-induced membrane fusion.

On the basis of the foregoing, two models are proposed of gp41-mediated membrane fusion which are schematically shown in FIG. 11A-B. The reason for
30 proposing two models is that the temporal nature of the interaction between the regions defined by DP107 and DP178 cannot, as yet, be pinpointed. Each model envisions two conformations for gp41 - one in a "native" state as it might be found on a resting virion. The other in a "fusogenic" state to reflect
35

conformational changes triggered following binding of gp120 to CD4 and just prior to fusion with the target cell membrane. The strong binding affinity between gp120 and CD4 may actually represent the trigger for the fusion process obviating the need for a pH change such as occurs for viruses that fuse within intracellular vesicles. The two major features of both models are: (1) the leucine zipper sequences (DP107) in each chain of oligomeric envelope are held apart in the native state and are only allowed access to one another in the fusogenic state so as to form the extremely stable coiled-coils, and (2) association of the DP178 and DP107 sites as they exist in gp41 occur either in the native or fusogenic state. FIG. 11A depicts DP178/DP107 interaction in the native state as a molecular class. On the other hand, if one assumes that the most stable form of the envelope occurs in the fusogenic state, the model in FIG. 11B can be considered.

When synthesized as peptides, both DP107 and DP178 are potent inhibitors of HIV infection and fusion, probably by virtue of their ability to form complexes with viral gp41 and interfere with its fusogenic process; e.g., during the structural transition of the viral protein from the native structure to the fusogenic state, the DP178 and DP107 peptides may gain access to their respective binding sites on the viral gp41, and exert a disruptive influence. DP107 peptides which demonstrate anti-HIV activity are described in Applicants' U.S. Patent No. 5,656,480 issued August 12, 1997.

As shown in the working examples, infra, a truncated recombinant gp41 protein corresponding the

2164698

ectodomain of gp41 containing both DP107 and DP178 domains (excluding the fusion peptide, transmembrane region and cytoplasmic domain of gp41) did not inhibit HIV-1 induced fusion. However, when a single mutation was introduced to disrupt the coiled-coil structure of the DP107 domain -- a mutation which results in a total loss of biological activity of DP107 peptides -- the inactive recombinant protein was transformed to an active inhibitor of HIV-1 induced fusion. This transformation may result from liberation of the potent DP178 domain from a molecular clasp with the leucine zipper, DP107 domain.

For clarity of discussion, the invention will be described for DP178 peptide inhibitors of HIV. However, the principles may be analogously applied to other fusogenic enveloped viruses, including but not limited to those viruses containing the peptides listed in Tables V through X, below.

5.1. DP-178 AND DP-178-LIKE PEPTIDES

The peptide DP-178 (SEQ ID:1) of the invention corresponds to amino acid residues 638 to 673 of the transmembrane protein gp41 from the HIV-1_{LAI} isolate, and has the 36 amino acid sequence (reading from amino to carboxy terminus):

NH₂-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-COOH (SEQ ID:1)

In addition to the full-length DP-178 (SEQ ID:1) 36-mer, the peptides of the invention may include truncations of the DP-178 (SEQ ID:1) peptide which exhibit antiviral activity. Such truncated DP-178 (SEQ ID:1) peptides may comprise peptides of between 3 and 36 amino acid residues (i.e., peptides ranging in size from a tripeptide to a 36-mer polypeptide), and

2164698

may include but are not limited to those listed in Tables I and II, below. Peptide sequences in these tables are listed from amino (left) to carboxy (right) terminus. "X" may represent an amino group ($-\text{NH}_2$) and "Z" may represent a carboxyl ($-\text{COOH}$) group.

5 Alternatively, as described below, "X" and/or "Z" may represent a hydrophobic group, an acetyl group, a Fmoc group, an amido group, or a covalently attached macromolecule.

10

15

20

25

30

35

2164698

TABLE I
DP-178 (SEQ ID:1) CARBOXY TRUNCATIONS

X-YTS-Z
 X-YTSL-Z
 X-YTSLI-Z
 X-YTSLIH-Z
 5 X-YTSLIHS-Z
 X-YTSLIHSL-Z
 X-YTSLIHSLI-Z
 X-YTSLIHSLIE-Z
 X-YTSLIHSLIEE-Z
 X-YTSLIHSLIEES-Z
 X-YTSLIHSLIEESQ-Z
 10 X-YTSLIHSLIEESQN-Z
 X-YTSLIHSLIEESQNNQ-Z
 X-YTSLIHSLIEESQNNQQ-Z
 X-YTSLIHSLIEESQNNQQE-Z
 X-YTSLIHSLIEESQNNQQEK-Z
 X-YTSLIHSLIEESQNNQQEKN-Z
 X-YTSLIHSLIEESQNNQQEKNE-Z
 X-YTSLIHSLIEESQNNQQEKNEQ-Z
 15 X-YTSLIHSLIEESQNNQQEKNEQE-Z
 X-YTSLIHSLIEESQNNQQEKNEQEEL-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELL-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLE-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELD-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDK-Z
 20 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKW-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWA-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWas-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWasL-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWasLW-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWasLWN-Z
 X-YTSLIHSLIEESQNNQQEKNEQEELLELDKWasLWNWF-Z
 25

The one letter amino acid code is used.

Additionally,

"X" may represent an amino group, a hydrophobic group,
 including but not limited to carbobenzoxy, dansyl, or
 30 T-butyloxycarbonyl; an acetyl group; a 9-
 fluorenylmethoxy-carbonyl (Fmoc) group; a
 macromolecular carrier group including but not limited
 to lipid-fatty acid conjugates, polyethylene glycol,
 or carbohydrates.

"Z" may represent a carboxyl group; an amido group; a
 T-butyloxycarbonyl group; a macromolecular carrier
 35 group including but not limited to lipid-fatty acid
 conjugates, polyethylene glycol, or carbohydrates.

2164698

TABLE II
DP-178 (SEQ ID:1) AMINO TRUNCATIONS

	X-NWF-Z
	X-WNWF-Z
	X-LWNWF-Z
	X-SLWNWF-Z
5	X-ASLWNWF-Z
	X-WASLWNWF-Z
	X-KWASLWNWF-Z
	X-DKWASLWNWF-Z
	X-LDKWASLWNWF-Z
	X-ELDKWASLWNWF-Z
	X-LELDKWASLWNWF-Z
10	X-LLELDKWASLWNWF-Z
	X-ELLELDKWASLWNWF-Z
	X-QELLELDKWASLWNWF-Z
	X-EQELLELDKWASLWNWF-Z
	X-NEQELLELDKWASLWNWF-Z
	X-KNEQELLELDKWASLWNWF-Z
	X-EKNEQELLELDKWASLWNWF-Z
	X-QEKNEQELLELDKWASLWNWF-Z
15	X-QQEKNEQELLELDKWASLWNWF-Z
	X-NQQEKNEQELLELDKWASLWNWF-Z
	X-QNQQEKNEQELLELDKWASLWNWF-Z
	X-SQNQQEKNEQELLELDKWASLWNWF-Z
	X-ESQNQQEKNEQELLELDKWASLWNWF-Z
	X-EESQNQQEKNEQELLELDKWASLWNWF-Z
	X-IEESQNQQEKNEQELLELDKWASLWNWF-Z
20	X-LIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-SLIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-HSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-IHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-LIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-SLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
	X-TSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
25	X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z

The one letter amino acid code is used.

Additionally,

30 "X" may represent an amino group, a hydrophobic group, including but not limited to carbobenzoxyl, dansyl, or T-butyloxycarbonyl; an acetyl group; a 9-fluorenylmethoxy-carbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

35 "Z" may represent a carboxyl group; an amido group; a T-butyloxycarbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

2164698

The antiviral peptides of the invention also include analogs of DP-178 and/or DP-178 truncations which may include, but are not limited to, peptides comprising the DP-178 (SEQ ID:1) sequence, or DP-178 truncated sequence, containing one or more amino acid substitutions, insertions and/or deletions. Analogs of DP-178 homologs, described below, are also within the scope of the invention. The DP-178 analogs of the invention exhibit antiviral activity, and may, further, possess additional advantageous features, such as, for example, increased bioavailability, and/or stability, or reduced host immune recognition.

HIV-1 and HIV-2 envelope proteins are structurally distinct, but there exists a striking amino acid conservation within the DP-178- corresponding regions of HIV-1 and HIV-2. The amino acid conservation is of a periodic nature, suggesting some conservation of structure and/or function. Therefore, one possible class of amino acid substitutions would include those amino acid changes which are predicted to stabilize the structure of the DP-178 peptides of the invention.

Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions consist of replacing one or more amino acids of the DP-178 (SEQ ID:1) peptide sequence with amino acids of similar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to aspartic acid (D) amino acid substitution. When only conserved substitutions are made, the resulting peptide is functionally equivalent to DP-178 (SEQ ID:1) or the DP-178 peptide from which it is derived. Non-conserved substitutions consist of replacing one or more amino acids of the DP-178 (SEQ ID:1) peptide sequence with amino acids possessing dissimilar charge, size, and/or hydrophobicity

2164698

characteristics, such as, for example, a glutamic acid (E) to valine (V) substitution.

5 Amino acid insertions may consist of single amino acid residues or stretches of residues ranging from 2 to 15 amino acids in length. One or more insertions may be introduced into DP-178 (SEQ ID:1), DP-178 fragments, analogs and/or DP-178 homologs (described below).

10 Deletions of DP-178 (SEQ ID:1), DP-178 fragments, analogs, and/or DP-178 homologs (described below) are also within the scope of the invention. Such deletions consist of the removal of one or more amino acids from the DP-178 or DP-178-like peptide sequence, with the lower limit length of the resulting peptide sequence being 4 to 6 amino acids. Such deletions may 15 involve a single contiguous or greater than one discrete portion of the peptide sequences.

The peptides of the invention may further include homologs of DP-178 (SEQ ID:1) and/or DP-178 truncations which exhibit antiviral activity. Such 20 DP-178 homologs are peptides whose amino acid sequences are comprised of the amino acid sequences of peptide regions of other (*i.e.*, other than HIV-1_{LAI}) viruses that correspond to the gp41 peptide region from which DP-178 (SEQ ID:1) was derived. Such 25 viruses may include, but are not limited to, other HIV-1 isolates and HIV-2 isolates. DP-178 homologs derived from the corresponding gp41 peptide region of other (*i.e.*, non HIV-1_{LAI}) HIV-1 isolates may include, for example, peptide sequences as shown below.

30

NH₂-YTNTIYTLLEESQNQQEKNEQELLELDKWASLWNWF-COOH (DP-185; SEQ ID:3);

35

NH₂-YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF-COOH (SEQ ID:4);

2164698

NH₂-YTSLIYSLLLEKSQIQQEKNEQELLELDKWASLWNWF-COOH (SEQ ID:5).

5 SEQ ID:3 (DP-185), SEQ ID:4, and SEQ ID:5 are derived from HIV-1_{SF2}, HIV-1_{RF}, and HIV-1_{MN} isolates, respectively. Underlined amino acid residues refer to those residues that differ from the corresponding position in the DP-178 (SEQ ID:1) peptide. One such DP-178 homolog, DP-185 (SEQ ID:3), is described in the Working Example presented in Section 6, below, where it is demonstrated that DP-185 (SEQ ID:3) exhibits
10 antiviral activity. The DP-178 homologs of the invention may also include truncations, amino acid substitutions, insertions, and/or deletions, as described above.

15 In addition, striking similarities, as shown in FIG. 1, exist within the regions of HIV-1 and HIV-2 isolates which correspond to the DP-178 sequence. A DP-178 homolog derived from the HIV-2_{NH2} isolate has the 36 amino acid sequence (reading from amino to
20 carboxy terminus):

NH₂-LEANISQSLEQAQIQQEKNMVELQKLNSWDVFTNWL-COOH (SEQ ID:7)

25 Table III and Table IV show some possible truncations of the HIV-2_{NH2} DP-178 homolog, which may comprise peptides of between 3 and 36 amino acid residues (i.e., peptides ranging in size from a tripeptide to a 36-mer polypeptide). Peptide sequences in these tables are listed from amino (left) to carboxy (right) terminus. "X" may represent an amino group (-NH₂) and
30 "Z" may represent a carboxyl (-COOH) group. Alternatively, as described below, "X" and/or "Z" may represent a hydrophobic group, an acetyl group, a Fmoc group, an amido group, or a covalently attached
35 macromolecule, as described below.

TABLE IIIHIV-2_{NIH} DP-178 homolog carboxy truncations.

X-LEA-Z
 X-LEAN-Z
 X-LEANI-Z
 X-LEANIS-Z
 5 X-LEANISQ-Z
 X-LEANISQS-Z
 X-LEANISQSL-Z
 X-LEANISQSLE-Z
 X-LEANISQSLEQ-Z
 X-LEANISQSLEQA-Z
 X-LEANISQSLEQAAQ-Z
 10 X-LEANISQSLEQAQI-Z
 X-LEANISQSLEQAQIQ-Z
 X-LEANISQSLEQAQIQQ-Z
 X-LEANISQSLEQAQIQQE-Z
 X-LEANISQSLEQAQIQQEK-Z
 X-LEANISQSLEQAQIQQEKN-Z
 X-LEANISQSLEQAQIQQEKNM-Z
 X-LEANISQSLEQAQIQQEKNMY-Z
 15 X-LEANISQSLEQAQIQQEKNMYE-Z
 X-LEANISQSLEQAQIQQEKNMYEL-Z
 X-LEANISQSLEQAQIQQEKNMYELQ-Z
 X-LEANISQSLEQAQIQQEKNMYELQK-Z
 X-LEANISQSLEQAQIQQEKNMYELQKL-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLN-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNS-Z
 20 X-LEANISQSLEQAQIQQEKNMYELQKLNSW-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWD-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWDV-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFT-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTN-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNW-Z
 X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL-Z
 25

The one letter amino acid code is used.

Additionally,

30 "X" may represent an amino group, a hydrophobic group, including but not limited to carbobenzoxy, dansyl, or T-butyloxycarbonyl; an acetyl group; a 9-fluorenylmethoxy-carbonyl (Fmoc) group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

35 "Z" may represent a carboxyl group; an amido group; a T-butyloxycarbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

TABLE IV

HIV-2_{NH2} DP-178 homolog amino truncations.

	X-NWL-Z
	X-TNWL-Z
	X-FTNWL-Z
5	X-VFTNWL-Z
	X-DVFTNWL-Z
	X-WDVFTNWL-Z
	X-SWDVFTNWL-Z
	X-NSWDVFTNWL-Z
	X-LNSWDVFTNWL-Z
	X-KLNSWDVFTNWL-Z
10	X-QKLNSWDVFTNWL-Z
	X-LQKLNSWDVFTNWL-Z
	X-ELQKLNSWDVFTNWL-Z
	X-YELQKLNSWDVFTNWL-Z
	X-MYELQKLNSWDVFTNWL-Z
	X-NMYELQKLNSWDVFTNWL-Z
	X-KNMYELQKLNSWDVFTNWL-Z
	X-EKNMYELQKLNSWDVFTNWL-Z
15	X-QEKNMYELQKLNSWDVFTNWL-Z
	X-QQEKNMYELQKLNSWDVFTNWL-Z
	X-IQQEKNMYELQKLNSWDVFTNWL-Z
	X-QIQQEKNMYELQKLNSWDVFTNWL-Z
	X-AQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-QAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-EAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-LEAQIQQEKNMYELQKLNSWDVFTNWL-Z
20	X-SLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-QSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-SQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-ISQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-NISQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-ANISQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
	X-EANISQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z
25	X-LEANISQSLEAQIQQEKNMYELQKLNSWDVFTNWL-Z

The one letter amino acid code is used.

Additionally,

30 "X" may represent an amino group, a hydrophobic group, including but not limited to carbobenzoxy, dansyl, or T-butyloxycarbonyl; an acetyl group; a 9-fluorenylmethoxy-carbonyl (Fmoc) group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

35 "Z" may represent a carboxyl group; an amido group; a T-butyloxycarbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

5.2. DP-107 and DP-178 ANALOGOUS ANTIVIRAL PEPTIDES

Peptide sequences functionally corresponding, and thus analogous to, the DP-178 sequences of the invention, described, above, in Section 5.1 may be found in other, non-HIV-1 envelope viruses. Further, peptide sequences functionally corresponding, and thus analogous to, DP-107, an HIV-1-derived antiviral peptide, may also be found in other, non-HIV-1 envelope viruses. DP-107 is a 38 amino acid peptide corresponding to residues 558 to 595 of HIV-1_{LA1} transmembrane (TM) gp41 protein, which exhibits potent anti-viral activity. DP-107 is more fully described in Applicant's U.S. Patent No. 5,656,480 issued August 12, 1997. These DP-107-like and DP-178-like analogous peptides and present in TM proteins of envelope viruses and preferably exhibit antiviral activity, most preferably antiviral activity which is specific to the virus in which their native sequences are found.

DP-107-like and DP-178-like peptides may be identified, for example, by utilizing a computer-assisted search strategy such as that described and demonstrated, below, in the Examples presented in Sections 9 through 16. The search strategy identifies regions in other viruses that are similar in predicted secondary structure to DP-107 and DP-178.

This search strategy is described fully, below, in the Example presented in Section 9. While this search strategy is based, in part, on a primary amino acid motif deduced from DP-107 and DP-178, it is not based solely on searching for primary amino acid sequence homologies, as such protein sequence homologies exist within, but not between major groups of viruses. For example, primary amino acid sequence homology is high within the TM protein of different

strains of HIV-1 or within the TM protein of different isolates of simian immunodeficiency virus (SIV).

Primary amino acid sequence homology between HIV-1 and SIV, however, is low enough so as not to be useful.

5 It is not possible, therefore, to find DP-107 or DP-178-like peptides within other viruses, whether structurally, or otherwise, based on primary sequence homology, alone.

Further, while it would be potentially useful to identify primary sequence arrangements of amino acids
10 based on the physical chemical characteristics of different classes of amino acids rather than based on the specific amino acids themselves, for instance, a by concentrating on the coiled-coil nature of the peptide sequence, a computer algorithm designed by
15 Lupas et al. to identify such coiled-coil propensities of regions within proteins (Lupas, A., et al., 1991 Science 252:1162-1164) is inadequate for identifying protein regions analogous to DP-107 or DP-178.

Specifically, analysis of HIV-1 gp160 (containing
20 both gp120 and gp41) using the Lupas algorithm does not identify the coiled-coil region within DP-107. It does, however, identify a region within DP-178 beginning eight amino acids N-terminal to the start of DP-178 and ending eight amino acids from the C-
25 terminus. The DP-107 peptide has been shown experimentally to form a stable coiled coil. A search based on the Lupas search algorithm, therefore, would not have identified the DP-107 coiled-coil region. Conversely, the Lupas algorithm identified the DP-178
30 region as a potential coiled-coil motif. However, the peptide DP-178 derived from this region failed to form a coiled coil in solution. A possible explanation for the inability of the Lupas search algorithm to accurately identify coiled-coil sequences within the
35 HIV-1 TM, is that the Lupas algorithm is based on the

structure of coiled coils from proteins that are not structurally or functionally similar to the TM proteins of viruses, antiviral peptides (e.g. DP-107 and DP-178) of which are an object of this invention.

5 The computer search strategy of the invention, as demonstrated in the Examples presented below, in Sections 9 through 16, successfully identifies regions of viral TM proteins similar to DP-107 or DP-178. This search strategy was designed to be used with a commercially-available sequence database packages,
10 preferably PC/Gene™. A series of motifs were designed and engineered to range in stringency from very strict to very broad, as discussed in Section 9.

Among the protein sequence search motifs which may be utilized in such a computer-assisted DP-107-like
15 and DP-178-like antiviral peptide search are the 107x178x4 motif, the ALLMOTI5 motif, and the PLZIP series of motifs, each of which is described in the Example presented in Section 9, below, with 107x178x4 being preferred.

20 Coiled-coiled sequences are thought to consist of heptad amino acid repeats. For ease of description, the amino acid positions within the heptad repeats are sometimes referred to as A through G, with the first position being A, the second B, etc. The motifs used
25 to identify DP-107-like and DP-178-like sequences herein are designed to specifically search for and identify such heptad repeats. In the descriptions of each of the motifs described, below, amino acids enclosed by brackets , i.e., [], designate the only
30 amino acid residues that are acceptable at the given position, while amino acids enclosed by braces, i.e., {}, designate the only amino acids which are unacceptable at the given heptad position. When a set of bracketed or braced amino acids is followed by a
35 number in parentheses i.e., (), it refers to the

number of subsequent amino acid positions for which the designated set of amino acids hold, e.g, a (2) means "for the next two heptad amino acid positions.

The ALLMOTI5 is written as follows:

5 {CDGHP}-{CFP}(2)-{CDGHP}-{CFP}(3)-
 {CDGHP}-{CFP}(2)-{CDGHP}-{CFP}(3)-
 {CDGHP}-{CFP}(2)-{CDGHP}-{CFP}(3)-
 {CDGHP}-{CFP}(2)-{CDGHP}-{CFP}(3)-
 {CDGHP}-{CFP}(2)-{CDGHP}-{CFP}(3)-

Translating this motif, it would read: "at the first (A) position of the heptad, any amino acid residue except C, D, G, H, or P is acceptable, at the next two (B,C) amino acid positions, any amino acid residue except C, F, or P is acceptable, at the fourth heptad position (D), any amino acid residue except C, D, G, H, or P is acceptable, at the next three (E, F, G) amino acid positions, any amino acid residue except C, F, or P is acceptable. This motif is designed to search for five consecutive heptad repeats (thus the repeat of the first line five times), meaning that it searches for 35-mer sized peptides. It may also be designed to search for 28-mers, by only repeating the initial motif four times. With respect to the ALLMOTI5 motif, a 35-mer search is preferred. Those viral sequences identified via such an ALLMOTI5 motif are listed in Table V, below, at the end of this Section. The viral sequences listed in Table V potentially exhibit antiviral activity, may be useful in the the identification of antiviral compounds, and are intended to be within the scope of the invention.

The 107x178x4 motif is written as follows:

30 [EFIKLNQSTVWY]-{CFMP}(2)-[EFIKLNQSTVWY]-{CFMP}(3)-
 [EFIKLNQSTVWY]-{CFMP}(2)-[EFIKLNQSTVWY]-{CFMP}(3)-
 [EFIKLNQSTVWY]-{CFMP}(2)-[EFIKLNQSTVWY]-{CFMP}(3)-
 [EFIKLNQSTVWY]-{CFMP}(2)-[EFIKLNQSTVWY]-{CFMP}(3)-

Translating this motif, it would read: "at the first (A) position of the heptad, any amino acid residue except E, F, I, K, L, N, Q, S, T, V, W, or Y

is acceptable, at the next two (B,C) amino acid positions, any amino acid residue except C, F, M or P is acceptable, at the fourth position (D), any amino acid residue except E, F, I, K, L, N, Q, S, T, V, W, or Y is acceptable, at the next three (E, F, G) amino acid positions, any amino acid residue except C, F, M or P is acceptable. This motif is designed to search for four consecutive heptad repeats (thus the repeat of the first line four times), meaning that it searches for 28-mer sized peptides. It may also be designed to search for 35-mers, by repeating the initial motif five times. With respect to the 107x178x4 motif, a 28-mer search is preferred. Those viral sequences identified via such a 107x178x4 motif are listed in Table V, below, at the end of this Section. The viral sequences listed in Table V potentially exhibit antiviral activity, may be useful in the the identification of antiviral compounds, and are intended to be within the scope of the invention.

The PLZIP series of motifs are as listed in FIG. 19. These motifs are designed to identify leucine zipper coiled-coil like heptads wherein at least one proline residue is present at some predefined distance N-terminal to the repeat. These PLZIP motifs find regions of proteins with similarities to HIV-1 DP-178 generally located just N-terminal to the transmembrane anchor. These motifs may be translated according to the same convention described above. Each line depicted in FIG. 19 represents a single, complete search motif. "X" in these motifs refers to any amino acid residue. In instances wherein a motif contains two numbers within parentheses, this refers to a variable number of amino acid residues. For example, X (1,12) is translated to "the next one to twelve amino acid residues, inclusive, may be any amino acid".

Tables VI through X, below, at the end of this

2164698

Section, list hits from such PLZIP motifs. The viral sequences listed in Table VI through X potentially exhibit antiviral activity, may be useful in the the identification of antiviral compounds, and are intended to be within the scope of the invention.

5 The Examples presented in Sections 17 and 18, below, demonstrate that respiratory syncytial virus and parainfluenza virus sequences identified via such a computer search exhibit antiviral and/or structural characteristics similar to those of DP-107 or DP-178.

10 The DP-107-like and DP-178-like analogous peptides may, further, contain any of the additional groups described for DP-178, above, in Section 5.1. For example, these peptides may include any of the additional amino-terminal groups which "X" of Tables I
15 through IV may represent, and may also include any of the carboxy-terminal groups which "Z" of Tables I through IV may represent.

20 Additionally, such DP-107-like and DP-178-like peptides may furthr include DP-107-like or DP-178-like peptides, such as those listed in Tables V through X, above, containing one or more amino acid substitutions, insertions, and/or deletions. Also, analogs of such DP-107-like and DP-178-like peptides are intended to be within the scope of the invention.
25 Such analogs of the invention may exhibit increased antiviral activity, and may, further, posses increased bioavailability, and/or stability, or reduced immune recognition.

30 The DP-107-like and DP-178-like amino acid substitutions, insertions and deletions, are as described for DP-178, above, in Section 5.1. Analog modifications are as described, below, in Section 5.3.

35

TABLE V

Search Results Summary for 107x178x4 and
ALLMOTI5 Motifs

[illegible]

PENV HV1H3	545-594	631-683	791-818			PENV HV1BN	501-590	609-708	763-831		
PENV HV1J3	556-605	642-694	802-829			PENV HV1BR	510-599	615-717	772-841		
PENV HV1JR		622-675	783-811			PENV HV1C4	510-606	620-724	778-855		
PENV HV1KB	555-596	637-677	776-824			PENV HV1EL	502-591	607-709	768-829		
PENV HV1MA	547-595	633-707	794-826			PENV HV1H2	506-594	610-712	767-836		
PENV HV1MF	543-592	629-681	789-816			PENV HV1H3	505-594	610-712	767-843		
PENV HV1MN	567-595	632-684	791-819			PENV HV1J3	517-605	622-723	778-843		
PENV HV1ND	536-583	621-673	783-813			PENV HV1JR	497-586	603-704	758-835		
PENV HV1OY	544-593	630-704	789-820			PENV HV1KB	511-545	555-599	618-718	772-848	
PENV HV1PV	545-594	631-683	791-818			PENV HV1MA	507-596	617-714	770-825		
PENV HV1RH	554-602	640-692	800-832			PENV HV1MF	503-592	622-710	765-841		
PENV HV1S1	536-585	622-674	782-809			PENV HV1MN	506-595	617-713	774-841		
PENV HV1S3	541-589	627-679	787-815			PENV HV1ND	495-584	601-702	757-825		
PENV HV1SC	545-593	631-683				PENV HV1OY	497-583	610-711	768-842		
PENV HV1W1	545-593	631-683	791-818			PENV HV1PV	505-584	610-712	767-843		
PENV HV1W2	536-584	622-674	782-809			PENV HV1RH	507-603	619-721	776-852		
PENV HV1Z2	542-591	628-680	790-820			PENV HV1S1	496-585	602-703	758-830		
PENV HV1Z6	545-593	630-682	792-822			PENV HV1S3	494-590	607-708	763-837		
PENV HV1Z8	573-601	634-678	797-828			PENV HV1SC	496-594	611-712	767-834		
PENV HV1ZH	545-594	627-686	791-823			PENV HV1W1	498-594	611-712	767-836		
PENV HV2BE	532-591	621-648	653-697			PENV HV1W2	499-584	602-703	758-827		
PENV HV2CA	534-593	623-650	655-699			PENV HV1Z2	502-591	607-709	764-831		
PENV HV2D1	523-550	555-582	644-688			PENV HV1Z6	504-593	609-711	766-840		
PENV HV2G1	524-551	556-583	613-640	645-693		PENV HV1Z8	512-601	617-675	682-718	774-831	
PENV HV2NZ	524-551	556-583	613-640	662-689		PENV HV1ZH	522-594	612-712	777-838		
PENV HV2RO	533-592	622-688				PENV HV2BE	510-595	617-680			
PENV HV2S2	527-554	559-586	648-682			PENV HV2CA	512-597	619-709			
PENV HV2S8	557-584	614-673				PENV HV2D1	501-586	608-698			
PENV HV2ST	527-554	559-586	648-692			PENV HV2G1	502-597	609-699			
PENV MCFE	473-512					PENV HV2NZ	488-587	609-699			
PENV MCFE3	488-515					PENV HV2RO	511-596	618-708			
PENV MLVAV	517-544					PENV HV2S2	505-590	612-702			
PENV MLVCB	510-539					PENV HV2S8	526-598	614-700			
PENV MLVF5	523-553					PENV HV2ST	506-590	612-702			
PENV MLVFF	523-553					PENV IPMAE	387-422	466-527			
PENV MLVFP	510-540					PENV JSRV	403-455	571-605			
PENV MLVKI	40-81					PENV MCFE	473-525	537-571			
PENV MLVMO	502-543					PENV MCFE3	474-526	538-572			
PENV MLVRD	497-538					PENV MLVAV	503-555	567-601			
PENV MLVRK	497-538					PENV MLVCB	498-550	562-596			
PENV MMTVB	458-485	562-589				PENV MLVF5	520-594	576-610			
PENV MMTVG	468-485	562-589				PENV MLVFF	520-594	576-610			
PENV MPMV	422-470					PENV MLVFP	520-594	576-610			
PENV MSVFB	57-84					PENV MLVHO	504-551	563-597			
PENV OMVVB	42-69	186-223	780-807			PENV MLVKI	40-92	104-138			
PENV RMCFV	487-517					PENV MLVMO	502-554	566-600			
						PENV MLVRD	497-549	561-595			

PENV SFV1	14-41	866-801					PENV MLVRK	497-549	561-595				
PENV SFV3L	18-46	319-367	673-700	863-898			PENV MMTVB	477-539	556-612				
PENV SIVA1	561-588	592-619	662-679	687-724			PENV MMTVG	477-539	556-612				
PENV SIVAG	566-593	597-624	658-685	703-730			PENV MPMV	408-474					
PENV SIVAI	548-603	634-708					PENV MSVFB	43-95	107-141				
PENV SIVAT	590-617	651-678					PENV OMVVB	22-84	185-223	684-746	780-816		
PENV SIVCZ	526-584	627-654					PENV RMCV	484-528	540-574				
PENV SIVGB	588-650	784-816					PENV RFFV	342-376					
PENV SIVM1	550-609	671-715					PENV SFV1	1-41	101-140	154-205	321-355	563-651	866-904
PENV SIVM2	156-215	277-288					PENV SFV3L	5-46	158-209	319-357	560-706	863-901	
PENV SIVMK	553-608						PENV SIVA1	269-310	551-623	643-693			
PENV SIVML	548-608						PENV SIVAG	556-628	651-699	808-852			
PENV SIVS4	553-612	642-669	691-718				PENV SIVAI	267-291	338-370	535-607	627-684	792-840	
PENV SIVSP	554-585	646-722					PENV SIVAT	264-288	549-621	644-692	796-833		
PENV SMRVH	400-462						PENV SIVCZ	263-281	330-365	512-584	669-703	803-837	
PENV SRV1	408-471						PENV SIVGB	568-664	677-725				
PENV VILV	773-800						PENV SIVM1	114-151	465-506	528-613	636-725	809-864	
PENV VILV1	780-807						PENV SIVM2	71-118	134-219	245-331			
PENV VILV2	782-809						PENV SIVMK	484-505	540-612	638-724			
PHEMA CVBLY	208-242						PENV SIVML	484-505	540-612	638-724			
PHEMA CVBM	208-242						PENV SIVS4	466-509	517-616	638-728	812-853		
PHEMA CVBQ	208-242						PENV SIVSP	470-513	521-620	642-732	811-848		
PHEMA CVHOC	208-242						PENV SMRVH	400-466					
PHEMA IAAIC	384-443						PENV SRV1	408-475					
PHEMA IADA1	381-451						PENV VILV	21-62	184-222	637-740	773-809		
PHEMA IADA2	423-453	499-543					PENV VILV1	21-62	184-222	643-746	780-816		
PHEMA IADA3	387-453						PENV VILV2	21-62	184-222	645-746	782-818		
PHEMA IADA4	418-478						PHEMA CVBLY	208-242					
PHEMA IADCZ	381-451						PHEMA CVBM	208-242					
PHEMA IADE1	402-453	506-533					PHEMA CVBQ	208-242					
PHEMA IADH1	371-437						PHEMA CVHOC	208-242					
PHEMA IADH2	371-437						PHEMA IAAIC	380-456					
PHEMA IADH3	371-437						PHEMA IABAN	384-440					
PHEMA IADH4	371-437						PHEMA IABUD	378-454					
PHEMA IADH5	371-437						PHEMA IACKA	378-454					
PHEMA IADH6	371-437						PHEMA IACKG	108-142	375-475	494-528			
PHEMA IADH7	371-437						PHEMA IACKP	380-452	487-532				
PHEMA IADIR	416-445						PHEMA IACKQ	380-452	487-532				
PHEMA IADM2	387-453						PHEMA IACKS	377-469	504-549				
PHEMA IADNZ	381-451						PHEMA IACKV	112-146	377-469				
							PHEMA IADA1	377-454					
							PHEMA IADA2	377-478	495-547				
							PHEMA IADA3	380-453					
							PHEMA IADA4	378-478	506-548				
							PHEMA IADCZ	378-454					
							PHEMA IADE1	21-55	377-472				
							PHEMA IADH1	364-440					

-35-

PHEMA_P11HW	79-110	368-393							PHEMA_INCP2	471-559								
PHEMA_P13B	86-93								PHEMA_INCP3	471-559								
PHEMA_P13H4	27-61								PHEMA_INCTA	471-559								
PHEMA_P13HA	27-61								PHEMA_INCYA	471-559								
PHEMA_P13HT	27-76								PHEMA_MEASE	48-90								
PHEMA_P13HU	23-70								PHEMA_MEASH	48-90								
PHEMA_P13HV	27-61								PHEMA_MEASI	48-87								
PHEMA_P13HW	27-61								PHEMA_MEASY	48-87								
PHEMA_P13HX	27-61								PHEMA_MUMPM	34-89								
PHEMA_RACVI	168-214	258-283							PHEMA_MUMPR	34-89								
PHEMA_SEND5	79-106								PHEMA_MUMPS	34-89								
PHEMA_SENDF	79-106								PHEMA_NDVA	8-52	477-529							
PHEMA_SENDH	79-106								PHEMA_NDVB	1-49								
PHEMA_SENDJ	79-106								PHEMA_NDVD	1-49								
PHEMA_SENDZ	79-106								PHEMA_NDVM	1-49								
PHEMA_SV41	22-52	394-421							PHEMA_NDVQ	1-49								
PHEMA_VACCC	119-146	175-202	216-243						PHEMA_NDVTG	1-49								
PHEMA_VACCI	109-146	175-202	216-243						PHEMA_NDVU	1-49								
PHEMA_VACCT	119-146	175-202	216-243						PHEMA_PHODV	38-73								
PHEMA_VACCV	109-146	175-202	216-242						PHEMA_P11HW	88-110								
PVENV_DHV11	318-368								PHEMA_P12H	247-281								
PVENV_EAV	120-147								PHEMA_P12HT	247-281								
PVENV_THOGV	313-347								PHEMA_P13B	38-83								
PVF03_VACCC	71-110	185-212							PHEMA_P13H4	13-110	394-428							
PVF03_VACCV	71-110	185-212							PHEMA_P13HA	20-110	394-428							
PVF05_VACCP	33-60								PHEMA_P13HT	13-110	394-428							
PVF05_VACCV	33-60								PHEMA_P13HU	13-110	394-428							
PVF11_VACCC	274-321								PHEMA_P13HV	13-110	394-428							
PVF11_VACCP	270-317								PHEMA_P13HW	13-110	394-428							
PVF12_VACCC	10-37	113-140	554-581						PHEMA_P13HX	13-110	394-428							
PVF12_VACCP	10-37	113-140	554-581						PHEMA_P14HA	54-88								
PVF16_VACCC	35-62	152-178							PHEMA_RACVI	168-214	258-280							
PVF16_VACCP	35-62	152-178							PHEMA_RINDK	48-87								
PVFP4_FOWPV	146-173								PHEMA_RINDL	48-87	191-225							
PVFUS_ORFNZ	59-68								PHEMA_SEND5	57-110								
PVFUS_VACCC	37-64								PHEMA_SENDF	57-110								
PVFUS_VACCV	37-64								PHEMA_SENDH	57-110								
PVG01_VACCC	225-252	301-335							PHEMA_SENDJ	57-110								
PVG01_VACCV	184-191	240-274							PHEMA_SENDZ	57-110								
PVG01_VARV	225-252	301-335							PHEMA_SV41	18-52	387-421							
PVG02_VACCV	98-123								PHEMA_SV6	27-82								
PVG02_VARV	98-123								PHEMA_SV6LN	27-82								
PVG03_HSVEB	146-176								PVENV_BEV	195-229								
PVG03_HSVEK	146-176								PVENV_DHV11	318-366								
PVG05_VACCC	48-75	131-161	225-269	355-389					PVENV_MCV1	262-288								
PVG05_VARV	48-75	124-161	255-289	355-389					PVENV_MCV2	262-288								
PVG07_H6V11	71-98								PVENV_THOGV	313-354								

PVG09_VACCC	308-338							PVENV_VACCC	257-295				
PVG09_VACCV	271-301							PVENV_VACCI	257-295				
PVG09_VARV	308-338							PVENV_VACCP	257-295				
PVG12_SPV1R	11-45							PVENV_VACCV	257-295				
PVG17_HSV11	177-204							PVFO1_VACCC	46-80	124-158			
PVG18_HSV11	174-208							PVFO1_VACCV	46-80	124-158			
PVG1_SPV1R	260-287							PVFO3_VACCC	71-110				
PVG1_SPV4	287-314	383-410						PVFO3_VACCV	71-110				
PVG22_HSV11	373-400	581-622	668-705	766-824				PVFO5_VACCC	81-129	282-320			
PVG24_HSV11	31-58							PVFO5_VACCP	81-129	282-320			
PVG28_HSV11	253-290	497-528						PVFO5_VACCV	81-129	283-321			
PVG2R_AMEPV	33-64	91-118						PVF11_VACCC	217-258	289-315			
PVG2_SPV1R	285-328							PVF11_VACCP	213-254	285-311			
PVG2_SPV4	148-173	175-205	262-310					PVF12_VACCC	1-67	102-143	189-236	350-388	544-581
PVG34_HSV11	95-122							PVF12_VACCP	1-67	102-143	189-236	350-388	544-581
PVG37_HSV11	442-468							PVF16_VACCC	155-194				
PVG38_HSV11	651-678	1088-1115						PVF16_VACCP	155-194				
PVG3L_AMEPV	2-29							PVFP3_FOWPV	1-43				
PVG3_SPV1R	15-49							PVFP4_FOWPV	139-173	239-273			
PVG3_SPV4	18-52	87-148						PVFP7_FOWPV	23-57				
PVG45_HSV9A	138-165							PVFPL_FOWP1	77-111				
PVG46_HSV11	142-169	346-373	887-924	973-1007				PVFUS_VACCC	30-64				
PVG48_HSV9A	380-394							PVFUS_VACCV	30-64				
PVG4R_AMEPV	4-31							PVG01_BPP22	84-135	400-468	475-513	608-659	
PVG4_SPV1R	116-146							PVG01_HSV11	271-306	512-563	591-647	730-784	
PVG51_HSV11	34-61	87-114						PVG01_VACCC	301-338				
PVG52_HSV9A	47-74							PVG01_VACCV	240-278				
PVG56_HSV11	582-609							PVG01_VARV	301-339				
PVG6_SPV1R	66-92							PVG03_HSV11	143-177				
PVG6_SPV4	56-83							PVG03_HSV11	143-177				
PVG63_HSV11	550-584							PVG03_VARV	64-88				
PVG64_HSV11	477-504							PVG05_VACCC	117-158	255-289	355-389		
PVG65_HSV11	1213-1254							PVG05_VARV	117-158	255-289	355-389		
PVG68_HSV11	362-406							PVG06_HSV11	61-109				
PVG67_HSV11	1342-1369							PVG07_HSV11	69-103				
PVG68_HSV11	261-288							PVG07_VACCC	114-175	324-358			
PVG72_HSV11	447-481							PVG07_VARV	114-175	324-358			
PVG75_HSV11	388-422							PVG08_VACCC	304-338				
PVG76_HSV11	200-227							PVG08_VACCV	267-301				
PVG7_SPV4	14-44							PVG09_VARV	304-338				
PVG1_IBVB	1230-1260	2408-2435						PVG10_HSV11	63-97				
PVG12_CVBF	399-426	642-676	1022-1084	1278-1305				PVG12_SPV1R	11-45				
PVG12_CVBL9	399-426		1022-1084	1278-1305				PVG16_HSV9A	58-95				
PVG12_CVBLY	399-426	642-676	1022-1084	1278-1305				PVG17_HSV11	92-129	177-211			
PVG12_CVBM	399-426	642-676	1022-1084	1278-1305				PVG18_HSV11	174-208	215-256			
PVG12_CVBQ	399-426	642-676	1022-1084	1278-1305				PVG1L_AMEPV	407-441				
PVG12_CVBV	399-426	642-676	1022-1084	1278-1305				PVG1_SPV1R	136-170	256-287	320-357		

PVGL2 CVH22	770-797	809-875	1056-1112			PVG1 SPV4	287-321	437-629	880-892	889-1055		
PVGL2 CVM4	643-684	1030-1092				PVG22 HSV11	117-168	74-108				
PVGL2 CVM45	36-63	591-632	978-1040			PVG24 HSV11	7-72					
PVGL2 CVM4H	502-543	889-951				PVG27 HSV11	184-219					
PVGL2 CVPFS	68-110	692-733	1072-1145	1353-1389		PVG28 HSV11	253-290					
PVGL2 CVPPU	68-107	690-731	1067-1143	1351-1387		PVG2R AMEPV	29-63	184-218				
PVGL2 CVPR8	468-509	845-921	1129-1165			PVG2 SPV1R	222-258	285-326				
PVGL2 CVPRM	468-509	845-921	1129-1165			PVG2 SPV4	255-310					
PVGL2 EBV	68-102					PVG33 HSV11	149-183					
PVGL2 FIPV	189-233	454-481	709-736	1072-1148	1356-1392	PVG34 HSV11	345-378					
PVGL2 IBV6	808-836	876-903	1067-1081			PVG35 HSV11	17-90					
PVGL2 IBVB	808-835	875-902	1058-1080			PVG37 HSV11	435-472					
PVGL2 IBVD2	808-836	876-903	1057-1081			PVG38 HSV11	84-118					
PVGL2 IBVK	808-835	875-902	1056-1080			PVG39 HSV11	124-158	266-300				
PVGL2 IBVM	808-835	875-902	1056-1080			PVG3 SPV1R	8-49	162-196	203-244			
PVGLB EBV	95-122	831-858				PVG3 SPV4	6-54	87-121				
PVGLB HCMVA	25-88	397-424	440-467	851-878		PVG43 HSV11	116-150	262-298	324-381	643-677		
PVGLB HCMVT	50-88	397-424	435-462	852-879		PVG45 HSVSA	121-162					
PVGLB HSVB1	427-464					PVG46 HSV11	45-86	939-1078	1251-1321			
PVGLB HSVB2	447-474					PVG48 HSV11	168-207					
PVGLB HSVBC	428-453					PVG48 HSVSA	360-417	611-666	733-767			
PVGLB HSVB1	443-470	934-961				PVG49 HSVSA	68-102					
PVGLB HSVB4	486-513	816-843				PVG4R AMEPV	4-38					
PVGLB HSVB4	443-470	934-961				PVG4 SPV4	88-130					
PVGLB HSVB	443-470	934-961				PVG51 HSV11	34-73	89-123				
PVGLB HSVEL	443-470	933-960				PVG51 HSVSA	28-70	123-157	162-196			
PVGLB HSVMD	93-120	362-379				PVG53 HSV11	67-127					
PVGLB MCMVS	381-408	441-475				PVG54 HSV11	355-396					
PVGLC HSV11	489-510					PVG55 HSV11	101-135					
PVGLC HSV1K	489-510					PVG55 HSVSA	126-178					
PVGLC HSVB	124-151					PVG56 HSV11	151-192	678-612	644-678	750-784	846-880	1111-1146
PVGLC HSVMB	63-87					PVG59 HSV11	10-72	89-123				
PVGLC HSVMG	62-86					PVG59 HSVSA	168-209					
PVGLC HSVMM	63-87					PVG5 SPV1R	65-103					
PVGLC VZVD	295-322					PVG61 HSV11	265-299					
PVGLC VZV8	295-322					PVG63 HSV11	546-584					
PVGLC HSV2	111-148					PVG65 HSV11	805-839	1213-1254				
PVGLF BRSA	38-65	154-202	216-243	442-469	488-531	PVG66 HSV11	154-188	328-410				
PVGLF BRVC	38-65	154-202	216-243	444-471	488-533	PVG67 HSV11	378-413	601-646	1321-1369	1478-1541		
PVGLF BRSVR	38-65	154-202	216-243	444-471	488-533	PVG68 HSV11	245-288					
PVGLF CDVO	252-283	340-367				PVG72 HSV11	447-484	723-757	812-849			
PVGLF HRSV1	38-65	154-203	442-471	488-515		PVG75 HSV11	271-305	388-422				
PVGLF HRSVA	38-65	154-202	213-243	488-518		PVG8 SPV1R	5-51					
PVGLF HRSVL	38-65	154-202	216-243	444-471	488-515	PVG1 IBVB	142-179	1233-1267	2118-2156	3388-3424	3475-3513	3761-3795
PVGLF HRSVR	38-65	154-202	213-243	442-471	488-518	PVG13 HCMVA	10-44					
PVGLF MEASE	228-262					PVGL2 CVBF	642-676	850-885	993-1088	1263-1305		
PVGLF MEASI	231-265					PVGL2 CVBL9	850-885	883-1109	1263-1306			

PVGLF MEASV	228-262						PVGL2 CVBLY	642-676	850-886	893-1108	1263-1305	
PVGLF MUMPM	20-54	447-486					PVGL2 CVBM	642-676	850-886	893-1108	1263-1305	
PVGLF MUMPR	20-54	447-486					PVGL2 CVBQ	642-676	850-886	893-1108	1263-1305	
PVGLF MUMPS	151-178	426-511					PVGL2 CVBV	642-676	850-886	893-1108	1263-1305	
PVGLF NDVA	151-178	426-512					PVGL2 CVH22	770-916	1055-1112			
PVGLF NDVB	151-178	426-512					PVGL2 CVM4	643-684	1001-1117	1270-1315		
PVGLF NDVI	151-178	426-512					PVGL2 CVMA6	691-632	949-1079	1218-1263		
PVGLF NDVM	151-178	426-512					PVGL2 CVMJH	602-643	860-876	1128-1174		
PVGLF NDVT	151-178	426-512					PVGL2 CVPFS	69-110	448-492	692-733	889-923	1352-1389
PVGLF NDVTG	151-178	426-512					PVGL2 CVPPU	69-110	448-480	690-731	887-921	1351-1387
PVGLF NDVU	151-178	426-512					PVGL2 CVPR8	224-258	468-508	666-699	816-862	1128-1165
PVGLF PHODV	36-63	221-262	309-336				PVGL2 CVPRM	224-258	468-508	666-699	816-862	1128-1165
PVGLF P11HC	147-174	210-266					PVGL2 EBV	68-102				
PVGLF P12H	90-117	141-175	238-268	483-528			PVGL2 FIPV	189-245	451-485	695-738	892-926	1355-1392
PVGLF P12HQ	90-117	141-175	238-268	483-528			PVGL2 IBV6	791-905	1057-1091			
PVGLF P12HT	90-117	141-175	238-268	483-528			PVGL2 IBVB	437-478	772-904	1056-1090		
PVGLF P13B	115-182	207-241	459-497				PVGL2 IBVD2	773-905	1057-1091			
PVGLF P13H4	115-182	207-241	457-497				PVGL2 IBVK	437-478	772-904	1056-1090		
PVGLF RINDK	224-265	458-485					PVGL2 IBVM	437-478	772-904	1056-1090		
PVGLF RINDL	224-285	458-506					PVGLB HCMVA	43-88	128-162	436-484	844-878	
PVGLF SEND5	122-149	211-245	480-507				PVGLB HCMVT	22-88	128-162	437-485	845-879	
PVGLF SENDF	122-149	211-245	480-507				PVGLB HSMV11	828-890				
PVGLF SENDH	122-149	211-245	480-507				PVGLB HSMV1F	827-889				
PVGLF SENDJ	122-149	211-245	480-507				PVGLB HSMV1K	827-889				
PVGLF SENDZ	122-149	211-245	480-507				PVGLB HSMV1P	828-890				
PVGLF SV41	144-185	241-269	459-496				PVGLB HSMV23	828-890				
PVGLF SV5	137-171	417-444					PVGLB HSMV2H	828-890				
PVGLF TRTV	124-161	193-200	457-484				PVGLB HSMV2S	817-871				
PVGLG BEFV	523-557						PVGLB HSMV8U	37-71	185-223			
PVGLG BR6VC	92-123						PVGLB HSMV81	859-913				
PVGLG HR6V1	63-83						PVGLB HSMV82	440-474	848-902			
PVGLG HR6V4	66-107						PVGLB HSMVBC	863-900				
PVGLG HR6V6	243-273						PVGLB HSMVE1	642-576	911-961			
PVGLG HR6V8	66-83						PVGLB HSMVE4	474-515	847-900			
PVGLG HSMVE4	271-298						PVGLB HSMVEA	642-576	911-961			
PVGLG HSMVEB	383-410						PVGLB HSMVEB	642-576	911-961			
PVGLG RABVT	489-519						PVGLB HSMVEL	642-576	910-960			
PVGLG VSVIG	472-499						PVGLB HSMVMD	390-435	648-683	787-845		
PVGLH EBV	549-579	619-648					PVGLB HSMVSA	240-288	406-447			
PVGLH HCMVA	107-135	270-287					PVGLB MCMV8	206-260	427-475	693-778	860-894	
PVGLH HCMVT	106-135						PVGLB PRVIF	847-891				
PVGLH HSMV6G	62-89	360-403					PVGLB VZVD	82-133	598-630	808-867		
PVGLH HSMV9A	368-415						PVGLC HSMV11	468-510				
PVGLI HCMVA	47-111						PVGLC HSMV1K	468-510				
PVGLM BUNGE	512-546	914-941	1128-1255				PVGLC HSMV2	442-476				
PVGLM BUNL7	913-950						PVGLC HSMV23	443-477				
PVGLM BUNYW	340-374	504-535	682-709				PVGLC HSMVBC	235-269				

PVGLM_DUGBV	945-972						PVGLC_HSVB	182-218				
PVGLM_HANTB	73-100	693-720					PVGLC_HSVMB	63-97				
PVGLM_HANTH	76-102						PVGLC_HSVMG	62-96				
PVGLM_HANTL	76-102						PVGLC_HSVMM	63-97				
PVGLM_HANTV	76-102						PVGLC_PRVF	183-236				
PVGLM_PHV	69-86						PVGLC_VZVD	280-321				
PVGLM_PUUMH	72-110						PVGLC_VZV8	280-321				
PVGLM_PUUMS	72-110						PVGLD_HSVEA	88-123				
PVGLM_SEOUR	73-100	513-540	694-721				PVGLD_HSVB	139-173				
PVGLM_SEOUS	73-100	513-540	694-721				PVGLD_HSVEK	139-173				
PVGLN_BEV	623-664						PVGLC_HSV11	111-146				
PVGLP_BEV	48-82	1146-1179	1184-1211	1505-1532			PVGLC_HSV2	111-159				
PVGLX_HSVB	17-44	413-444					PVGLF_BRSVA	146-202	504-546			
PVGLX_PVR1	427-461						PVGLF_BRSVC	146-202	267-302	506-547		
PVGLY_JUNIN	14-41						PVGLF_BRSVR	146-202	267-302	506-564		
PVGLY_LASSG	86-113						PVGLF_CDVO	228-297	340-381	568-602		
PVGLY_MOPEI	86-113	316-346					PVGLF_HRSV1	116-203	267-302	506-548		
PVGLY_P1ARV	334-375						PVGLF_HRSVA	116-202	267-302	506-549		
PVGLY_TACV	109-136	315-350					PVGLF_HRSVL	116-202	267-302	506-547		
PVGLY_TACV6	303-338						PVGLF_HRSVR	116-202	267-302	506-549		
PVGLY_TACV7	302-337						PVGLF_MEASE	116-184	228-269	452-500		
PVGLY_TACVT	303-338						PVGLF_MEASI	116-187	231-272	456-503		
PVGLZ_HSVEK	17-44						PVGLF_MEASY	116-184	228-269	452-500		
PVGNM_BPMV	403-430						PVGLF_MUMPM	20-54	103-179	235-272	447-502	
PVGNM_CPSMV	192-221						PVGLF_MUMPR	20-54	103-179	235-272	447-502	
PVGP8_EBV	104-149						PVGLF_MUMPS	20-54	103-179	235-272	447-502	
PVM1_REOVL	280-317						PVGLF_NDVA	117-182	231-272	426-512		
PVM21_REOVD	625-662						PVGLF_NDVB	122-182	231-272	426-517		
PVM22_REOVD	624-661						PVGLF_NDVI	133-182	238-272	426-517		
PVM2_REOVJ	624-661						PVGLF_NDVM	117-182	231-272	426-512		
PVM3_REOVD	159-186	343-370	458-483	631-680			PVGLF_NDVT	117-182	231-272	426-517		
PVMA2_BRSVA	124-152						PVGLF_NDVTG	122-182	231-272	426-517		
PVMA2_HRSVA	124-161						PVGLF_NDVU	122-182	231-272	426-512		
PVMAT_BRSVA	218-246						PVGLF_PHODV	28-63	197-268	309-350	633-681	
PVMAT_HRSVA	218-248						PVGLF_P11HC	123-174	207-267	458-503		
PVMAT_INCJJ	151-185						PVGLF_P12H	93-183	477-528			
PVMAT_NDVA	247-274						PVGLF_P12HG	93-183	477-528			
PVMAT_P12HT	96-123						PVGLF_P12HT	93-185	477-528			
PVMAT_P13B	201-231						PVGLF_P13B	117-182	207-241	458-518		
PVMAT_P13H4	201-231						PVGLF_P13H4	117-182	207-241	462-532		
PVMAT_SV41	323-353						PVGLF_RINDK	112-180	224-266	448-493		
PVME1_CVBIM	176-209						PVGLF_RINDL	112-180	224-266	448-506		
PVME1_CVTKE	176-208						PVGLF_SENDE	127-188	211-271	463-533		
PVME1_IBV6	21-48	184-218					PVGLF_SENDF	127-188	211-271	463-533		
PVME1_IBVB	21-48	184-218					PVGLF_SENDH	127-188	218-271	463-533		
PVME1_IBVB2	21-48	184-218					PVGLF_SENDJ	127-188	211-271	463-533		
PVME1_IBVK		184-218					PVGLF_SENDZ	127-188	211-271	463-533		

PVMP_CAMVC		220-254	273-324		PVGLF_SV41	98-186	454-508				
PVMP_CAMVD	28-58	220-254	273-324		PVGLF_SV5	103-171	241-275	451-487			
PVMP_CAMVE		227-254	273-324		PVGLF_TRTV	105-161	190-224	457-498			
PVMP_CAMVN		220-254	273-324		PVGLG_BEV	508-612					
PVMP_CAMVS		220-254	273-324		PVGLG_BRVVC	30-70	104-138				
PVMP_CAMVW		220-254	273-324		PVGLG_HRSV1	30-81					
PVMP_CERV	28-53	100-127			PVGLG_HRSV2	30-85					
PVMP_SOCMV	4-31	78-118			PVGLG_HRSV3	30-85					
PVMSA_HP8HE	284-328				PVGLG_HRSV4	30-107					
PVMT1_DHV11	38-85	237-264			PVGLG_HRSV5	30-85					
PVMT8_MYXVL	163-180				PVGLG_HRSV6	30-85					
PVMT9_MYXVL	465-492				PVGLG_HRSV7	30-85					
					PVGLG_HRSV8	30-81					
					PVGLG_HRSVA	30-67					
					PVGLG_HRSVL	25-85					
					PVGLG_HSVE4	271-305					
					PVGLG_SIGMA	344-381	464-498				
					PVGLG_SYN	488-523					
					PVGLG_VHSVO	363-397					
					PVGLG_VSVIG	476-510					
					PVGLH_EBV	53-87	160-201	336-380	653-694		
					PVGLH_HCMVA	103-137	270-311	693-741			
					PVGLH_HCMVT	102-136	692-740				
					PVGLH_HSV11	447-481					
					PVGLH_HSV1E	447-481					
					PVGLH_HSV6G	357-406					
					PVGLH_HSVBC	364-416					
					PVGLH_HSVE4	334-378	414-455				
					PVGLH_HSVEB	327-372	407-448				
					PVGLH_HSVSA	32-88	374-453	684-712			
					PVGLH_MCMV8	440-474					
					PVGLH_PRVKA	226-260					
					PVGLH_PRVN3	226-260					
					PVGLH_PRVRI	226-260					
					PVGLH_VZVD	455-506					
					PVGLI_HCMVA	47-111	323-358				
					PVGLM_BUNGE	512-567	685-737	1228-1262			
					PVGLM_BUNL7	643-677	918-950				
					PVGLM_BUNSH	643-677					
					PVGLM_BUNYW	340-374	604-663	905-939			
					PVGLM_DUGBV	837-889	1239-1300				
					PVGLM_HANTB	693-727					
					PVGLM_HANTH	72-106					
					PVGLM_HANTL	72-106					
					PVGLM_HANTV	72-106					
					PVGLM_PHV	73-111					
					PVGLM_PTPV	149-251					

[illegible]

2164698

TABLE VI

Search Results Summary for PCTLZIP,
P1CTLZIP, and P2CTLZIP Motifs

PCTLZIP		P1CTLZIP					P2CTLZIP		
LIBRARY FILE		LIBRARY FILE					LIBRARY FILE		
PENV FOAMV	481-496	PENV BIV06		434-450			PENV BIV06	526-542	
PENV HV1MA	438-453	PENV BIV27		463-478			PENV BIV27	554-571	
PENV HV1MF	183-198	PENV FOAMV		481-496	864-880		PENV FENV1	30-47	630-647
PENV HV1RH	445-460	PENV HV1KB		752-768			PENV FIVPE	781-798	
PENV HV1SC	186-201	PENV HV1MA		437-453			PENV FIVSD	779-798	
PENV HV1Z2	123-138	PENV HV1MF		183-198			PENV FIVT2	780-797	
PENV HV1ZH	438-453	PENV HV1RH		444-460			PENV FLVCA	38-55	624-641
PENV HV2BE	750-765	PENV HV1S1		738-754			PENV FLVGL	606-622	
PENV HV2D1	741-756	PENV HV1SC		186-201			PENV FLVLB	625-642	
PENV HV2G1	741-756	PENV HV1Z2		123-138			PENV FLVSA	602-618	
PENV HV2NZ	742-757	PENV HV1Z3		117-133			PENV FOAMV	710-727	957-974
PENV HV2RO	751-766	PENV HV1ZH		437-453			PENV FSVGA	625-642	
PENV HV2SB	743-758	PENV HV2BE		750-765			PENV FSVGB	606-622	
PENV HV2ST	745-760	PENV HV2D1		741-756			PENV FSVSM	608-625	
PENV JSRV	104-118	PENV HV2G1		741-756			PENV HV1QY	123-140	
PENV MMTVB	618-633	PENV HV2NZ		742-757			PENV HV1Z2	410-427	
PENV MMTVG	618-633	PENV HV2RO		751-766			PENV HV1Z3	164-171	
PENV SIVMK	139-154	PENV HV2SB		743-758			PENV HV2CA	750-767	
PENV SIVML	139-154	PENV HV2ST		745-760			PENV MCFF	600-617	
PHEMA CVBLY	391-406	PENV JSRV		104-118	541-557		PENV MCFF3	601-618	
PHEMA CVBM	391-406	PENV MCFF		397-413			PENV MLVAV	630-647	
PHEMA CVBQ	391-406	PENV MCFF3		397-413			PENV MLVCB	625-642	
PHEMA CVHOC	391-406	PENV MLVAV		427-443			PENV MLVFB	638-656	
PHEMA CVMA5	402-417	PENV MLVCB		422-438			PENV MLVFF	639-656	
PHEMA CVMS	403-418	PENV MLVHO		423-439			PENV MLVFP	639-656	
PHEMA INBAA	295-310	PENV MLVMO		426-442			PENV MLVHO	626-643	
PHEMA INBBE	303-318	PENV MLVRD		424-440			PENV MLVKI	167-184	
PHEMA INBBO	293-308	PENV MLVRK		424-440			PENV MLVMO	629-646	
PHEMA INBEN	301-316	PENV MMTVB		618-633			PENV MLVRD	624-641	
PHEMA INBFU	286-301	PENV MMTVG		618-633			PENV MLVRK	624-641	
PHEMA INBGL	296-311	PENV SFV1		864-880			PENV MSVFB	170-187	
PHEMA INBHK	293-308	PENV SFV3L		861-877			PENV RMCFFV	603-620	
PHEMA INBIB	288-303	PENV SIVGB		93-109			PENV SFV1	710-727	957-974
PHEMA INBID	288-314	PENV SIVMK		139-154	802-818		PENV SFV3L	707-724	954-971
PHEMA INBLE	302-317	PENV SIVML		139-154	801-817		PENV SIVM1	766-783	
PHEMA INBMD	292-307	PENV SIVS4		806-822			PENV SIVMK	765-782	
PHEMA INBME	296-311	PENV SIVSP		810-826			PENV SIVML	764-781	
PHEMA INBNA	288-303	PHEMA CDVO		36-52			PENV SIVS4	769-786	
PHEMA INBOR	301-316	PHEMA CVBLY		391-406			PENV SIVSP	773-790	
PHEMA INBSI	301-316	PHEMA CVBM		391-406			PENV SMRVH	536-553	
PHEMA INBSJ	286-313	PHEMA CVBQ		391-406			PENV SMSAV	42-59	
PHEMA INBUS	294-308	PHEMA CVHOC		391-406			PHEMA CDVO	36-53	200-217
PHEMA INBVI	296-311	PHEMA CVMA5		402-417			PHEMA CVBLY	391-406	
PHEMA INBVK	303-318	PHEMA CVMS		403-418			PHEMA CVBM	391-406	
PHEMA INBYB	286-301	PHEMA IAAIC		237-253			PHEMA CVBQ	391-406	

2164698

PHEMA_MUMPM	133-148		PHEMA_IABAN	221-237			PHEMA_CVHOC	391-408	
PHEMA_MUMPR	133-148		PHEMA_IABUD	234-250			PHEMA_IAAIC	322-339	
PHEMA_MUMPS	133-148		PHEMA_IACKA	234-250			PHEMA_IABAN	308-323	
PHEMA_PI1HW	345-360		PHEMA_IACKG	231-247			PHEMA_IABUD	320-337	
PHEMA_PI2H	65-80		PHEMA_IACKV	230-246			PHEMA_IACKA	320-337	
PHEMA_PI2HT	65-80		PHEMA_IADA1	234-250			PHEMA_IACKG	316-333	
PHEMA_RINDK	368-383		PHEMA_IADA3	237-253			PHEMA_IACKP	302-319	
PHEMA_SV5	7-94		PHEMA_IADCZ	234-250			PHEMA_IACKQ	302-319	
PHEMA_SV5CM	7-94		PHEMA_IADH1	221-237			PHEMA_IACKS	319-336	
PHEMA_SV5CP	7-94		PHEMA_IADH2	221-237			PHEMA_IACKV	315-332	
PHEMA_SV5LN	7-94		PHEMA_IADH3	221-237			PHEMA_IADA1	320-337	
PVENV_DHV11	42-57		PHEMA_IADH4	221-237			PHEMA_IADA3	322-338	
PVFP7_CAPVK	89-104		PHEMA_IADH5	221-237			PHEMA_IADCZ	320-337	
PVFUS_VACC6	72-87		PHEMA_IADH6	221-237			PHEMA_IADH1	308-323	
PVG01_BPP22	242-257		PHEMA_IADH7	221-237			PHEMA_IADH2	308-323	
PVG01_HSVEB	169-184		PHEMA_IADM2	237-253			PHEMA_IADH3	308-323	
PVG01_HSVI1	210-225	317-332	PHEMA_IADNZ	234-250			PHEMA_IADH4	308-323	
PVG08_BPT4	184-199		PHEMA_IAEN6	221-237			PHEMA_IADH6	308-323	
PVG07_BPT4	885-900		PHEMA_IAEN7	237-253			PHEMA_IADH7	308-323	
PVG08_HSVI1	134-149		PHEMA_IAPFR	230-246			PHEMA_IADM2	322-338	
PVG10_BPPH2	183-198		PHEMA_Iahal	236-252			PHEMA_IADNZ	320-337	
PVG10_BPPZA	183-198		PHEMA_IAHAR	235-251			PHEMA_IADU3	322-339	
PVG10_HSVSA	108-124		PHEMA_Iahc6	230-246			PHEMA_IAEN6	308-323	
PVG16_BPP1	81-96		PHEMA_Iahc7	230-246			PHEMA_IAEN7	322-339	
PVG18_BPT4	468-483		PHEMA_Iahcd	230-246			PHEMA_IAPFR	315-332	
PVG25_BPT4	97-112		PHEMA_Iahde	230-246			PHEMA_IAGRE	320-337	
PVG28_HSVI1	20-36		PHEMA_Iahfo	236-252			PHEMA_IAGU2	320-337	
PVG30_BPPH8	11-84		PHEMA_Iahk6	236-252			PHEMA_IAGUA	319-336	
PVG36_BPOX2	22-37		PHEMA_Iahk7	236-252			PHEMA_Iahal	321-338	
PVG36_HSV6A	108-123		PHEMA_Iahle	230-246			PHEMA_Iahc6	315-332	
PVG37_BPT2	1253-1268		PHEMA_Iahlo	230-246			PHEMA_Iahc7	315-332	
PVG37_HSVI1	284-289		PHEMA_Iahmi	236-252			PHEMA_Iahcd	315-332	
PVG55_HSVI1	22-37	143-168	PHEMA_Iahnm	236-252			PHEMA_Iahde	315-332	
PVG56_HSVI1	268-283		PHEMA_Iahro	236-252			PHEMA_Iahfo	321-338	
PVG58_HSVI1	102-117		PHEMA_Iahsa	236-252			PHEMA_Iahk6	321-338	
PVG59_HSVI1	267-282		PHEMA_Iahsp	230-246			PHEMA_Iahk7	321-338	
PVG65_HSVI1	518-533		PHEMA_Iahsw	230-246			PHEMA_Iahle	315-332	
PVG9_BPPH2	234-249		PHEMA_Iahte	236-252			PHEMA_Iahlo	315-332	
PVG9_BPPZA	234-249		PHEMA_Iahto	236-252			PHEMA_Iahmi	321-338	
PVG9_SPV1R	57-72		PHEMA_Iahur	236-252			PHEMA_Iahnm	321-338	
PVGF_BPPHX	234-249		PHEMA_Iakie	235-251			PHEMA_Iahnn	315-332	
PVGL2_CVBF	264-279		PHEMA_Ialen	235-251			PHEMA_Iahpr	315-332	
PVGL2_CVBL9	264-279		PHEMA_Iamaa	233-249			PHEMA_Iahro	321-338	
PVGL2_CVBLY	264-279		PHEMA_Iamab	238-254			PHEMA_Iahsa	321-338	
PVGL2_CVBM	264-279		PHEMA_Iamao	237-253			PHEMA_Iahsp	315-332	
PVGL2_CVBQ	264-279		PHEMA_Iame1	237-253			PHEMA_Iahsw	315-332	
PVGL2_CVBV	264-279		PHEMA_Iame2	237-253			PHEMA_Iahte	321-338	

PVGL2_CVPF3	442-457		PHEMA_IAME6	221-237				PHEMA_IAHTO	321-338	
PVGL2_CVPPU	440-455	504-519	PHEMA_IAMIN	85-101	231-247			PHEMA_IAHUR	321-338	
PVGL2_CVPR8	218-233		PHEMA_IANT6	237-253				PHEMA_IJAP	317-334	
PVGL2_CVPRM	218-233		PHEMA_IACU7	221-237				PHEMA_IAMAA	319-336	
PVGL2_IBV6	1056-1071		PHEMA_IARUD	234-250				PHEMA_IAMAB	324-341	
PVGL2_IBVB	1055-1070		PHEMA_IASE2	234-250				PHEMA_IAMAO	322-339	
PVGL2_IBVD2	1056-1071		PHEMA_IASH2	234-250				PHEMA_IAME1	322-339	
PVGL2_IBVK	1055-1070		PHEMA_IASTA	230-246				PHEMA_IAME2	322-339	
PVGL2_IBVM	1055-1070		PHEMA_IATAI	236-251				PHEMA_IAME6	306-323	
PVGLB_HSVSA	701-716		PHEMA_IATKM	234-250				PHEMA_IAMIN	316-333	
PVGLB_PRVIF	203-218		PHEMA_IATKO	233-249				PHEMA_IANT6	322-339	
PVGLC_HSVBC	475-490		PHEMA_IATKR	230-246				PHEMA_IAPIL	320-337	
PVGLC_HSVE4	444-459		PHEMA_IATKW	229-245				PHEMA_IACU7	306-323	
PVGLC_HSVB	427-442		PHEMA_IAUDO	237-253				PHEMA_IARUD	320-337	
PVGLC_PRVIF	446-461		PHEMA_IAUSS	236-251				PHEMA_IASE2	320-337	
PVGLD_HSV11	79-94		PHEMA_IAV17	238-254				PHEMA_IASH2	321-338	
PVGLD_HSV2	79-94		PHEMA_IAXIA	236-251				PHEMA_IASTA	315-332	
PVGLF_BR6VA	265-280		PHEMA_IAZCO	237-253				PHEMA_IATKM	320-337	
PVGLF_BR6VC	265-280		PHEMA_IASH2	221-237				PHEMA_IAUDO	322-339	380-397
PVGLF_BR6VR	265-280		PHEMA_IASH3	221-237				PHEMA_IAV17	323-340	
PVGLF_HR6V1	265-280		PHEMA_IAZUK	237-253				PHEMA_IAZCO	322-338	
PVGLF_HR6VA	265-280		PHEMA_INBAA	116-131	285-310			PHEMA_IASH2	306-323	
PVGLF_HR6VL	265-280		PHEMA_INBBE	123-139	303-318			PHEMA_IASH3	306-323	
PVGLF_HR6VR	265-280		PHEMA_INBBO	116-132	283-308			PHEMA_IAZUK	322-339	
PVGLF_MUMPS	5-94		PHEMA_INBEN	123-139	301-316			PHEMA_MUMPM	101-118	
PVGLI_VZVD	278-293		PHEMA_INBFU	108-124	288-301			PHEMA_MUMPR	101-118	
PVGLM_HANTB	900-915		PHEMA_INBGL	119-135	296-311			PHEMA_MUMPS	101-118	
PVGLM_PTPV	743-758		PHEMA_INBHK	116-132	283-308			PHEMA_NDVA	93-110	
PVGLM_SEOUR	901-916		PHEMA_INBIB	108-124	288-303			PHEMA_NDV8	93-110	
PVGLM_SEOUS	900-915		PHEMA_INBID	120-136	289-314			PHEMA_NDVO	93-110	
PVGLY_LAS8G	426-441		PHEMA_INBLE	123-139	302-317			PHEMA_NDVH	93-110	
PVGLY_LAS6J	427-442		PHEMA_INBMD	113-129	292-307			PHEMA_NDVI	93-110	
PVGLY_MOPEI	425-440		PHEMA_INBME	116-132	296-311			PHEMA_NDVM	93-110	
PVM3_REQVD	521-536		PHEMA_INBNA	108-124	288-303			PHEMA_NDVQ	93-110	
PVMSA_HPBGS	380-395		PHEMA_INBOR	123-139	301-316			PHEMA_NDVTG	93-110	
PVMSA_HPBV8	187-202		PHEMA_INB8I	123-139	301-316			PHEMA_NDVU	93-110	
PVMSA_WHV1	378-393		PHEMA_INBSJ	119-135	298-313			PHEMA_PHODV	36-53	
PVMSA_WHV59	383-398		PHEMA_INBUS	116-132	294-309			PHEMA_P11HW	486-503	
PVMSA_WHV7	383-398		PHEMA_INBVI	116-132	296-311			PHEMA_P13B	111-128	
PVMSA_WHV8	383-398		PHEMA_INBVK	123-139	303-318			PHEMA_P13H4	111-128	
PVMSA_WHVBI	383-398		PHEMA_INBYB	108-124	286-301			PHEMA_P13HA	111-128	
PVMSA_WHVW6	234-249		PHEMA_MUMPM	133-148				PHEMA_P13HT	111-128	
PVMT2_IAANN	25-40		PHEMA_MUMPR	133-148				PHEMA_P13HU	111-128	
PVMT2_IABAN	25-40		PHEMA_MUMPS	133-148				PHEMA_P13HV	111-128	
PVMT2_IAFOW	25-40		PHEMA_P11HW	345-360				PHEMA_P13HW	111-128	
PVMT2_IAPPR	25-40		PHEMA_P12H	65-81				PHEMA_P13HX	111-128	
PVMT2_IAPFW	25-40		PHEMA_P12HT	65-81				PHEMA_P14HA	50-67	

2164698

PVMT2_IAL1	25-40		PHEMA_P13B	324-340					PHEMA_SV41	85-102	
PVMT2_IAL2	25-40		PHEMA_P13H4	324-340					PHEMA_SV5	84-101	
PVMT2_IAMAN	25-40		PHEMA_P13HA	324-340					PHEMA_SV5CM	84-101	
PVMT2_IAPUE	25-40		PHEMA_P13HT	324-340					PHEMA_SV5CP	84-101	
PVMT2_IASIN	25-40		PHEMA_P13HU	324-340					PHEMA_SV5LN	84-101	
PVMT2_IAUDO	25-40		PHEMA_P13HV	324-340					PVFO5_VACCC	280-287	
PVMT2_IAWIL	25-40		PHEMA_P13HW	324-340					PVFO5_VACCP	280-287	
PVMT9_MYXVL	226-241		PHEMA_P13HX	324-340					PVFO5_VACCV	281-288	
			PHEMA_RINDK	368-383					PVFO8_VACCC	176-193	
			PHEMA_SV5	7-84					PVFO8_VACCV	176-183	
			PHEMA_SV5CM	7-84					PVG27_HSVSA	208-226	
			PHEMA_SV5CP	7-84					PVG28_HSVI1	173-190	
			PHEMA_SV5LN	7-84					PVG39_HSVI1	648-665	
			PVENV_DHV11	42-57					PVG43_HSVI1	108-126	521-538
			PVENV_EAV	25-41					PVG67_HSVI1	171-188	
			PVFP2_FOWPV	88-104					PVG72_HSVI1	1252-1289	
			PVFP7_CAPVK	88-104					PVGF1_IBVB	3073-3090	
			PVFUS_VACCB	72-87					PVGL2_IBV6	1094-1111	
			PVG01_HSVB	168-184					PVGLB_HSVE1	738-753	
			PVG01_HSVI1	208-225	317-332				PVGLB_HSVE4	675-692	
			PVG08_HSVI1	134-149					PVGLB_HSVEA	738-753	
			PVG10_HSVSA	108-124					PVGLB_HSVB	738-753	
			PVG11_HSVI1	103-119					PVGLB_HSVEL	738-753	
			PVG12_HSVI1	270-286					PVGLB_ILTV6	597-614	
			PVG1_SPV1R	76-92					PVGLB_ILTV8	607-624	
			PVG29_HSVI1	20-35					PVGLB_ILTVT	607-624	
			PVG36_BPOX2	22-37					PVGLC_PRVIF	180-197	
			PVG36_HSVSA	108-123					PVGLE_VZVD	489-488	
			PVG37_HSVI1	284-299					PVGLF_SV5	401-418	
			PVG41_HSVI1	244-260					PVGLH_HCMVA	365-382	
			PVG46_HSVI1	1244-1260					PVGLH_HCMVT	364-381	
			PVG55_HSVI1	22-37	143-158				PVGLH_HSVI1	245-262	803-820
			PVG56_HSVI1	268-283					PVGLH_HSV1E	245-262	803-820
			PVG58_HSVI1	101-117					PVGLI_HSVI1	43-60	
			PVG58_HSVSA	130-146	330-346				PVGLM_BUNL7	81-98	
			PVG59_HSVI1	267-282					PVGLM_BUNSH	81-98	
			PVG65_HSVI1	362-378	518-533				PVGLM_PUUMH	712-729	
			PVG71_HSVSA	89-105					PVGLM_PUUMS	712-729	
			PVG9_BPPH2	234-249					PVGLM_RVFX	344-361	
			PVG9_BPPZA	234-249					PVGLM_RVFXZ	344-361	
			PVG9_SPV1R	57-72					PVGLY_LASSG	12-94	
			PVGF1_IBVB	2210-2226					PVGLY_LASSJ	12-94	
			PVGL2_CVBF	123-139	174-190	264-279			PVGLY_LYCVA	12-94	
			PVGL2_CVBL9	123-139	174-190	264-279			PVGLY_LYCVW	12-94	
			PVGL2_CVBLV	123-139	174-190	264-279			PVGLY_MOPEI	12-94	
			PVGL2_CVBM	123-139	174-190	264-279			PVM1_REOVD	280-287	
			PVGL2_CV8Q	31-47	123-139	174-190	264-279		PVM1_REOVL	280-287	

-51-

2164698 **TABLE VII**

Search Results Summary for P3CTLZIP, P4CTLZIP,
P5CTLZIP, and P6CTLZIP Motifs

-55-

PVMO1_VACCV	83-101	126-144	PVGL2_CVM4	999-1018	PVENV_THOGV	356-378	PHEMA_P12H	13-34	
PVM1_REOVD	227-246		PVGL2_CVMA5	947-966	PVG01_VACCC	298-318	PHEMA_P12HT	13-34	
PVM1_REOVL	227-246		PVGL2_CVMJH	868-877	PVG01_VACCV	237-257	PHEMA_SV5	7-28	379-400
PVMAT_HRSVA	44-62		PVGL2_CVPF5	84-83	PVG01_VARV	298-318	PHEMA_SV5CM	7-28	379-400
PVMAT_NDVA	180-208		PVGL2_CVPPU	84-83	PVG08_VACCC	31-51	PHEMA_SV5CP	7-28	379-400
PVMAT_NDVB	180-208		PVGL2_CVPR8	814-833	PVG06_VARV	31-51	PHEMA_SV6LN	7-28	379-400
PVMP_CAMVC	183-201		PVGL2_CVPRM	814-833	PVG09_BPPF1	25-45	PVG01_HSVB	169-180	
PVMP_CAMVD	183-201		PVGL2_FIPV	1041-1080	PVG12_HSVI1	151-171	PVG01_HSVI1	589-610	
PVMP_CAMVE	183-201		PVGL2_IBV6	588-607	PVG22_HSVI1	300-320	PVG23_HSVI1	314-335	
PVMP_CAMVN	183-201		PVGL2_IBVB	587-606	PVG39_HSVI1	648-668	PVG37_BPOX2	66-86	
PVMP_CAMVS	183-201		PVGL2_IBVD2	588-607	PVG51_HSVI1	29-49	PVG43_HSVI1	167-176	
PVMP_CAMVW	183-201		PVGL2_IBVK	587-606	PVG63_HSVI1	338-356	PVG55_HSVI1	288-309	
PVMP_FMVD	180-198		PVGL2_IBVM	587-606	PVG65_HSVI1	117-137	PVG55_HSVSA	85-106	
			PVGLB_HCMVA	708-725	PVG74_HSV8A	124-144	PVG58_HSVI1	1155-1176	
			PVGLB_HCMVT	707-726	PVGL2_IBV6	328-348	PVG58_HSVSA	268-287	
			PVGLB_HSV6U	117-136	PVGL2_IBVB	327-347	PVG80_HSVI1	30-51	
			PVGLB_ILTV6	256-275	PVGL2_IBVD2	328-348	PVG83_HSVI1	238-259	
			PVGLB_ILTVS	268-285	PVGL2_IBVD3	328-348	PVG81_IBVB	1858-1877	
			PVGLB_ILTVT	268-285	PVGL2_IBVK	327-347	PVG83_HCMVA	167-178	
			PVGLC_HSV11	3-94	PVGL2_IBVM	327-347	PVGL2_CVBF	1259-1280	
			PVGLC_HSV1K	3-94	PVGL2_IBVU2	310-330	PVGL2_CVBL9	1259-1280	
			PVGLC_HSVBC	476-494	PVGLB_EBV	732-752	PVGL2_CVBLV	1259-1280	
			PVGLQ_CHAV	436-455	PVGLB_HCMVA	760-770	PVGL2_CVBM	1259-1280	
			PVGLQ_RABVH	372-391	PVGLB_HCMVT	761-771	PVGL2_CVBO	1259-1280	
			PVGLI_HSVB	44-63	PVGLB_HSV23	79-98	PVGL2_CVBV	1259-1280	
			PVGLI_VZVD	278-297	PVGLB_HSV2H	79-98	PVGL2_CVM4	1317-1338	
			PVGLM_BUNGE	117-136	PVGLB_HSV2S	65-85	PVGL2_CVMA5	1265-1286	
			PVGLM_PHV	152-171	PVGLB_HSV6U	72-92	PVGL2_CVMJH	1176-1197	
			PVGLM_PTPV	997-1016	PVGLB_HSVB2	279-299	PVGLB_HSVI1	83-104	
			PVGLM_PUUMH	155-174	PVGLB_HSV8A	63-83	PVGLB_HSV1F	82-103	
			PVGLM_PUUMS	155-174	PVGLB_MCMVS	738-758	PVGLB_HSV1K	82-103	
			PVGLM_RVFEV	830-849	PVGLF_P13H4	283-303	PVGLB_HSV1P	83-104	
			PVGLM_RVFEVZ	830-849	PVGLQ_RABVE	454-474	PVGLB_MCMVS	136-156	
			PVGLM_UUK	855-874	PVGLQ_RABVH	454-474	PVGLC_PRVIF	448-467	
			PVGLY_LYCVW	89-108	PVGLQ_RABVP	454-474	PVGLF_CDVO	338-357	
			PVGNB_CPMV	1165-1184	PVGLQ_RABV6	454-474	PVGLF_MEASE	224-245	
			PVM3_REOVD	521-540	PVGLQ_RABVT	454-474	PVGLF_MEASI	227-248	
			PVME1_CVBM	171-180	PVGLH_MCMVS	670-690	PVGLF_MEASY	224-245	
			PVME1_CVH22	136-155	PVGLM_BUNL7	1326-1345	PVGLF_MUMPM	448-467	
			PVME1_CVPF5	174-193	PVGLM_BUNSH	1326-1345	PVGLF_MUMPR	448-467	
			PVME1_CVPPU	174-193	PVGLM_BUNYW	998-1016	PVGLF_MUMPS	448-467	
			PVME1_CVPRM	174-193	PVGLM_HANTB	998-1019	PVGLF_PHODV	305-326	
			PVME1_CVTKE	171-190	PVGLM_HANTH	1000-1020	PVGLF_P11HC	458-477	
					PVGLM_HANTL	1001-1021	PVGLF_P12H	450-471	
					PVGLM_HANTV	1001-1021	PVGLF_P12HG	450-471	
					PVGLM_RVFEVZ	1158-1176	PVGLF_P12HT	450-471	
					PVGLM_SEOUR	1000-1020	PVGLF_P13B	405-426	453-474

TABLE VIII

Search Results Summary for P7CTLZIP,
P8CTLZIP, and P9CTLZIP Motifs

-60-

PHEMA_IAVI7	38-60	PVGL2_IBVK	195-218	PVGLB_HSVMD	589-613				
PHEMA_IAX31	37-59	PVGL2_IBVM	195-218	PVGLB_ILTV6	597-621				
PHEMA_IAXCO	37-59	PVGL2_IBVU1	178-201	PVGLB_ILTVS	607-631				
PHEMA_IAXH2	21-43	PVGL2_IBVU2	178-201	PVGLB_ILTVT	607-631				
PHEMA_IAXH3	21-43	PVGL2_IBVU3	178-201	PVGLB_HSV11	413-437				
PHEMA_IAXUK	37-59	PVGLB_HCMVA	535-558	PVGLB_VZVD	489-493				
PHEMA_PHODV	38-58	PVGLB_HCMVT	536-559	PVGLF_SV6	401-425				
PHEMA_P12H	65-87	PVGLB_HSV6A	483-508	PVGLH_HCMVA	574-598				
PHEMA_P12HT	65-87	PVGLB_MCMVS	566-589	PVGLH_HCMVT	673-697				
PVPF7_CAPVK	88-111	PVGLC_HSV11	467-490	PVGLH_HSV11	443-467	803-827			
PVFUS_VACC8	72-94	PVGLC_HSV1K	467-490	PVGLH_HSV1E	443-467	803-827			
PVG01_HSVI1	317-339	PVGLC_HSV2	435-458	PVGLM_BUNL7	31-55				
PVG03_VACCC	50-72	PVGLC_HSV23	436-459	PVGLM_BUNSH	31-55				
PVG03_VARV	50-72	PVGLM_BUNL7	1387-1410	PVGLM_HANTH	694-718				
PVG04_VACCC	11-33	PVGLM_BUNSH	1387-1410	PVGLM_RVFX	344-368				
PVG04_VARV	11-33	PVGLM_UUK	966-989	PVGLM_RVFXZ	344-368				
PVG19_HSVI1	88-110	PVGLY_JUNIN	12-35	PVGLM_UUK	561-585				
PVG28_HSVI1	173-185	PVGLY_LASSG	12-35	PVGNM_CPMV	311-335				
PVG29_HSVI1	20-42	PVGLY_LASSJ	12-35	PVGP2_EBV	657-681				
PVG46_HSVI1	134-158	PVGLY_LYCV A	12-35	PVGP3_EBV	854-878				
PVG48_HSV6A	71-93	PVGLY_LYCVW	12-35	PVM1_REOVD	280-304				
PVG58_HSV6A	266-288	PVGLY_MOPEI	12-35	PVM1_REOVL	280-304				
PVG59_HSVI1	267-289	PVGLY_TACV	12-35	PVM21_REOVD	168-192				
PVG5_SPV4	42-64	PVGLY_TACV6	12-35	PVM22_REOVD	168-192				
PVG80_HSVI1	53-75	PVGLY_TACV7	12-35	PVM2_REOVJ	168-192				
PVG65_HSVI1	1347-1389	PVGLY_TACVT	12-35	PVM2_REOVL	168-192				
PVG8_SPV1R	60-82	PVGNM_CPMV	741-764	PVMAT_MEAS1	87-111				
PVGL2_IBV6	1056-1078	PVM1_REOVD	324-347	PVMAT_SSPVB	314-338				
PVGL2_IBVB	1056-1077	PVM1_REOVL	464-477	PVME1_CVBM	137-161				
PVGL2_IBVD2	1056-1078	PVMAT_MUMPS	227-250	PVME1_CVHOC	137-161				
PVGL2_IBVK	1055-1077	PVMSA_HPBD8	268-292	PVME1_CVTKE	137-161				
PVGL2_IBVM	1055-1077	PVMSA_HPBD8	268-291	PVME1_IBV6	74-98				
PVGLB_HSV6U	117-139	PVMSA_HPBDU	231-254	PVME1_IBVB	74-98				
PVGLB_HSVB2	745-767	PVMSA_HPBDW	289-292	PVME1_IBVB2	74-98				
PVGLC_HSVMB	399-421	PVMSA_HPBE	236-259	PVME1_IBVK	74-98				
PVGLC_HSVMB	388-420			PVMSA_HPBG8	271-295				
PVGLC_HSVMM	399-421			PVMSA_WHV1	269-293				
PVGLF_BR6VA	265-287	482-504		PVMSA_WHV59	274-298				
PVGLF_BR6VC	484-508			PVMSA_WHV7	274-298				
PVGLF_BR6VR	484-508			PVMSA_WHV8	274-298				
PVGLF_HRSV1	484-508			PVMSA_WHV8I	274-298				
PVGLF_HRSVA	484-506			PVMSA_WHVW6	125-149				
PVGLF_HRSVL	484-506								
PVGLF_HRSVR	484-506								
PVGLF_TRTV	452-474								
PVGLG_IHNV	77-99								
PVGLG_VHSV0	406-428								

[illegible]

TABLE IX

Search Results Summary for P12CTLZIP Motif

-64-

[illegible]

PHEMA_IAHNM	236-262	321-338
PHEMA_IAHNN	315-332	
PHEMA_IAHPR	315-332	
PHEMA_IAHRO	236-262	321-338
PHEMA_IAHSA	236-262	321-338
PHEMA_IAHSP	230-246	315-332
PHEMA_IAHSW	230-246	315-332
PHEMA_IAHTE	236-262	321-338
PHEMA_IAHTO	236-262	321-338
PHEMA_IAHUR	236-262	321-338
PHEMA_IAJAP	317-334	
PHEMA_IAMAA	197-223	318-336
PHEMA_IAMAB	202-228	324-341
PHEMA_IAMAO	37-59	322-339
PHEMA_IAME1	37-59	322-339
PHEMA_IAME2	37-59	322-339
PHEMA_IAME6	21-43	
PHEMA_IAMIN	85-101	231-247
PHEMA_IANT6	37-59	322-339
PHEMA_IAPIL	320-337	
PHEMA_IQU7	21-43	306-323
PHEMA_IARUD	320-337	
PHEMA_IASE2	320-337	
PHEMA_IASH2	321-338	
PHEMA_IASTA	230-246	315-332
PHEMA_IATAI	33-55	320-337
PHEMA_IATKI	233-249	
PHEMA_IATKR	230-246	
PHEMA_IATKW	228-245.	
PHEMA_IAUDO	37-59	322-339
PHEMA_IIV7	38-60	323-340
PHEMA_IAX31	37-59	
PHEMA_IAZCO	37-59	322-339
PHEMA_IAPH2	21-43	306-323
PHEMA_IAPH3	21-43	306-323
PHEMA_IAZUK	37-59	322-339
PHEMA_INBAA	115-131	295-310
PHEMA_INBBE	123-139	303-318
PHEMA_INBBQ	116-132	293-308
PHEMA_INBEN	123-139	301-316
PHEMA_INBFU	108-124	286-301
PHEMA_INBGL	119-135	296-311
PHEMA_INBHK	116-132	293-308
PHEMA_INBIB	108-124	288-303
PHEMA_INBID	120-136	288-314
PHEMA_INBLE	123-139	302-317
PHEMA_INBMD	113-129	292-307

2164698

-68-

[illegible]

[illegible]

[illegible]

[illegible]

-73-

PVGLM_PTPV	743-785	997-1016	1275-1302						
PVGLM_PUUMH	155-174	509-525	712-728						
PVGLM_PUUMS	155-174	509-525	712-729	1092-1117					
PVGLM_RVFFV	53-80	344-368	830-856						
PVGLM_RVFFZ	53-80	344-368	830-856	1158-1178					
PVGLM_SEOUR	355-371	693-718	901-916	1000-1020					
PVGLM_SEOUS	355-371	692-717	800-815	999-1019					
PVGLM_UUK	561-585	655-674	828-842	925-952	966-989				
PVGLP_BEV	430-452	869-885	1099-1124	1548-1568					
PVGLX_PVRRI	149-176								
PVGLY_JUNIN	12-38								
PVGLY_LA5SG	12-38	237-258	426-448						
PVGLY_LA5GJ	12-38	238-259	427-449						
PVGLY_LYCVA	12-38								
PVGLY_LYCVW	12-38	89-108							
PVGLY_MOPEI	12-38	425-447							
PVGLY_PIARV	12-38	441-466							
PVGLY_TACV	12-38								
PVGLY_TACV6	12-38								
PVGLY_TACV7	12-38								
PVGLY_TACVT	12-38								
PVGNB_CPMV	141-161	569-594	757-783	1110-1135	1165-1184				
PVGNM_BPMV	678-698								
PVGNM_CPMV	311-335	741-764	1021-1037						
PVGP2_EBV	657-681								
PVGP3_EBV	854-878								
PVGP6_EBV	67-68								
PVM01_VACCC	134-159	177-195	281-302						
PVM01_VACCV	83-108	128-144	230-251						
PVM1_REOVD	141-168	227-246	280-304	324-347	414-436	454-477			
PVM1_REOVL	141-168	227-246	280-304	414-436	454-477				
PVM21_REOVD	168-192								
PVM22_REOVD	168-192								
PVM2_REOVJ	168-192								
PVM2_REOVL	168-192								
PVM3_REOVD	304-328	521-540							
PVMAT_BR5VA	37-62								
PVMAT_COVO	148-165	283-309							
PVMAT_HRSVA	44-62	138-160							
PVMAT_LPMV	311-338								
PVMAT_MEASE	283-309								
PVMAT_MEASH	283-309								
PVMAT_MEASI	87-111								
PVMAT_MEASU	283-309								
PVMAT_MUMPS	191-207	227-250	310-330						
PVMAT_NDVA	135-151	190-208	309-329						
PVMAT_NDV8	135-151	190-208	309-329						

-75-

2164698

[illegible]

2164698

TABLE X

Search Results Summary for P23CTLZIP Motif

2164698

P23LZIPC									
LIBRARY FILE									
PENV AVISU	98-136								
PENV BAEVM	202-240	528-554							
PENV BIV06	434-472	526-553	628-659						
PENV BIV27	554-582	657-688							
PENV CAEVG	44-78								
PENV EIAV1	795-828								
PENV EIAV2	795-828								
PENV EIAV3	795-828								
PENV EIAV5	798-829								
PENV EIAV9	795-828								
PENV EIAVC	795-828								
PENV EIAVW	795-828								
PENV EIAVY	795-828								
PENV FIVPE	128-168								
PENV FIVT2	48-74								
PENV FLVGL	447-475								
PENV FLVLB	487-495								
PENV FLV8A	444-472								
PENV FOAMV	44-78	481-519	552-584						
PENV FR8FB	315-350								
PENV F8VGA	467-495								
PENV F8VGB	447-475								
PENV F8V8M	450-478								
PENV F8V8T	467-495								
PENV GALV	519-554								
PENV HV1A2	729-762								
PENV HV1B1	730-763								
PENV HV1B8	725-758								
PENV HV1BN	743-781								
PENV HV1BR	735-768								
PENV HV1C4	742-776								
PENV HV1EL	254-285	727-760							
PENV HV1H2	730-763								
PENV HV1H3	730-763								
PENV HV1J3	741-774								
PENV HV1JR	722-755								
PENV HV1KB	552-586	752-780							
PENV HV1MA	258-289	733-766							
PENV HV1MF	728-761								
PENV HV1MN	392-430	731-764							
PENV HV1ND	248-279								
PENV HV1OY	729-762								
PENV HV1PV	730-763								
PENV HV1RH	738-772								
PENV HV18C	730-763								

2164698

PENV HV1W1	730-763				
PENV HV1W2	721-764				
PENV HV1Z2	264-286	727-760			
PENV HV1Z3	250-281				
PENV HV1Z6	255-286	729-762			
PENV HV1Z8	265-296				
PENV HV2BE	761-811				
PENV HV2D1	772-802				
PENV HV2G1	772-802				
PENV HV2NZ	777-814				
PENV HV2SB	743-776				
PENV JSRV	288-332	484-515			
PENV MMTVB	435-472				
PENV MMTVG	435-472				
PENV RSVF	533-570				
PENV SFV1	44-78	492-530			
PENV SFV3L	48-82	550-588			
PENV SIVCZ	745-776				
PENV SIVGB	247-277	353-386			
PENV SIVM1	766-800				
PENV SIVMK	765-799				
PENV SIVML	511-545	764-798			
PENV SIVS4	458-486				
PENV SIVSP	462-490	810-840			
PHEMA CDVO	200-234				
PHEMA IABUD	23-55				
PHEMA IACKA	23-55				
PHEMA IACKV	517-547				
PHEMA IADA1	23-55				
PHEMA IADCZ	23-55				
PHEMA IADH6	293-323				
PHEMA IADNZ	23-55				
PHEMA IAFPR	16-51				
PHEMA IAGRE	23-55				
PHEMA IAMAA	22-54				
PHEMA IAMAB	27-59				
PHEMA IARUD	23-55				
PHEMA IASE2	23-55				
PHEMA IASTA	517-547				
PHEMA MUMPM	19-52	101-132			
PHEMA MUMPR	19-52	101-132			
PHEMA MUMPS	19-52	101-132			
PHEMA NDVA	60-88				
PHEMA NDVB	60-88				
PHEMA NDVD	60-88				
PHEMA NDVH	60-88				
PHEMA NDVI	60-88				

2164698

PHEMA_NDVM	60-88						
PHEMA_NDVQ	60-88						
PHEMA_NDVTG	60-88						
PHEMA_NDVU	60-88						
PHEMA_P11HW	29-60	198-233					
PHEMA_P12H	13-46	334-369					
PHEMA_P12HT	13-46	334-369					
PHEMA_P13B	184-231						
PHEMA_P13H4	184-231						
PHEMA_P13HA	184-231						
PHEMA_P13HT	184-231						
PHEMA_P13HU	184-231						
PHEMA_P13HV	184-231						
PHEMA_P13HW	184-231						
PHEMA_P13HX	184-231						
PHEMA_P14HA	245-280	338-378					
PHEMA_RACVI	255-283						
PHEMA_RINDL	282-313						
PHEMA_SENDE	16-54	198-233					
PHEMA_SENDF	16-54	198-233					
PHEMA_SENDH	16-54	198-233					
PHEMA_SENDJ	16-54	198-233					
PHEMA_SENDZ	23-54	198-233					
PHEMA_6V41	55-84	330-385					
PHEMA_6V5	7-35						
PHEMA_6V5CM	7-41						
PHEMA_6V5CP	7-41						
PHEMA_6V5LN	7-35						
PHEMA_VACCC	258-284						
PHEMA_VACCI	258-284						
PHEMA_VACCT	258-284						
PHEMA_VACCV	258-284						
PVENV_BEV	18-51	87-117					
PVENV_DHV11	297-335						
PVENV_MCV1	203-238						
PVENV_MCV2	203-238						
PVENV_VACCC	208-241						
PVENV_VACCI	208-241						
PVENV_VACCP	208-241						
PVENV_VACCV	208-241						
PVF03_VACCC	2-40	61-93					
PVF03_VACCV	2-40	61-93					
PVFP1_FOWPV	287-330						
PVFP4_FOWPV	237-267						
PVFP7_CAPVK	89-118						
PVFU8_VACCC	28-61						
PVFU8_VACCV	28-61						

PVG01_HSVI1	317-346				
PVG02_HSVB	163-186				
PVG02_VACCV	92-120				
PVG02_VARV	92-120				
PVG03_HSVI1	108-136				
PVG06_HSVI1	54-83				
PVG06_VACCC	99-136				
PVG06_VARV	98-136				
PVG07_VACCC	113-145				
PVG07_VARV	113-145				
PVG09_VACCC	303-338				
PVG09_VACCV	266-301				
PVG09_VARV	303-338				
PVG11_HSVI1	150-183				
PVG12_HSVI1	206-243				
PVG12_HSVSA	68-106				
PVG1_SPV1R	254-292	303-337	414-452		
PVG22_HSVI1	300-337	647-678			
PVG23_HSVI1	70-108				
PVG26_HSVI1	94-125				
PVG27_HSVSA	36-74				
PVG28_HSVI1	481-521				
PVG26_HSVSA	7-40				
PVG2R_AMEPV	180-217				
PVG2_SPV4	209-244				
PVG35_HSVI1	15-46	190-226			
PVG36_HSVSA	151-185				
PVG38_HSVI1	543-577	648-682			
PVG40_HSVSA	187-216				
PVG41_HSVI1	11-45	202-233			
PVG42_HSVI1	91-125				
PVG43_HSVI1	109-140	157-185			
PVG46_HSVI1	688-925				
PVG48_HSVSA	329-357				
PVG50_HSVSA	113-141				
PVG51_HSVI1	28-64	84-120			
PVG52_HSVI1	66-134				
PVG55_HSVI1	100-129				
PVG56_HSVI1	631-667	1091-1126			
PVG58_HSVI1	342-375	480-508			
PVG58_HSVSA	25-60	195-233			
PVG59_HSVI1	82-118				
PVG61_HSVI1	76-109				
PVG64_HSVI1	55-89	363-401	420-452		
PVG65_HSVI1	801-836	1290-1326			
PVG67_HSVI1	150-188	1150-1185			
PVG6_SPV1R	60-89				

2164698

PVG71_H8V8A	128-168					
PVG72_H8V11	445-478	720-761	1158-1189	1262-1285		
PVG75_H8V11	263-291	387-422				
PVG76_H8V11	187-221					
PVG7_8PV1R	18-46					
PVGF1_IBVB	1719-1747	1856-1891	2108-2146	3601-3633		
PVGH3_HCMVA	80-116	167-185				
PVGL2_CVBF	1259-1294					
PVGL2_CVBL9	661-681	1259-1294				
PVGL2_CVBLY		1259-1294				
PVGL2_CVBM		1259-1294				
PVGL2_CVBQ		1259-1294				
PVGL2_CVBV		1259-1294				
PVGL2_CVH22	1053-1088					
PVGL2_CVM4	1267-1304					
PVGL2_CVMA5	1215-1252					
PVGL2_CVMJH	1120-1163					
PVGL2_CVPF8	632-665	738-764	1328-1363			
PVGL2_CVPPU	630-663	734-762	1326-1361			
PVGL2_CVPR8	512-540	1104-1139				
PVGL2_CVPRM	408-441	1104-1139				
PVGL2_FIPV	635-668	739-767	1331-1366			
PVGL2_IBVB	153-188					
PVGLB_HCMVA	116-147	706-743				
PVGLB_HCMVT	116-147	707-744				
PVGLB_H8V6U	72-110					
PVGLB_H8VB1	264-288					
PVGLB_H8VB2	264-289	745-774				
PVGLB_H8VBC	253-287					
PVGLB_ILTV8	442-472					
PVGLB_ILTV9	462-482					
PVGLB_ILTVT	462-482					
PVGLB_MCMV9	136-163	738-776				
PVGLC_H8V11	467-500					
PVGLC_H8V1K	467-500					
PVGLC_H8V2	435-465					
PVGLC_H8V23	436-466					
PVGLC_H8VBC	475-507					
PVGLC_VZVD	351-388	513-548				
PVGLC_VZV8	351-388	513-548				
PVGLD_H8VEA	340-370					
PVGLD_H8VEB	41-70	390-420				
PVGLD_H8VEK	41-70	390-420				
PVGLE_H8VE4	95-125					
PVGLE_H8VEB	63-100	390-420				
PVGLE_H8VEL	63-100	382-422				
PVGLE_PRVRI	332-369					

PVGLF BR9VA	266-301	482-511			
PVGLF BR9VC	484-513				
PVGLF BR9VR	484-513				
PVGLF CDVO	562-588				
PVGLF HRSV1	484-513				
PVGLF HRSVA	484-513				
PVGLF HRSVL	484-513				
PVGLF HRSVR	484-513				
PVGLF MEASE	224-256	451-484			
PVGLF MEAB1	227-259	454-487			
PVGLF MEASY	224-256	451-484			
PVGLF MUMPM	446-474				
PVGLF MUMPR	446-474				
PVGLF MUMPS	5-38	446-474			
PVGLF NDVI	132-165				
PVGLF PHODV	531-585				
PVGLF PI1HC	456-484				
PVGLF PI3B	453-481				
PVGLF PI3H4	453-481				
PVGLF RINDK	220-252	447-480			
PVGLF RINDL	220-252	447-480			
PVGLF SEND5	460-488				
PVGLF SENDF	460-488				
PVGLF SENDH	460-488				
PVGLF SENDJ	460-488				
PVGLF SENDZ	460-488				
PVGLF SV5	446-474				
PVGLF TRTV	452-481				
PVGLG H9VEB	327-364				
PVGLG SYN	524-553				
PVGLG VSVIG	450-488				
PVGLG VSVJO	457-482				
PVGLG VSVQ	450-488				
PVGLG VSVSJ	450-488				
PVGLH HCMVA	691-719				
PVGLH HCMVT	690-718				
PVGLH H9V8G	640-677				
PVGLH H9VE4	814-850				
PVGLH H9VEB	807-843				
PVGLI HCMVA	158-194				
PVGLM BUNGE	197-227	438-468	982-1020	1049-1084	
PVGLM BUNL7	190-220				
PVGLM BUNSH	190-220	344-381			
PVGLM BUNYW	193-228	434-472	823-854		
PVGLM DUGBV	244-273	637-672	886-915	935-965	1403-1441
PVGLM HANTB	610-641	1081-1119			
PVGLM HANTH	188-222	612-643	1082-1120		

PVGLM HANTL	188-222	612-643	1083-1121	
PVGLM HANTV	188-222	612-643	1083-1121	
PVGLM PHV	616-649	1088-1121		
PVGLM PTPV	949-982	1275-1308		
PVGLM PUUMH	620-653	1082-1125		
PVGLM PUUMS	620-653	1082-1125		
PVGLM RVFV	620-653	830-863		
PVGLM RVFVZ	620-653	830-863	1156-1185	
PVGLM SEOUR	606-641	1082-1120		
PVGLM SEOUS	610-641	1081-1119		
PVGLM UUK	431-468	966-995		
PVGLP BEV	1491-1526			
PVGLY JUNIN	12-45			
PVGLY LASSG	237-265			
PVGLY LASSJ	238-268			
PVGLY PIARV	12-50			
PVGLY TACV	12-50			
PVGLY TACV5	12-50	89-124		
PVGLY TACV7	12-50	89-124		
PVGLY TACVT	12-50	89-124		
PVGNB CPMV	1527-1555			
PVGNM BPMV	137-167	280-327	837-868	
PVGNM CPMV	208-242	741-771		
PVGNM CFSMV	50-86	478-515		
PVGNM RCMV	768-799			
PVGP2 EBV	78-111			
PVGP3 EBV	78-111			
PVM1 REOVD	280-318	324-361		
PVM1 REOVL	280-318			
PVM21 REOVD	168-199			
PVM22 REOVD	168-199			
PVM2 REOVJ	168-199			
PVM2 REOVL	168-199			
PVM3 REOVD	333-364			
PVMAT SV5	308-342			
PVMAT TRTV	122-150			
PVME1 CVBM	64-102			
PVME1 CVHOC	64-102			
PVME1 CVMA5	65-103			
PVME1 CVMJH	65-103			
PVME1 CVTKE	64-102			
PVME1 EBV	178-213			
PVMP CERV	63-126			
PVMP SOCMV	66-98	273-303		
PVMSA HP8DB	201-238	268-302		
PVMSA HP8DC	194-227	268-301		
PVMSA HP8DU	157-190	231-264		

[illegible]

5.3. SYNTHESIS OF PEPTIDES

The peptides of the invention may be synthesized or prepared by techniques well known in the art. See, for example, Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman and Co., NY.

5

Short peptides, for example, can be synthesized on a solid support or in solution. Longer peptides may be made using recombinant DNA techniques. Here, the nucleotide sequences encoding the peptides of the invention may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, NY.

10

15

The peptides of the invention may alternatively be synthesized such that one or more of the bonds which link the amino acid residues of the peptides are non-peptide bonds. These alternative non-peptide bonds may be formed by utilizing reactions well known to those in the art, and may include, but are not limited to imino, ester, hydrazide, semicarbazide, and azo bonds, to name but a few. In yet another embodiment of the invention, peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxy, dansyl, or t-butyloxycarbonyl groups, may be added to the peptides' amino termini. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the peptides' amino termini. (See "X" in Tables I to IV, above.) Additionally, the hydrophobic group, t-

20

25

30

35

2164698

butyloxycarbonyl, or an amido group may be added to the peptides' carboxy termini. (See "Z" in Tables I to IV, above.) Further, the peptides of the invention may be synthesized such that their steric configuration is altered. For example, the D-isomer of one or more of the amino acid residues of the peptide may be used, rather than the usual L-isomer. Still further, at least one of the amino acid residues of the peptides of the invention may be substituted by one of the well known non-naturally occurring amino acid residues. Alterations such as these may serve to increase the stability, bioavailability and/or inhibitory action of the peptides of the invention.

Any of the peptides described above may, additionally, have a non-peptide macromolecular carrier group covalently attached to their amino and/or carboxy termini. Such macromolecular carrier groups may include, for example, lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates. "X", in Tables I to IV, above, may therefore additionally represent any of the above macromolecular carrier groups covalently attached to the amino terminus of a peptide. Likewise, "Z", in Tables I to IV, may additionally represent any of the macromolecular carrier groups described above.

5.4. ASSAYS FOR ANTIVIRAL ACTIVITY

The antiviral activity exhibited by the peptides of the invention may be measured, for example, by easily performed in vitro assays, such as those described below, which can test the peptides' ability to inhibit syncytia formation, or their ability to inhibit infection by cell-free virus. Using these assays, such parameters as the relative antiviral activity of the peptides, exhibit against a given strain of virus and/or the strain specific inhibitory

activity of the peptide can be determined. A cell fusion assay may be utilized to test the peptides' ability to inhibit HIV-induced syncytia formation in vitro. Such an assay may comprise culturing uninfected CD-4⁺ cells (such as Molt or CEM cells, for example) in the presence of chronically HIV-infected cells and a peptide to be assayed. For each peptide, a range of peptide concentrations may be tested. This range should include a control culture wherein no peptide has been added. Standard conditions for culturing, well known to those of ordinary skill in the art, are used. After incubation for an appropriate period (24 hours at 37°C, for example) the culture is examined microscopically for the presence of multinucleated giant cells, which are indicative of cell fusion and syncytia formation.

A reverse transcriptase (RT) assay may be utilized to test the peptides' ability to inhibit infection of CD-4⁺ cells by cell-free HIV. Such an assay may comprise culturing an appropriate concentration (i.e., TCID₅₀) of virus and CD-4⁺ cells in the presence of the peptide to be tested. Culture conditions well known to those in the art are used. As above, a range of peptide concentrations may be used, in addition to a control culture wherein no peptide has been added. After incubation for an appropriate period (e.g., 7 days) of culturing, a cell-free supernatant is prepared, using standard procedures, and tested for the presence of RT activity as a measure of successful infection. The RT activity may be tested using standard techniques such as those described by, for example, Goff et al. (Goff, S. et al., 1981, J. Virol. 38:239-248) and/or Willey et al. (Willey, R. et al., 1988, J. Virol. 62:139-147).

Standard methods which are well-known to those of skill in the art may be utilized for assaying non-retroviral activity. See, for example, Pringle et al. (Pringle, C.R. et al., 1985, J. Medical Virology 17:377-386) for a discussion of respiratory syncytial virus and parainfluenza virus activity assay techniques. Further, see, for example, "Zinsser Microbiology", 1988, Joklik, W.K. et al., eds., Appleton & Lange, Norwalk, CT, 19th ed., for a general review of such techniques.

5.5. USES OF THE PEPTIDES OF THE INVENTION

The DP-178 (SEQ ID:1) peptides of the invention, and DP-178 fragments, analogs, and homologs, exhibit potent antiviral activity. The DP-107-like and DP-178-like peptides of the invention preferably exhibit antiviral activity. As such, the peptides may be used as inhibitors of human and non-human viral and retroviral, especially HIV, transmission to uninfected cells.

The human retroviruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to all strains of HIV-1 and HIV-2 and the human T-lymphocyte viruses (HTLV-I and II). The non-human retroviruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to bovine leukosis virus, feline sarcoma and leukemia viruses, simian immunodeficiency, sarcoma and leukemia viruses, and sheep progress pneumonia viruses.

Non retroviral viruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to human respiratory syncytial virus, canine distemper virus, newcastle disease virus, human parainfluenza virus, and influenza

2164698

viruses. Further, any virus or retrovirus containing peptides listed in Tables V through X above, may be inhibited by the peptides of the invention.

As discussed more fully, below, in Section 5.5.1 and in the Example presented, below, in Section 8, DP-107 and DP-178, and DP-107-like and DP-178-like peptides form non-covalent protein-protein interactions which are required for normal activity of the virus. Thus, the peptides of the invention may also be utilized as components in assays for the identification of compounds that interfere with such protein-protein interactions and may, therefore, act as antiviral agents. These assays are discussed, below, in Section 5.5.1.

5.5.1. ANTIVIRAL COMPOUND SCREENING SCREENING
ASSAYS FOR COMPOUNDS THAT INTERACT WITH
THE PKD1 GENE PRODUCT

As demonstrated in the Example presented in Section 8, below, DP-107 and DP-178 portions of the TM protein gp41 form non-covalent protein-protein interactions. As also demonstrated, the maintenance of such interactions is necessary for normal viral infectivity. Thus, compounds which bind DP-107, bind DP-178, and/or act to disrupt normal DP-107/DP-178 protein-protein interactions may act as potent antiviral agents. Described below are assays for the identification of such compounds. Note that, while, for ease and clarity of discussion, DP-107 and DP-178 peptides will be used as components of the assays described, but it is to be understood that any of the DP-107-like or DP-178-like peptides described, above, in Sections 5.1 and 5.2 may also be utilized as part of these screens for antiviral compounds.

Compounds which may be tested for an ability to bind DP-107, DP-178, and/or disrupt DP-107/DP-178 interactions, and which therefore, potentially

represent antiviral compounds, include, but are not limited to, peptides made of D- and/or L-configuration amino acids (in, for example, the form of random peptide libraries; see Lam, K.S. et al., 1991, Nature 354:82-84), phosphopeptides (in, for example, the form of random or partially degenerate, directed phosphopeptide libraries; see, for example, Songyang, Z. et al., 1993, Cell 72:767-778), antibodies, and small organic or inorganic molecules. Synthetic compounds, natural products, and other sources of potentially effective materials may be screened in a variety of ways, as described in this Section. The compounds, antibodies, or other molecules identified may be tested for an ability to inhibit viral activity, utilizing, for example, viral assays such as those described, above, in Section 5.4.

Among the peptides which may be tested are soluble peptides comprising DP-107 and/or DP-178 domains, and peptides comprising DP-107 and/or DP-178 domains having one or more mutations within one or both of the domains, such as the M41-P peptide described, below, in the Example presented in Section 8, which contains a isoleucine to proline mutation within the DP-178 sequence.

In one embodiment of such screening methods is a method for identifying a compound to be tested for antiviral ability comprising:

- (a) exposing at least one compound to a peptide comprising a DP-107 peptide for a time sufficient to allow binding of the compound to the DP-107 peptide;
- (b) removing non-bound compounds; and
- (c) determining the presence of the compound bound to the DP-107 peptide, thereby identifying an agent to be tested for antiviral ability.

In a second embodiment of such screening methods is a method for identifying a compound to be tested for antiviral ability comprising:

5 (a) exposing at least one compound to a peptide comprising a DP-178 peptide for a time sufficient to allow binding of the compound to the DP-178 peptide;

(b) removing non-bound compounds; and

10 (c) determining the presence of the compound bound to the DP-178 peptide, thereby identifying an agent to be tested for antiviral ability.

One method utilizing these types of approaches that may be pursued in the isolation of such DP-107-binding or DP-178-binding compounds is an assay which
15 would include the attachment of either the DP-107 or the DP-178 peptide to a solid matrix, such as, for example, agarose or plastic beads, microtiter plate wells, petri dishes, or membranes composed of, for example, nylon or nitrocellulose. In such an assay
20 system, either the DP-107 or DP-178 protein may be anchored onto a solid surface, and the compound, or test substance, which is not anchored, is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The
25 anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the protein and drying.

30 Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the labeled
35 compound is added to the coated surface containing the anchored DP-107 or DP-178 peptide. After the reaction

is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways.

5 Where the compound is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the labeled component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using
10 a labeled antibody specific for the compound (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, such an assay can be conducted in a liquid phase, the reaction products separated from
15 unreacted components, and complexes detected; e.g., using an immobilized antibody specific for DP-107 or DP-178, whichever is appropriate for the given assay, or an antibody specific for the compound, i.e., the test substance, in order to anchor any complexes
20 formed in solution, and a labeled antibody specific for the other member of the complex to detect anchored complexes.

By utilizing procedures such as this, large numbers of types of molecules may be simultaneously
25 screened for DP-107 or DP-178-binding capability, and thus potential antiviral activity.

Further, compounds may be screened for an ability to inhibit the formation of or, alternatively, disrupt DP-107/DP-178 complexes. Such compounds may then be
30 tested for antiviral capability. For ease of description, DP-107 and DP-178 will be referred to as "binding partners." Compounds that disrupt such interactions may exhibit antiviral activity. Such compounds may include, but are not limited to
35

molecules such as antibodies, peptides, and the like described above.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the DP-107 and DP-178 peptides involves
5 preparing a reaction mixture containing peptides under conditions and for a time sufficient to allow the two peptides to interact and bind, thus forming a complex. In order to test a compound for disruptive activity, the reaction is conducted in the presence and absence
10 of the test compound, i.e., the test compound may be initially included in the reaction mixture, or added at a time subsequent to the addition of one of the binding partners; controls are incubated without the test compound or with a placebo. The formation of any
15 complexes between the binding partners is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound indicates that the compound interferes with the interaction of the DP-107 and
20 DP-178 peptides.

The assay for compounds that interfere with the interaction of the binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring one of the
25 binding partners onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be
30 varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence
35 of the test substance; i.e., by adding the test

substance to the reaction mixture prior to or simultaneously with the binding partners. On the other hand, test compounds that disrupt preformed complexes, e.g. compounds with higher binding constants that displace one of the binding partners from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, one binding partner, e.g., either the DP-107 or DP-178 peptide, is anchored onto a solid surface, and its binding partner, which is not anchored, is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody specific for the protein may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the binding partner of the immobilized species is added to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the binding partner was pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the binding partner is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for

2164698

the binding partner (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed
5 complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g.,
10 using an immobilized antibody specific for one binding partner to anchor any complexes formed in solution, and a labeled antibody specific for the other binding partner to detect anchored complexes. Again, depending upon the order of addition of reactants to
15 the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a
20 preformed complex of the DP-107 and DP-178 peptides is prepared in which one of the binding partners is labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 by Rubenstein which utilizes this
25 approach for immunoassays). The addition of a test substance that competes with and displaces one of the binding partners from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt DP-107/
30 DP-178 protein-protein interaction can be identified.

5.5 PHARMACEUTICAL FORMULATIONS, DOSAGES AND MODES OF ADMINISTRATION

With respect to HIV, the peptides of the
35 invention may be used as a therapeutic in the

treatment of AIDS. The peptides of the invention may be administered using techniques well known to those in the art. Preferably, agents are formulated and administered systemically. Techniques for formulation and administration may be found in "Remington's
5 Pharmaceutical Sciences", 18th ed., 1990, Mack Publishing Co., Easton, PA. Suitable routes may include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including
10 intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few. Most preferably, administration is intravenous. For injection, the agents of the invention may be
15 formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated
20 are used in the formulation. Such penetrants are generally known in the art.

In addition, the peptides may be used as a prophylactic measure in previously uninfected individuals after acute exposure to an HIV virus.
25 Examples of such prophylactic use of the peptides may include, but are not limited to, prevention of virus transmission from mother to infant and other settings where the likelihood of HIV transmission exists, such as, for example, accidents in health care settings
30 wherein workers are exposed to HIV-containing blood products. The peptides of the invention in such cases may serve the role of a prophylactic vaccine, wherein the host raises antibodies against the peptides of the invention, which then serve to neutralize HIV viruses
35 by, for example, inhibiting further HIV infection.

Administration of the peptides of the invention as a prophylactic vaccine, therefore, would comprise administering to a host a concentration of peptides effective in raising an immune response which is sufficient to neutralize HIV, by, for example,
5 inhibiting HIV ability to infect cells. The exact concentration will depend upon the specific peptide to be administered, but may be determined by using standard techniques for assaying the development of an immune response which are well known to those of
10 ordinary skill in the art. The peptides to be used as vaccines are usually administered intramuscularly.

The peptides may be formulated with a suitable adjuvant in order to enhance the immunological response. Such adjuvants may include, but are not
15 limited to mineral gels such as aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols, polyanions; other peptides; oil emulsions; and potentially useful human adjuvants such as BCG and Corynebacterium parvum. Many methods may
20 be used to introduce the vaccine formulations described here. These methods include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and intranasal routes.

25 Alternatively, an effective concentration of polyclonal or monoclonal antibodies raised against the peptides of the invention may be administered to a host so that no uninfected cells become infected by HIV. The exact concentration of such antibodies will
30 vary according to each specific antibody preparation, but may be determined using standard techniques well known to those of ordinary skill in the art. Administration of the antibodies may be accomplished using a variety of techniques, including, but not
35 limited to those described in this section.

Effective dosages of the peptides of the invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Given the data
5 presented below in Section 6, DP-178, for example, may prove efficacious in vivo at doses required achieve circulating levels of 10ng per ml of peptide.

A therapeutically effective dose refers to that amount of the compound sufficient to result in
10 amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or
15 experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and
20 therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such
25 compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any
30 compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50
35 (i.e., the concentration of the test compound which achieves a half-maximal disruption of the PTK/adaptor

2164698

protein complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for
5 example, by high performance liquid chromatography (HPLC).

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl
10 et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1).

It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ
15 dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the oncogenic disorder of interest
20 will vary with the severity of the condition to be treated and to the route of administration. The dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that
25 discussed above may be used in veterinary medicine.

As demonstrated in the Example presented below in Section 6, the antiviral activity of the peptides of the invention may show a pronounced type and subtype specificity, i.e., specific peptides may be effective
30 in inhibiting the activity of only specific viruses. This feature of the invention presents many advantages. One such advantage, for example, lies in the field of diagnostics, wherein one can use the antiviral specificity of the peptide of the invention
35 to ascertain the identity of a viral isolate. With

respect to HIV, one may easily determine whether a viral isolate consists of an HIV-1 or HIV-2 strain. For example, uninfected CD-4⁺ cells may be co-infected with an isolate which has been identified as containing HIV the DP-178 (SEQ ID:1) peptide, after
5 which the retroviral activity of cell supernatants may be assayed, using, for example, the techniques described above in Section 5.2. Those isolates whose retroviral activity is completely or nearly completely inhibited contain HIV-1. Those isolates whose viral
10 activity is unchanged or only reduced by a small amount, may be considered to not contain HIV-1. Such an isolate may then be treated with one or more of the other DP-178 peptides of the invention, and subsequently be tested for its viral activity in order
15 to determine the identify of the viral isolate.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the
20 invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be
25 formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups,
30 slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective
35 amount to achieve its intended purpose. Determination

2164698

of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable
5 pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated
10 for oral administration may be in the form of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing,
15 dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally,
20 suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes.
25 Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the
30 solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid
35 excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding

suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

6. EXAMPLE: DP-178 (SEQ ID:1) IS A POTENT
INHIBITOR OF HIV-1 INFECTION

In this example, DP-178 (SEQ ID:1) is shown to be a potent inhibitor of HIV-1 mediated CD-4⁺ cell-cell fusion and infection by cell free virus. In the fusion assay, this peptide completely blocks virus induced syncytia formation at concentrations of from 1-10 ng/ml. In the infectivity assay the inhibitory concentration is somewhat higher, blocking infection at 90ng/ml. It is further shown that DP-178 (SEQ ID:1) shows that the antiviral activity of DP-178 (SEQ ID:1) is highly specific for HIV-1. Additionally, a synthetic peptide, DP-185 (SEQ ID:3), representing a HIV-1-derived DP-178 homolog is also found to block HIV-1-mediated syncytia formation.

15

6.1. MATERIALS AND METHODS

6.1.1. PEPTIDE SYNTHESIS

Peptides were synthesized using Fast Moc chemistry on an Applied Biosystems™ Model 431A peptide synthesizer. Amidated peptides were prepared using Rink resin (Advanced Chemtech) while peptides containing free carboxy termini were synthesized on Wang (p-alkoxy-benzyl-alcohol) resin (Bachem). First residues were double coupled to the appropriate resin and subsequent residues were single coupled. Each coupling step was followed by acetic anhydride capping. Peptides were cleaved from the resin by treatment with trifluoroacetic acid (TFA) (10ml), H₂O (0.5ml), thioanisole (0.5ml), ethanedithiol (0.25ml), and crystalline phenol (0.75g). Purification was carried out by reverse phase HPLC. Approximately 50mg samples of crude peptide were chromatographed on a Waters Delta Pak™ C18 column (19mm x 30cm, 15μ spherical) with a linear gradient; H₂O/acetonitrile

35

0.1% TFA. Lyophilized peptides were stored desiccated and peptide solutions were made in water at about 1mg/ml. Electrospray mass spectrometry yielded the following results: DP-178 (SEQ ID:1):4491.87 (calculated 4491.94); DP-180 (SEQ ID:2):4491.45 (calculated 4491.94); DP-185 (SEQ ID:3):not done (calculated 4546.97).

6.1.2. VIRUS

The HIV-1_{LAI} virus was obtained from R. Gallo (Popovic, M. et al., 1984, Science 224:497-508) and propagated in CEM cells cultured in RPMI 1640 containing 10% fetal calf serum. Supernatant from the infected CEM cells was passed through a 0.2 μ m filter and the infectious titer estimated in a microinfectivity assay using the AA5 cell line to support virus replication. For this purpose, 25 μ l of serial diluted virus was added to 75 μ l AA5 cells at a concentration of 2×10^5 /ml in a 96-well microtitre plate. Each virus dilution was tested in triplicate. Cells were cultured for eight days by addition of fresh medium every other day. On day 8 post infection, supernatant samples were tested for virus replication as evidenced by reverse transcriptase activity released to the supernatant. The TCID₅₀ was calculated according to the Reed and Muench formula (Reed, L.J. et al., 1938, Am. J. Hyg. 27:493-497). The titer of the HIV-1_{LAI} and HIV-1_{MN} stocks used for these studies, as measured on the AA5 cell line, was approximately 1.4×10^6 and 3.8×10^4 TCID₅₀/ml, respectively.

6.1.3. CELL FUSION ASSAY

Approximately 7×10^4 Molt cells were incubated with 1×10^4 CEM cells chronically infected with the HIV-1_{LAI} virus in 96-well plates (one-half area cluster plates; Costar, Cambridge, MA) in a final volume of

100 μ l culture medium as previously described
 (Matthews, T.J. et al., 1987, Proc. Natl. Acad. Sci.
 USA 84: 5424-5428). Peptide inhibitors were added in
 a volume of 10 μ l and the cell mixtures were incubated
 for 24 hr. at 37°C. At that time, multinucleated
 5 giant cells were estimated by microscopic examination
 at a 40x magnification which allowed visualization of
 the entire well in a single field.

6.1.4. CELL FREE VIRUS INFECTION ASSAY

10 Synthetic peptides were incubated at 37°C with
 either 247 TCID₅₀ (for experiment depicted in FIG. 2),
 or 62 TCID₅₀ (for experiment depicted in FIG.3) units
 of HIV-1_{LAI} virus or 25 TCID₅₀ units of HIV-2_{NH2} and CEM
 CD4⁺ cells at peptide concentrations of 0, 0.04, 0.4,
 15 4.0, and 40 μ g/ml for 7 days. The resulting reverse
 transcriptase (RT) activity in counts per minute was
 determined using the assay described, below, in
 Section 6.1.5. See, Reed, L.J. et al., 1938, Am. J.
 Hyg. 27: 493-497 for an explanation of TCID₅₀
 20 calculations.

6.1.5. REVERSE TRANSCRIPTASE ASSAY

The micro-reverse transcriptase (RT) assay was
 adapted from Goff et al. (Goff, S. et al., 1981, J.
 25 Virol. 38:239-248) and Willey et al. (Willey, R. et
al., 1988, J. Virol. 62:139-147). Supernatants from
 virus/cell cultures are adjusted to 1% Triton-X100™. A
 10 μ l sample of supernatant was added to 50 μ l of RT
 cocktail in a 96-well U-bottom microtitre plate and
 30 the samples incubated at 37°C for 90 min. The RT
 cocktail contained 75mM KCl, 2mM dithiothreitol, 5mM
 MgCl₂, 5 μ g/ml poly A (Pharmacia, cat. No. 27-4110-01),
 0.25 units/ml oligo dT (Pharmacia, cat. No. 27-7858-
 01), 0.05% NP40, 50mM Tris-HCl, pH 7.8, 0.5 μ M non-
 35

radioactive dTTP, and 10 μ Ci/ml ³²P-dTTP (Amersham, cat. No. PB.10167).

After the incubation period, 40 μ l of reaction mixture was applied to a Schleicher and Schuell (S+S) NA45 membrane (or DE81 paper) saturated in 2 x SSC
 5 buffer (0.3M NaCl and 0.003M sodium citrate) held in a S+S Minifold over one sheet of GB003 (S+S) filter paper, with partial vacuum applied. Each well of the minifold was washed four times with 200 μ l 2xSSC, under full vacuum. The membrane was removed from the
 10 minifold and washed 2 more times in a pyrex dish with an excess of 2xSSC. Finally, the membrane was drained on absorbent paper, placed on Whatman #3 paper, covered with Saran wrap[™], and exposed to film overnight at -70°C.

15

6.2. RESULTS

6.2.1. PEPTIDE INHIBITION OF INFECTED CELL-INDUCED SYNCYTIA FORMATION

20 The initial screen for antiviral activity assayed peptides' ability to block syncytium formation induced by overnight co-cultivation of uninfected Molt4 cells with chronically HIV-1 infected CEM cells. The results of several such experiments are presented
 25 herein. In the first of these experiments, serial DP-178 (SEQ ID:1) peptide concentrations between 10 μ g/ml and 12.5ng/ml were tested for blockade of the cell fusion process. For these experiments, CEM cells chronically infected with either HIV-1_{LAI}, HIV-1_{MN}, HIV-
 30 1_{RF}, or HIV-1_{SF2} virus were cocultivated overnight with uninfected Molt 4 cells. The results (FIG. 4A) show that DP-178 (SEQ ID:1) afforded complete protection against each of the HIV-1 isolates down to the lowest concentration of DP-178 (SEQ ID:1) used. For HIV_{LAI}
 35 inhibition, the lowest concentration tested was

12.5ng/ml; for all other HIV-1 viruses, the lowest concentration of DP-178 (SEQ ID:1) used in this study was 100ng/ml. A second peptide, DP-180 (SEQ ID:2), containing the same amino acid residues as DP-178 (SEQ ID:1) but arranged in a random order exhibited no evidence of anti-fusogenic activity even at the high concentration of 40µg/ml (FIG. 4A). These observations indicate that the inhibitory effect of DP-178 (SEQ ID:1) is primary sequence-specific and not related to non-specific peptide/protein interactions. The actual endpoint (i.e., the lowest effective inhibitory concentration) of DP-178 inhibitory action is within the range of 1-10 ng/ml.

The next series of experiments involved the preparation and testing of a DP-178 (SEQ ID:1) homolog for its ability to inhibit HIV-1-induced syncytia formation. As shown in FIG. 1, the sequence of DP-185 (SEQ ID:3) is slightly different from DP-178 (SEQ ID:1) in that its primary sequence is taken from the HIV-1_{SP2} isolate and contains several amino acid differences relative to DP-178 (SEQ ID:1) near the N terminus. As shown in FIG. 4A, DP-185 (SEQ ID:3), exhibits inhibitory activity even at 312.5ng/ml, the lowest concentration tested.

The next series of experiments involved a comparison of DP-178 (SEQ ID:1) HIV-1 and HIV-2 inhibitory activity. As shown in FIG. 5, DP-178 (SEQ ID:1) blocked HIV-1-mediated syncytia formation at peptide concentrations below 1ng/ml. DP-178 (SEQ ID:1) failed, however, to block HIV-2 mediated syncytia formation at concentrations as high as 10µg/ml. This striking 4 log selectivity of DP-178 (SEQ ID:1) as an inhibitor of HIV-1-mediated cell fusion demonstrates an unexpected HIV-1 specificity in the action of DP-178 (SEQ ID:1). DP-178 (SEQ ID:1) inhibition of HIV-1-mediated cell fusion, but the

peptide's inability to inhibit HIV-2 mediated cell fusion in the same cell type at the concentrations tested provides further evidence for the high degree of selectivity associated with the antiviral action of DP-178 (SEQ ID:1).

5

6.2.2. PEPTIDE INHIBITION OF INFECTION BY
CELL-FREE VIRUS

DP-178 (SEQ ID:1) was next tested for its ability to block CD-4⁺ CEM cell infection by cell free HIV-1 virus. The results, shown in FIG. 2, are from an experiment in which DP-178 (SEQ ID:1) was assayed for its ability to block infection of CEM cells by an HIV-1_{LAI} isolate. Included in the experiment were three control peptides, DP-116 (SEQ ID:9), DP-125 (SEQ ID:8), and DP-118 (SEQ ID:10). DP-116 (SEQ ID:9) represents a peptide previously shown to be inactive using this assay, and DP-125 (SEQ ID:8; Wild, C. et al., 1992, Proc. Natl. Acad, Sci. USA 89:10,537) and DP-118 (SEQ ID:10) are peptides which have previously been shown to be active in this assay. Each concentration (0, 0.04, 0.4, 4, and 40 μ g/ml) of peptide was incubated with 247 TCID₅₀ units of HIV-1_{LAI} virus and CEM cells. After 7 days of culture, cell-free supernatant was tested for the presence of RT activity as a measure of successful infection. The results, shown in FIG. 2, demonstrate that DP-178 (SEQ ID:1) inhibited the de novo infection process mediated by the HIV-1 viral isolate at concentrations as low as 90ng/ml (IC₅₀=90ng/ml). In contrast, the two positive control peptides, DP-125 (SEQ: ID:8) and DP-118 (SEQ ID:10), had over 60-fold higher IC₅₀ concentrations of approximately 5 μ g/ml.

In a separate experiment, the HIV-1 and HIV-2 inhibitory action of DP-178 (SEQ ID:1) was tested with CEM cells and either HIV-1_{LAI} or HIV-2_{NIH2}. 62 TCID₅₀

2164698

HIV-1_{LAI} or 25 GCID₅₀ HIV-2_{NIHZ} were used in these experiments, and were incubated for 7 days. As may be seen in FIG. 3, DP-178 (SEQ ID:1) inhibited HIV-1 infection with an IC₅₀ of about 31ng/ml. In contrast, DP-178 (SEQ ID:1) exhibited a much higher IC₅₀ for HIV-2_{NIHZ}, thus making DP-178 (SEQ ID:1) two logs more potent as a HIV-1 inhibitor than a HIV-2 inhibitor. This finding is consistent with the results of the fusion inhibition assays described, above, in Section 6.2.1, and further supports a significant level of selectivity (i.e., for HIV-1 over HIV-2).

7. EXAMPLE: THE HIV-1 INHIBITOR, DP-178 (SEQ ID:1) IS NON-CYTOXIC

In this Example, the 36 amino acid synthetic peptide inhibitor DP-178 (SEQ ID:1) is shown to be non-cytotoxic to cells in culture, even at the highest peptide concentrations (40μg/ml) tested.

7.1. MATERIALS AND METHODS

Cell proliferation and toxicity assay:
Approximately 3.8x10⁵ CEM cells for each peptide concentration were incubated for 3 days at 37°C in T25 flasks. Peptides tested were DP-178 (SEQ ID:1) and DP-116 (SEQ ID:9), as described in FIG. 1. The concentrations of each peptide used were 0, 2.5, 10, and 40μg/ml. Cell counts were taken at incubation times of 0, 24, 48, and 72 hours.

7.2. RESULTS

Whether the potent HIV-1 inhibitor DP-178 (SEQ ID:1) exhibited any cytotoxic effects was assessed by assaying the peptide's effects on the proliferation and viability of cells in culture. CEM cells were incubated in the presence of varying concentrations of DP-178 (SEQ ID:1), and DP-116 (SEQ ID:9), a peptide

2164698

previously shown to be ineffective as a HIV inhibitor (Wild, C. et al., 1992, Proc. Natl. Acad. Sci. USA 89:10,537-10,541). Additionally, cells were incubated in the absence of either peptide.

5 The results of the cytotoxicity study demonstrate
that DP-178 (SEQ ID:1) exhibits no cytotoxic effects
on cells in culture. As can be seen, below, in Table
XI, even the proliferation and viability
10 characteristics of cells cultured for 3 days in the
presence of the highest concentration of DP-178 (SEQ
ID:1) tested (40 μ g/ml) do not significantly differ
from the DP-116 (SEQ ID:9) or the no-peptide controls.
The cell proliferation data is also represented in
15 graphic form in FIG. 6. As was demonstrated in the
Working Example presented above in Section 6, DP-178
(SEQ ID:1) completely inhibits HIV-1 mediated syncytia
formation at peptide concentrations between 1 and
10ng/ml, and completely inhibits cell-free viral
infection at concentrations of at least 90ng/ml.
Thus, this study demonstrates that even at peptide
20 concentrations greater than 3 log higher than the HIV
inhibitory dose, DP-178 (SEQ ID:1) exhibits no cytotoxic
effects.

TABLE XI

25

		% Viability at time (hours)			
<u>Peptide</u>	<u>Peptide Concentration μg/ml</u>	<u>0</u>	<u>24</u>	<u>48</u>	<u>72</u>
30 DP178 (SEQ ID:1)	40	98	97	95	97
	10	98	97	98	98
	2.5	98	93	96	96

35

2164698

5	DP116 (SEQ ID:9)	40	98	95	98	97
		10	98	95	93	98
		2.5	98	96	98	99
	No Peptide	0	98	97	99	98
10	8. <u>EXAMPLE: THE INTERACTION OF DP178 AND DP107</u>					
15	Soluble recombinant forms of gp41 used in the example described below provide evidence that the DP178 peptide associates with a distal site on gp41 whose interactive structure is influenced by the DP107 leucine zipper motif. A single mutation disrupting the coiled-coil structure of the leucine zipper domain transformed the soluble recombinant gp41 protein from an inactive to an active inhibitor of HIV-1 fusion. This transformation may result from liberation of the potent DP178 domain from a molecular clasp with the leucine zipper, DP107, determinant. The results also indicate that the anti-HIV activity of various gp41 derivatives (peptides and recombinant proteins) may be due to their ability to form complexes with viral gp41 and interfere with its fusogenic process.					
20						
25						

8.1. MATERIALS AND METHODS

8.1.1. CONSTRUCTION OF FUSION PROTEINS AND GP41 MUTANTS

30 Construction of fusion proteins and mutants shown in FIG. 7 was accomplished as follows: the DNA sequence corresponding to the extracellular domain of gp41 (540-686) was cloned into the Xmn I site of the expression vector pMal-p2 (New England Biolab) to give
 35 M41. The gp41 sequence was amplified from pgtat

2164698

(Malim et al., 1988, Nature 355: 181-183) by using polymerase chain reaction (PCR) with upstream primer 5'-ATGACGCTGACGGTACAGGCC-3' (primer A) and downstream primer 5'-TGACTAAGCTTAATACCACAGCCAATTTGTTAT-3' (primer B). M41-P was constructed by using the T7-Gen
5 in vitro mutagenesis kit from United States Biochemicals (USB) following the supplier's instructions. The mutagenic primer (5'-GGAGCTGCTTGGGGCCCCAGAC-3') introduces an Ile to Pro mutation in M41 at position 578. M41Δ107 was made
10 using a deletion mutagenic primer 5'-CCAAATCCCCAGGAGCTGCTCGAGCTGCACTATACCAGAC-3' (primer C) following the USB T7-Gen mutagenesis protocol. M41Δ178 was made by cloning the DNA fragment corresponding to gp41 amino acids 540-642 into the Xmn
15 I site of pMal-p2. Primer A and 5'-ATAGCTTCTAGATTAATTGTTAATTTCTCTGTCCC-3' (primer D) were used in the PCR with the template pgtat to generate the inserted DNA fragments. M41-P was used as the template with primer A and D in PCR to generate M41-
20 PΔ178. All inserted sequences and mutated residues were checked by restriction enzyme analysis and confirmed by DNA sequencing.

25 8.1.2. PURIFICATION AND CHARACTERIZATION OF FUSION PROTEINS

The fusion proteins were purified according to the protocol described in the manufacturer's brochure of protein fusion and purification systems from New England Biolabs (NEB). Fusion proteins (10 ng) were
30 analyzed by electrophoresis on 8% SDS polyacrylamide gels. Western blotting analysis was performed as described by Sambrook et al, 1989, Molecular Cloning: A Laboratory Manual, 2d Ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Ch. 18,
35 pp. 64-75. An HIV-1 positive serum diluted 1000-fold,

2164698

or a human Fab derived from repertoire cloning was used to react with the fusion proteins. The second antibody was HRP-conjugated goat antihuman Fab. An ECL Western blotting detection system (Amersham) was used to detect the bound antibody. A detailed
5 protocol for this detection system was provided by the manufacturer. Rainbow molecular weight marker (Amersham) were used to estimate the size of fusion proteins.

10 8.1.3. CELL FUSION ASSAYS FOR ANTI-HIV ACTIVITY

Cell fusion assays were performed as previously described (Matthews et al., 1987, Proc. Natl. Acad. Sci. USA 84: 5424-5481). CEM cells (7×10^4) were
15 incubated with HIV-1_{III} chronically infected CEM cells (10^4) in 96-well flat-bottomed half-area plates (Costar) in 100 μ l culture medium. Peptide and fusion proteins at various concentrations in 10 μ l culture medium were incubated with the cell mixtures at 37°C for 24 hours. Multinucleated syncytia were estimated
20 with microscopic examination. Both M41 and M41-P did not show cytotoxicity at the concentrations tested and shown in FIG. 8.

Inhibition of HIV-1 induced cell-cell fusion activity was carried out in the presence of 10 nM
25 DP178 and various concentrations of M41 Δ 178 or M41-PA178 as indicated in FIG. 9. There was no observable syncytia in the presence of 10 nM DP178. No peptide or fusion protein was added in the control samples.

30 8.1.4. ELISA ANALYSIS OF DP178 BINDING TO THE LEUCINE ZIPPER MOTIF OF GP41

The amino acid sequence of DP178 used is:
YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF. For enzyme
linked immunoassay (ELISA), M41 Δ 178 or M41-PA178 (5
35 μ g/ml) in 0.1M NaHCO₃, pH 8.6, were coated on 96 wells

Linbro™ ELISA plates (Flow Lab, Inc.) overnight. Each well was washed three times with distilled water then blocked with 3% bovine serum albumin (BSA) for 2 hours. After blocking, peptides with 0.5% BSA in TBST (40 mM Tris-HCl pH7.5, 150 mM NaCl, 0.05% Tween 20™) were added to the ELISA plates and incubated at room temperature for 1 hour. After washing three times with TBST, Fab-d was added at a concentration of 10 ng/ml with 0.5% BSA in TBST. The plates were washed three times with TBST after incubation at room temperature for 1 hour. Horse radish peroxidase (HRP) conjugated goat antihuman Fab antiserum at a 2000 fold dilution in TBST with 0.5% BSA was added to each well and incubated at room temperature for 45 minutes. The plates were then washed four times with TBST. The peroxidase substrate o-phenylene diamine (2.5 mg/ml) and 0.15% H₂O₂ were added to develop the color. The reaction was stopped with an equal volume of 4.5 N H₂SO₄ after incubation at room temperature for 10 minutes. The optical density of the stopped reaction mixture was measured with a micro plate reader (Molecular Design) at 490 nm. Results are shown in FIG. 10.

8.2. RESULTS

8.2.1. THE EXPRESSION AND CHARACTERIZATION OF THE ECTODOMAIN OF GP41

As a step toward understanding the roles of the two helical regions in gp41 structure and function, the ectodomain of gp41 was expressed as a maltose binding fusion protein (M41) (Fig. 7). The fusogenic peptide sequence at the N-terminal of gp41 was omitted from this recombinant protein and its derivatives to improve solubility. The maltose binding protein facilitated purification of the fusion proteins under relatively mild, non-denaturing conditions. Because

2164698

the M41 soluble recombinant gp41 was not glycosylated, lacked several regions of the transmembrane protein (i.e., the fusion peptide, the membrane spanning, and the cytoplasmic domains), and was expressed in the absence of gp120, it was not expected to precisely reflect the structure of native gp41 on HIV-1 virions. Nevertheless, purified M41 folded in a manner that preserved certain discontinuous epitopes as evidenced by reactivity with human monoclonal antibodies, 98-6, 126-6, and 50-69, previously shown to bind conformational epitopes on native gp41 expressed in eukaryotic cells (Xu et al., 1991, J. Virol. 65: 4832-4838; Chen, 1994, J. Virol. 68:2002-2010). Thus, at least certain regions of native gp41 defined by these antibodies appear to be reproduced in the recombinant fusion protein M41. Furthermore, M41 reacted with a human recombinant Fab (Fab-d) that recognizes a conformational epitope on gp41 and binds HIV-1 virions as well as HIV-1 infected cells but not uninfected cells as analyzed by FACS. Deletion of either helix motif, i.e., DP107 or DP178, of the M41 fusion protein eliminated reactivity with Fab-d. These results indicate that both helical regions, separated by 60 amino acids in the primary sequence, are required to maintain the Fab-d epitope.

8.2.2. ANTI-HIV ACTIVITY OF THE RECOMBINANT ECTODOMAIN OF GP41

The wild type M41 fusion protein was tested for anti-HIV-1 activity. As explained, supra, synthetic peptides corresponding to the leucine zipper (DP107) and the C-terminal putative helix (DP178) show potent anti-HIV activity. Despite inclusion of both these regions, the recombinant M41 protein did not affect

HIV-1 induced membrane fusion at concentrations as high as 50 μ M (Table XII, below).

TABLE XII

**DISRUPTION OF THE LEUCINE ZIPPER OF
GP41 FREES THE ANTI-HIV MOTIF**

	<u>DP107</u>	<u>DP178</u>	<u>M41</u>	<u>M41-P</u>	<u>M41-PΔ178</u>
Cell fusion (IC ₅₀)	1 μ M	1 nM	> 50 μ M	83 nM	> 50 μ M
Fab-D binding (k _D)	-	-	3.5x10 ⁻⁹	2.5x10 ⁻⁸	-
HIV infectiv- ity (IC ₅₀)	1 μ M	80 nM	> 16 μ M	66 nM	> 8 μ M
The affinity constants of Fab-d binding to the fusion proteins were determined using a protocol described by B. Friguet et al., 1985, J. Immunol. Method. 77:305-319. - = No detectable binding of Fab-d to the fusion proteins. <i>Antiviral Infectivity Assays.</i> 20 μl of serially diluted virus stock was incubated for 60 minutes at ambient temperature with 20 μl of the indicated concentration of purified recombinant fusion protein in RPMI 1640 containing 10% fetal bovine serum and antibiotics in a 96-well microtiter plate. 20 μl of CEM4 cells at 6 x 10⁵ cells/ml were added to each well, and cultures were incubated at 37°C in a humidified CO₂ incubator. Cells were cultured for 9 days by the addition of fresh medium every 2 to 30 days. On days 5, 7, and 9 postinfection, supernatant samples were assayed for reverse transcriptase (RT) activity, as described below, to monitor viral replication. The 50% tissue culture infectious dose (TCID₅₀) was calculated for each condition according to the formula of Reed & Muench, 1937, Am. J. Hyg. 27:493-497. RT activity was determined by a modification of the published methods of Goff et al., 1981, J. Virol. 38:239-248 and Willey et al., 1988, J. Virol. 62:139-147 as described in Chen et al., 1993, AIDS Res. Human Retroviruses 9:1079-1086.					

Surprisingly, a single amino acid substitution, proline in place of isoleucine in the middle of the leucine zipper motif, yielded a fusion protein (M41-P)

which did exhibit antiviral activity (Table XII and Fig. 8). As seen in Table XII, M41-P blocked syncytia formation by 90% at approximately 85 nM and neutralized HIV-1_{III} infection by 90% at approximately 70 nM concentrations. The anti-HIV-1 activity of M41-P appeared to be mediated by the C-terminal helical sequence since deletion of that region from M41-P yielded an inactive fusion protein, M41-PA178 (Table XII). That interpretation was reinforced by experiments demonstrating that a truncated fusion protein lacking the DP178 sequence, M41Δ178, abrogated the potent anti-fusion activity of the DP178 peptide in a concentration-dependent manner (FIG. 9). The same truncated fusion protein containing the proline mutation disrupting the leucine zipper, M41-PA178, was not active in similar competition experiments (FIG. 9). The results indicate that the DP178 peptide associates with a second site on gp41 whose interactive structure is dependent on a wild type leucine zipper sequence. A similar interaction may occur within the wild type fusion protein, M41, and act to form an intramolecular clasp which sequesters the DP178 region, making it unavailable for anti-viral activity.

A specific association between these two domains is also indicated by other human monoclonal Fab-d studies. For example, Fab-d failed to bind either the DP178 peptide or the fusion protein M41Δ178, but its epitope was reconstituted by simply mixing these two reagents together (FIG. 10). Again, the proline mutation in the leucine zipper domain of the fusion protein, M41-PA178, failed to reconstitute the epitope in similar mixing experiments.

9. EXAMPLE: METHOD FOR COMPUTER-ASSISTED
IDENTIFICATION OF DP-107-LIKE
AND DP-178-LIKE SEQUENCES

A number of known coiled-coil sequences have been well described in the literature and contain heptad repeat positioning for each amino acid. Coiled-coil nomenclature labels each of seven amino acids of a heptad repeat A through G, with amino acids A and D tending to be hydrophobic positions. Amino acids E and G tend to be charged. These four positions (A, D, E, and G) form the amphipathic backbone structure of a monomeric alpha-helix. The backbones of two or more amphipathic helices interact with each other to form di-, tri-, tetrameric, etc., coiled-coil structures. In order to begin to design computer search motifs, a series of well characterized coiled coils were chosen including yeast transcription factor GCN4, Influenza Virus hemagglutinin loop 36, and human proto-oncogenes c-Myc, c-Fos, and c-Jun. For each peptide sequence, a strict homology for the A and D positions, and a list of the amino acids which could be excluded for the B, C, E, F, and G positions (because they are not observed in these positions) was determined. Motifs were tailored to the DP-107 and DP-178 sequences by deducing the most likely possibilities for heptad positioning of the amino acids of HIV-1 Bru DP-107, which is known to have coiled-coil structure, and HIV-1 Bru DP-178, which is still structurally undefined. The analysis of each of the sequences is contained in FIG. 12. For example, the motif for GCN4 was designed as follows:

1. The only amino acids (using standard single letter amino acid codes) found in the A or D positions of GCN4 were [LMNV].
2. All amino acids were found at B, C, E, F, and G positions except {CFGIMPTW}.

2164698

3. The PESEARCH motif would, therefore, be written as follows:

[LMNV]-{CFGIMPTW}(2)-[LMNV]-{CFGIMPTW}(3)-
 [LMNV]-{CFGIMPTW}(2)-[LMNV]-{CFGIMPTW}(3)-
 [LMNV]-{CFGIMPTW}(2)-[LMNV]-{CFGIMPTW}(3)-
 5 [LMNV]-{CFGIMPTW}(2)-[LMNV]-{CFGIMPTW}(3)

Translating or reading the motif: "at the first A position either L, M, N, or V must occur; at positions B and C (the next two positions) accept everything
 10 except C, F, G, I, M, P, T, or W; at the D position either L, M, N, or V must occur; at positions E, F, and G (the next 3 positions) accept everything except C, F, G, I, M, P, T, or W." This statement is contained four times in a 28-mer motif and five times
 15 in a 35-mer motif. The basic motif key then would be: [LMNV]-{CFGIMPTW}. The motif keys for the remaining well described coiled-coil sequences are summarized in FIG. 12.

The motif design for DP-107 and DP-178 was
 20 slightly different than the 28-mer model sequences described above due to the fact that heptad repeat positions are not defined and the peptides are both longer than 28 residues. FIG. 13 illustrates several possible sequence alignments for both DP-107 and DP-
 25 178 and also includes motif designs based on 28^{-mer}, 35^{-mer}, and full-length peptides. Notice that only slight differences occur in the motifs as the peptides are lengthened. Generally, lengthening the base peptide results in a less stringent motif. This is
 30 very useful in broadening the possibilities for identifying DP-107-or DP-178-like primary amino acid sequences referred to in this document as "hits".

In addition to making highly specific motifs for each type peptide sequence to be searched, it is also
 35 possible to make "hybrid" motifs. These motifs are

made by "crossing" two or more very stringent motifs to make a new search algorithm which will find not only both "parent" motif sequences but also any peptide sequences which have similarities to one, the other, or both "parents". For example, in Table 3 the "parent" sequence of GCN4 is crossed with each of the possible "parent" motifs of DP-107. Now the hybrid motif must contain all of the amino acids found in the A and D positions of both parents, and exclude all of the amino acids not found in either parent at the other positions. The resulting hybrid from crossing GCN4 or [LMNV]{CFGIMPTW} and DP-107 (28-mer with the first L in the D position) or [ILQT]{CDFIMPST}, is [ILMNQTV]{CFIMPT}. Notice that now only two basic hybrid motifs exist which cover both framing possibilities, as well as all peptide lengths of the parent DP-107 molecule. FIG. 15 represents the hybridizations of GCN4 with DP-178. FIG. 16 represents the hybridizations of DP-107 and DP-178. It is important to keep in mind that the represented motifs, both parent and hybrid, are motif keys and not the depiction of the full-length motif needed to actually do the computer search.

Hybridizations can be performed on any combination of two or more motifs. Table 5 summarizes several three-motif hybridizations including GCN4, DP-107 (both frames), and DP-178 (also both frames). Notice that the resulting motifs are now becoming much more similar to each other. In fact, the first and third hybrid motifs are actually subsets of the second and fourth hybrid motifs respectively. This means that the first and third hybrid motifs are slightly more stringent than the second and fourth. It should also be noted that with only minor changes in these four motifs, or by hybridizing them, a single motif could be obtained

which would find all of the sequences. However, it should be remembered that stringency is also reduced. Finally, the most broad-spectra and least-stringent hybrid motif is described in FIG. 18 which summarizes the hybridization of GCN4, DP-107 (both frames), DP-178 (both frames), c-Fos, c-Jun, c-Myc, and Flu loop 36.

A special set of motifs was designed based on the fact that DP-178 is located only approximately ten amino acids upstream of the transmembrane spanning region of gp41 and just C-terminal to a proline which separates DP-107 and DP-178. It has postulated that DP-178 may be an amphipathic helix when membrane associated, and that the proline might aid in the initiation of the helix formation. The same arrangement was observed in Respiratory Syncytial Virus; however, the DP-178-like region in this virus also had a leucine zipper just C-terminal to the proline. Therefore, designed N-terminal proline-leucine zipper motifs were designed to analyze whether any other viruses might contain this same pattern. The motifs are summarized in FIG. 19.

The PC/Gene protein database contains 5879 viral amino acid sequences (library file PVIRUSES; CD-ROM release 11.0). Of these, 1092 are viral envelope or glycoprotein sequences (library file PVIRUSE1). Tables V through X contain lists of protein sequence names and motif hit locations for all the motifs searched.

10. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION OF DP-107 AND DP-178-LIKE SEQUENCES IN HUMAN IMMUNODEFICIENCY VIRUS

FIG. 20 represents search results for HIV-1 BRU isolate gp41 (PC/Gene protein sequence PENV_HV1BR). Notice that the hybrid motif which crosses DP-107 and

DP-178 (named 107x178x4; the same motif as found in FIG. 16 found three hits including amino acids 550-599, 636-688, and 796-823. These areas include DP-107 plus eight N-terminal and four C-terminal amino acids; DP-178 plus seven N-terminal and ten C-terminal amino acids; and an area inside the transmembrane region (cytoplasmic). FIG. 20 also contains the results obtained from searching with the motif named ALLMOTI5, for which the key is found in FIG. 17 ({CDGHP}{CFP}x5). This motif also found three hits including DP-107 (amino acids 510-599), DP-178 (615-717), and a cytoplasmic region (772-841). These hits overlap the hits found by the motif 107x178x4 with considerable additional sequences on both the amino and carboxy termini. This is not surprising in that 107x178x4 is a subset of the ALLMOTI5 hybrid motif. Importantly, even though the stringency of ALLMOTI5 is considerably less than 107x178x4, it still selectively identifies the DP-107 and DP-178 regions of gp41 shown to contain sequences for inhibitory peptides of HIV-1. The results of these two motif searches are summarized in Table V under the PC/Gene protein sequence name PENV HV1BR. The proline-leucine zipper motifs also gave several hits in HIV-1 BRU including 503-525 which is at the very C-terminus of gp120, just upstream of the cleavage site (P7LZIPC and P12LZIPC); and 735-768 in the cytoplasmic domain of gp41 (P23LZIPC). These results are found in Tables VIII, IX, and X under the same sequence name as mentioned above. Notice that the only area of HIV-1 BRU which is predicted by the Lupas algorithm to contain a coiled-coil region, is from amino acids 635-670. This begins eight amino acids N-terminal to the start and ends eight amino acids N-terminal to the end of DP-178. DP-107, despite the fact that it is a known coiled coil, is

2164698

not predicted to contain a coiled-coil region using the Lupas method.

11. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION
OF DP-107-LIKE AND DP-178-LIKE
SEQUENCES IN HUMAN RESPIRATORY
SYNCYTIAL VIRUS

FIG. 21 represents search results for Human Respiratory Syncytial Virus (RSV; Strain A2) fusion glycoprotein F1 (PC/Gene protein sequence name PVGLF_HRSVA). Motif 107x178x4 finds three hits including amino acids 152-202, 213-243, and 488-515. The arrangement of these hits is similar to what is found in HIV-1 except that the motif finds two regions with similarities to DP-178, one just downstream of what would be called the DP-107 region or amino acids 213-243, and one just upstream of the transmembrane region (also similar to DP-178) or amino acids 488-515. Motif ALLMOTI5 also finds three areas including amino acids 116-202, 267-302, and 506-549. The proline-leucine zipper motifs also gave several hits including amino acids 205-221 and 265-287 (P1LZIPC 265-280, P12LZIPC), and 484-513 (P7LZIPC and P12LZIPC 484-506, P23LZIPC). Notice that the PLZIP motifs also identify regions which share location similarities with DP-178 of HIV-1.

12. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION OF
DP-107-LIKE AND DP-178-LIKE SEQUENCES
IN SIMIAN IMMUNODEFICIENCY VIRUS

Motif hits for Simian immunodeficiency Virus gp41 (AGM3 isolate; PC/Gene protein sequence name PENV_SIVAG) are shown in FIG. 22. Motif 107x178x4 finds three hits including amino acids 566-593, 597-624, and 703-730. The first two hits only have three amino acids between them and could probably be combined into one hit from 566-624 which would

represent a DP-107-like hit. Amino acids 703 to 730 would then represent a DP-178-like hit. ALLMOTI5 also finds three hits including amino acids 556-628 (DP-107-like), 651-699 (DP-178-like), and 808-852 which represents the transmembrane spanning region. SIV
5 also has one region from 655-692 with a high propensity to form a coiled coil as predicted by the Lupas algorithm. Both 107x178x4 and ALLMOTI5 motifs find the same region. SIV does not have any PLZIP motif hits in gp41.

10

13. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION OF
DP-107-LIKE AND DP-178 LIKE SEQUENCES
IN CANINE DISTEMPER VIRUS

Canine Distemper Virus (strain Onderstepoort)
15 fusion glycoprotein F1 (PC/Gene Protein sequence name PVGLF_CDVO) has regions similar to Human RSV which are predicted to be DP-107-like and DP-178-like (FIG. 23). Motif 107x178x4 highlights one area just C-terminal to the fusion peptide at amino acids 252-293. Amino
20 acids 252-286 are also predicted to be coiled coil using the Lupas algorithm. Almost 100 amino acids C-terminal to the first region is a DP-178-like area at residues 340-367. ALLMOTI5 highlights three areas of interest including: amino acids 228-297, which
25 completely overlaps both the Lupas prediction and the DP-107-like 107x178x4 hit; residues 340-381, which overlaps the second 107x178x4 hit; and amino acids 568-602, which is DP178-like in that it is located just N-terminal to the transmembrane region. It also
30 overlaps another region (residues 570-602) predicted by the Lupas method to have a high propensity to form a coiled coil. Several PLZIP motifs successfully identified areas of interest including P6 and P12LZIPC which highlight residues 336-357 and 336-361
35 respectively; P1 and P12LZIPC which find residues 398-

2164698

414; and P12 and P23LZIPC which find residues 562-589 and 562-592 respectively.

14. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION OF
DP-107-LIKE AND DP-178-LIKE SEQUENCES
IN NEWCASTLE DISEASE VIRUS

FIG. 24 shows the motif hits found in Newcastle Disease Virus (strain Australia-Victoria/32; PC Gene protein sequence name PVGLF_NDVA). Motif 107x178x4 finds two areas including a DP-107-like hit at amino acids 151-178 and a DP-178-like hit at residues 426-512. ALLMOTI5 finds three areas including residues 117-182, 231-272, and 426-512. The hits from 426-512 include a region which is predicted by the Lupas method to have a high coiled-coil propensity (460-503). The PLZIP motifs identify only one region of interest at amino acids 273-289 (P1 and 12LZIPC).

15. EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION
OF DP-107-LIKE AND DP-178-LIKE
SEQUENCES IN HUMAN PARAINFLUENZA VIRUS

Both motifs 107x178x4 and ALLMOTI5 exhibit DP-107-like hits in the same region, 115-182 and 117-182 respectively, of Human Parainfluenza Virus (strain NIH 47885; PC/Gene protein sequence name PVGLF_p13H4; (FIG. 25). In addition, the two motifs have a DP-178-like hit just slightly C-terminal at amino acids 207-241. Both motifs also have DP-178-like hits nearer the transmembrane region including amino acids 457-497 and 462-512 respectively. Several PLZIP motif hits are also observed including 283-303 (P5LZIPC), 283-310 (P12LZIPC), 453-474 (P6LZIPC), and 453-481 (P23LZIPC). The Lupas algorithm predicts that amino acids 122-176 have a propensity to form a coiled-coil.

16. **EXAMPLE: COMPUTER-ASSISTED IDENTIFICATION OF
DP-107-LIKE AND DP-178-LIKE SEQUENCES OF
INFLUENZA A VIRUS**

FIG. 26 illustrates the Lupas prediction for a coiled coil in Influenza A Virus (strain A/Aichi/2/68) at residues 379-436, as well as the motif hits for 107x178x4 at amino acids 387-453, and for ALLMOTI5 at residues 380-456. Residues 383-471 (38-125 of HA2) were shown by Carr and Kim to be an extended coiled coil when under acidic pH (Carr and Kim, 1993, Cell 73: 823-832). The Lupas algorithm predicts a coiled-coil at residues 379-436. All three methods successfully predicted the region shown to actually have coiled-coil structure; however, ALLMOTI5 predicted the greatest portion of the 88 residue stretch.

17. **EXAMPLE: RSV ANTIVIRAL COMPOUNDS**

In the Example presented herein, respiratory syncytial virus (RSV) peptide sequences identified by utilizing the computer-assisted coiled-coil peptide sequence searches described in Example 9, above, are shown to encode peptide domains that exhibit structural similarity to actual, known coiled-coil peptides, and are, additionally found to exhibit antiviral activity.

17.1 **MATERIALS AND METHODS**

Structural analyses consisted of circular dichroism (CD) studies, which were conducted according to the methods described in the Applicants' U.S. Patent No. 5,464,933 issued on November 7, 1995.

Anti-RSV antiviral activity was assayed as described in Pringle, C.R. et al., 1985, J. Medical Vir. 17:377-386.

2164698

5 A 48 amino acid RSV F2 peptide and a 53 amino acid RSV T67 peptide are utilized which span sequences that were identified via the computer assisted peptide sequence search strategies described in Example 9, above. See FIG. 21 for the exact position of these sequences and for the motifs utilized.

17.2 RESULTS

10 35-mer oligopeptides were synthesized which constituted portions of the 48 amino acid RSV F2 peptide sequence (FIG. 27) and portions of the 53 amino acid RSV T67 peptide sequence (FIG. 28). The oligopeptides were assayed, via CD analysis, for structural similarity to known coiled-coil structures, and for anti-RSV activity. As shown in FIGS. 27 and 15 28, a number of these oligopeptides exhibited substantial coiled-coil structural similarity and/or antiviral activity.

Thus, the computer assisted searches described, herein, in Example 9, for example, successfully 20 identified viral peptide domains that represent highly promising anti-RSV antiviral compounds.

18. EXAMPLE: HPF3 ANTIVIRAL COMPOUNDS

25 In the Example presented herein, human parainfluenza virus 3 (HPF3) peptide sequences identified by utilizing the computer-assisted coiled-coil peptide sequence searches described in Example 9, above, are shown to encode peptide domains that exhibit structural similarity to actual, known coiled-coil 30 peptides, and are, additionally found to exhibit antiviral activity.

18.1 MATERIALS AND METHODS

35 Structural analyses consisted of circular dichroism (CD) studies, which were conducted according

to the methods described in the Applicants' U.S. Patent No. 5,464,933 issued on November 7, 1995.

Anti-HPF3 antiviral activity was assayed as described in Pringle, C.R. et al., 1985, J. Medical Vir. 17:377-386.

5 A 56 amino acid and 70 amino acid HPF3 peptide are utilized which span sequences that were identified via the computer assisted peptide sequence search strategies described in Example 9, above. See FIG. 25 for the exact positions of these sequences and for the
10 motifs utilized.

18.2 RESULTS

35-mer oligopeptides were synthesized which constituted portions of the 56 amino acid HPF3 peptide
15 sequence (FIG. 29) and portions of the 70 amino acid HPF3 peptide sequence (FIG. 30). The oligopeptides were assayed, via CD analysis, for structural similarity to known coiled-coil structures, and for anti-HPF3 activity. As shown in FIGS. 29 and 30, a
20 number of these oligopeptides exhibited substantial coiled-coil structural similarity and/or antiviral activity.

Thus, the computer assisted searches described, herein, in Example 9, for example, successfully
25 identified viral peptide domains that represent highly promising anti-HPF3 antiviral compounds.

The present invention is not to be limited in scope by the specific embodiments described which are
30 intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will
35 become apparent to those skilled in the art from the

2164698

foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

5

10

15

20

25

30

35

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. An isolated peptide having a formula selected from:
 YTSLIHSLIEESQNQQEKNEQEELLELDKWASLWNWF SEQ ID NO: 1 (DP-178);
 YTNTIYTLLEESQNQQEKNEQEELLELDKWASLWNWF SEQ ID NO: 3 (DP-185);
 YTGIIYNLLEESQNQQEKNEQEELLELDKWANLWNWF SEQ ID NO: 4; or
 YTSLIYSLLEKSQTQQEKNEQEELLELDKWASLWNWF SEQ ID NO: 5;
 wherein the peptide comprises an amino terminal X and a carboxy terminal Z in which:

X comprises an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, a hydrophobic group, or a non-peptide macromolecular carrier group; and

Z comprises a carboxyl group, an amido group, a hydrophobic group, or a non-peptide macromolecular carrier group.

2. The isolated peptide of claim 1, having an amino acid sequence YTSLIHSLIEESQNQQEKNEQEELLELDKWASLWNWF.

3. The isolated peptide of claim 1, having an amino acid sequence YTNTIYTLLEESQNQQEKNEQEELLELDKWASLWNWF.

4. The isolated peptide of claim 1, having an amino acid sequence YTGIIYNLLEESQNQQEKNEQEELLELDKWANLWNWF.

5. The isolated peptide of claim 1, having an amino acid sequence YTSLIYSLLEKSQTQQEKNEQEELLELDKWASLWNWF.

6. The peptide according to any one of claims 1-5, wherein X is an amino group and Z is a carboxyl group.

7. The peptide of any one of claims 1-5, wherein X is a carbobenzoxyl, dansyl or t-butyloxycarbonyl hydrophobic group; and Z is a t-butyloxycarbonyl hydrophobic group or an amido group.

8. The peptide of any one of claims 1- 5, wherein X is an acetyl group or a 9-fluorenylmethoxy-carbonyl group; and Z is a t-butyloxycarbonyl hydrophobic group or an amido group.

9. The peptide of claim 8, wherein X is an acetyl group and Z is an amido group.

10. The peptide of any one of claims 1-5, wherein the macromolecular carrier group is a lipid-fatty acid conjugate, a polyethylene glycol, or a carbohydrate moiety.

11. The peptide according to any one of claims 1-10, wherein one bond linking adjacent amino acids is a non-peptide bond and the non-peptide bond is an imino, ester, hydrazine, semicarbazide, or azo bond.

12. The peptide according to any one of claims 1-11, wherein at least one amino acid residue is in a D-isomer configuration.

13. The peptide according to any one of claims 1-12, for use as an antiviral agent in the treatment of HIV infection.

14. Use of the peptide of any one of claims 1-12, for the manufacture of a medicament for use as an antiviral agent in the treatment of HIV infection.

15. The use according to claim 13 or 14, wherein the treatment comprises inhibiting the virus, in the presence of a

cell, with an effective concentration of the peptide for a sufficient period so that HIV transmission to the cell is inhibited.

16. A pharmaceutical composition, comprising the peptide of any one of claims 1-12, and a pharmaceutically acceptable carrier.

17. The peptide of claim 2, wherein X is an acetyl group and Z is an amido group.

18. Use of the peptide of claim 17, for the manufacture of a medicament for use as an antiviral agent in the treatment of HIV infection.

19. The use according to claim 18, wherein the treatment comprises inhibiting the virus, in the presence of a cell, with an effective concentration of the peptide for a sufficient period so that HIV transmission to the cell is inhibited.

20. A pharmaceutical composition, comprising the peptide of claim 17 and a pharmaceutically acceptable carrier.

21. An isolated nucleic acid encoding the peptide of claim 1, 3, 4, or 5.

22. An isolated nucleic acid encoding the peptide of claim 2.

23. A process for preparing the peptide of claim 1, 2, 3, 4, or 5 comprising:

- i) synthesizing the peptide; and
- ii) isolating the peptide.

24. The process of claim 23, wherein X is a hydrophobic group.

25. The process of claim 24, wherein the hydrophobic group X is carbobenzoxyl, dansyl, or t-butyloxycarbonyl.

26. The process of claim 23, wherein Z is a hydrophobic group.

27. The process of claim 26, wherein the hydrophobic group Z is t-butyloxycarbonyl.

28. The process of claim 23, wherein X is a macromolecular carrier group.

29. The process of claim 28, wherein the macromolecular carrier group X is a lipid-fatty acid conjugate, a polyethylene glycol, or a carbohydrate moiety.

30. The process of claim 23, wherein Z is a macromolecular carrier group.

31. The process of claim 30, wherein the macromolecular carrier group Z is a lipid-fatty acid conjugate, a polyethylene glycol, or a carbohydrate moiety.

32. The process of claim 23, wherein at least one bond linking adjacent amino acid residues is a non-peptide bond.

33. The process of claim 32, wherein the non-peptide bond is an imino, ester, hydrazine, semicarbazide, or azo bond.

34. The process of claim 23, wherein at least one amino acid residue is in a D-isomer configuration.

35. A process for preparing a peptide having the following formula:

X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z (SEQ ID NO:1),
wherein the peptide amino acid residues are presented by the single-letter code, further wherein:

X is an acetyl group; and

Z is an amido group comprising:

- i) synthesizing the peptide; and
- ii) isolating the peptide.

36. The process of any one of claims 23-35, further comprising formulating said peptide into a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

37. The peptide of claim 17, for the use as an antiviral agent in the treatment of HIV infection.

HIV1LAI	(DP-178; SEQ ID:1)	YTSLIHSLIEESQSQKEKNEQELLELDKWASLWNWF
HIV1SF2	(DP-185; SEQ ID:3)	YTNTIYNLLEESQSQKEKNEQELLELDKWASLWNWF
HIV1RF	(SEQ ID:4)	YTGIIYNLLEESQSQKEKNEQELLELDKWANLWNWF
HIV1MN	(SEQ ID:5)	YTSLIYSLLEKSQTQKEKNEQELLELDKWASLWNWF
HIV2ROD	(SEQ ID:6)	LEANISKSLEQAIIQKEKNMYELQKLSWDIFGNWF
HIV2NIHZ	(SEQ ID:7)	LEANISQSLEQAIIQKEKNMYELQKLSWDVFTNWL
DP180	(SEQ ID:2)	SSESFTLLEQWNNMKQLAEQWLEQINEKHYLEDIS
DP118	(SEQ ID:10)	QQLLDVVKRQQEMLRLTVWGTKNLQARVTAIEKYLKDQ
DP125	(SEQ ID:8)	CGGNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQ
DP116	(SEQ ID:9)	LQARILAVERYLKDQQQ

FIG.1



2164698

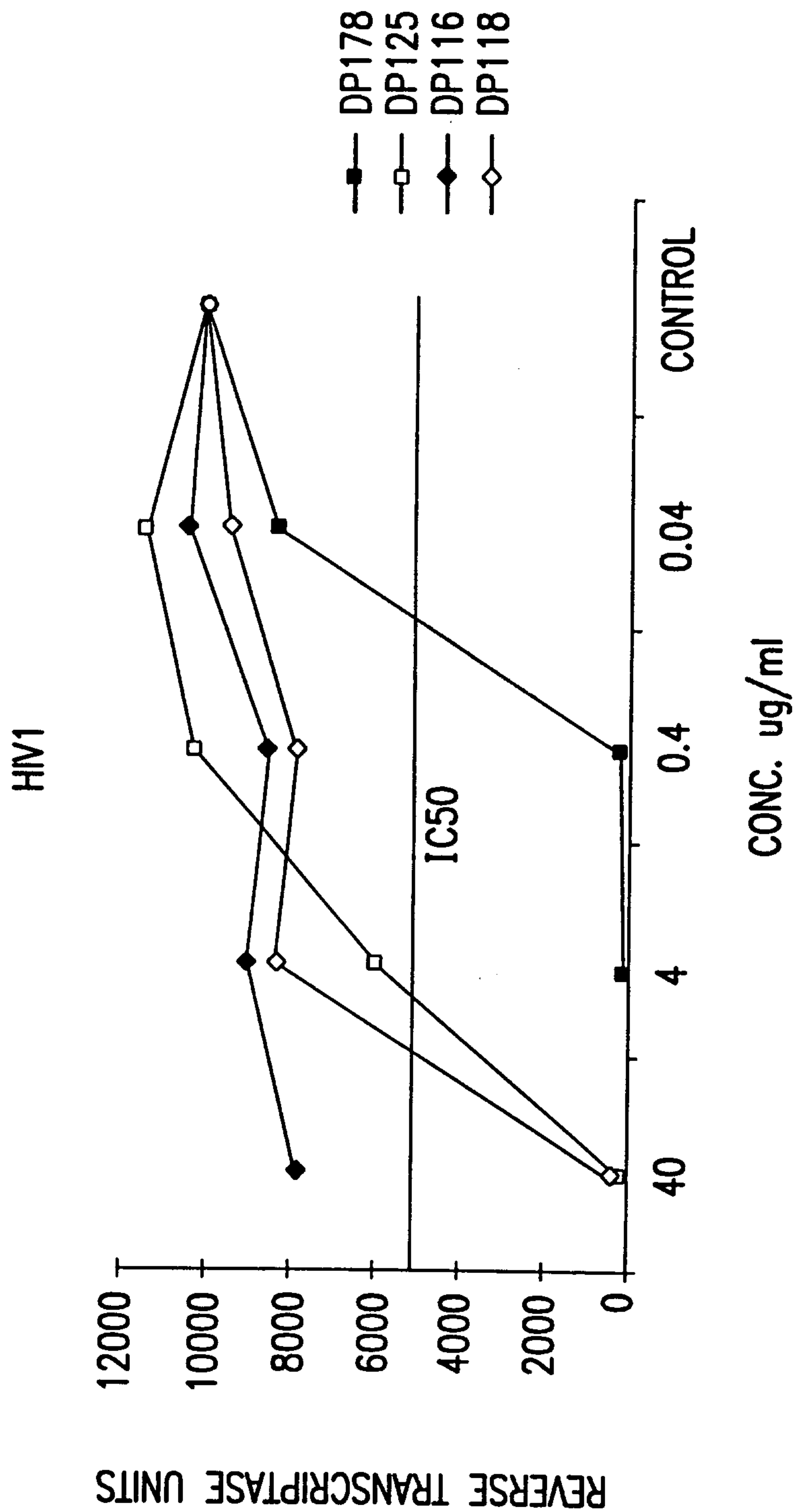


FIG.2

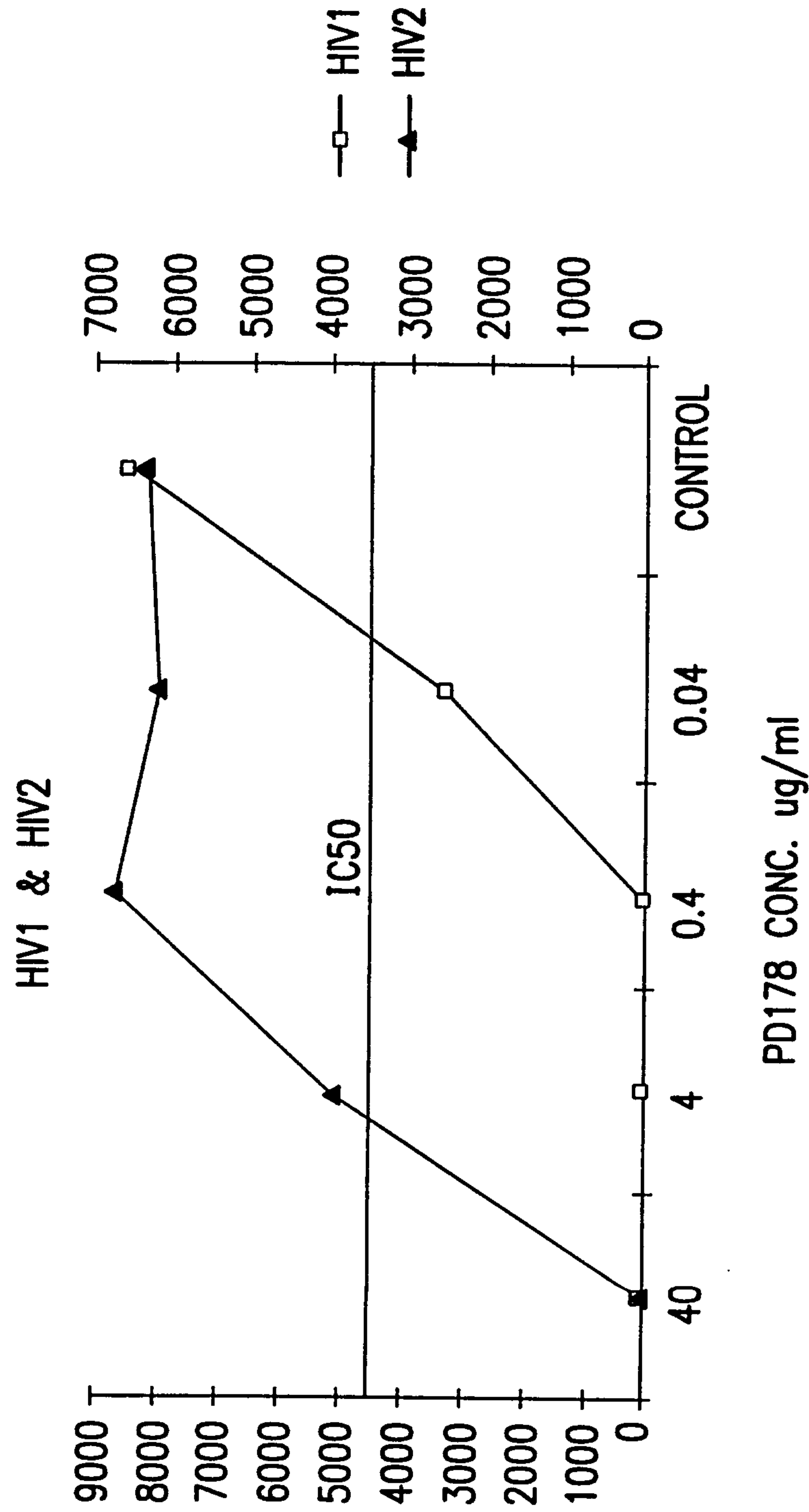


FIG.3

Number of Syncytia/well: concentration in $\mu\text{g/ml}$ (micrograms/ml)									
DP178	10	5	1	0.2	0.1	0.05	0.025	0.0125	Control
<i>Syncytia</i>									
HIV1LAT	0	0	0	0	0	0	0	0	67
HIV1MN	0	0	0	0	0	ND	ND	ND	34
HIV1RF	0	0	0	0	0	ND	ND	ND	65
HIV1SF2	0	0	0	0	0	ND	ND	ND	58
DP125	10	5	1	0.2	0.1	0.05	0.025	0.0125	Control
<i>Syncytia</i>									
HIV1LAT	0	0	54	69	80	75	79	82	67
HIV1MN	0	0	30	36	ND	ND	ND	ND	34
HIV1RF	0	0	67	63	ND	ND	ND	ND	65
HIV1SF2	0	0	9	66	ND	ND	ND	ND	58
DP116	10	5	1	0.2	0.1	0.05	0.025	0.0125	Control
<i>Syncytia</i>									
HIV1LAT	75	ND	ND	ND	ND	ND	ND	ND	67
HIV1MN	35	ND	ND	ND	ND	ND	ND	ND	34
HIV1RF	81	ND	ND	ND	ND	ND	ND	ND	65
HIV1SF2	81	ND	ND	ND	ND	ND	ND	ND	58

FIG.4A

DP180	40	20	10	5	2.5	1.25	0.625	0.3125	Control
<i>Syncytia</i>									
HIV1LAT	50	>45	>45	>45	>45	>45	>45	>45	58
DP185	40	20	10	5	2.5	1.25	0.625	0.3125	Control
<i>Syncytia</i>									
HIV1LAT	0	0	0	0	0	0	0	ND	60

FIG.4B
4 / 31

2164698

<u>HIV1</u>								
<u>Number of Syncytia/well: concentration in ng/ml (nanograms/ml)</u>								
DP178	20	10	5	2.5	1.25	0.625	0.3125	Control
<u>Syncytia</u> <u>HIV1</u>	0	0	0	0	0	14	20	48
DP116	20	10	5	2.5	1.25	0.625	0.3125	Control
<u>Syncytia</u> <u>HIV1</u>	ND	48	ND	ND	ND	ND	ND	ND
<u>HIV2</u>								
<u>Number of Syncytia/well: concentration in μg/ml (micrograms/ml)</u>								
DP178	20	10	5	2.5	1.25	0.625	0.3125	Control
<u>Syncytia</u> <u>HIV2</u>	50	54	55	57	63	77	78	76
DP116	20	10	5	2.5	1.25	0.625	0.3125	Control
<u>Syncytia</u> <u>HIV2</u>	ND	58	ND	ND	ND	ND	ND	ND

FIG.5

5 / 31

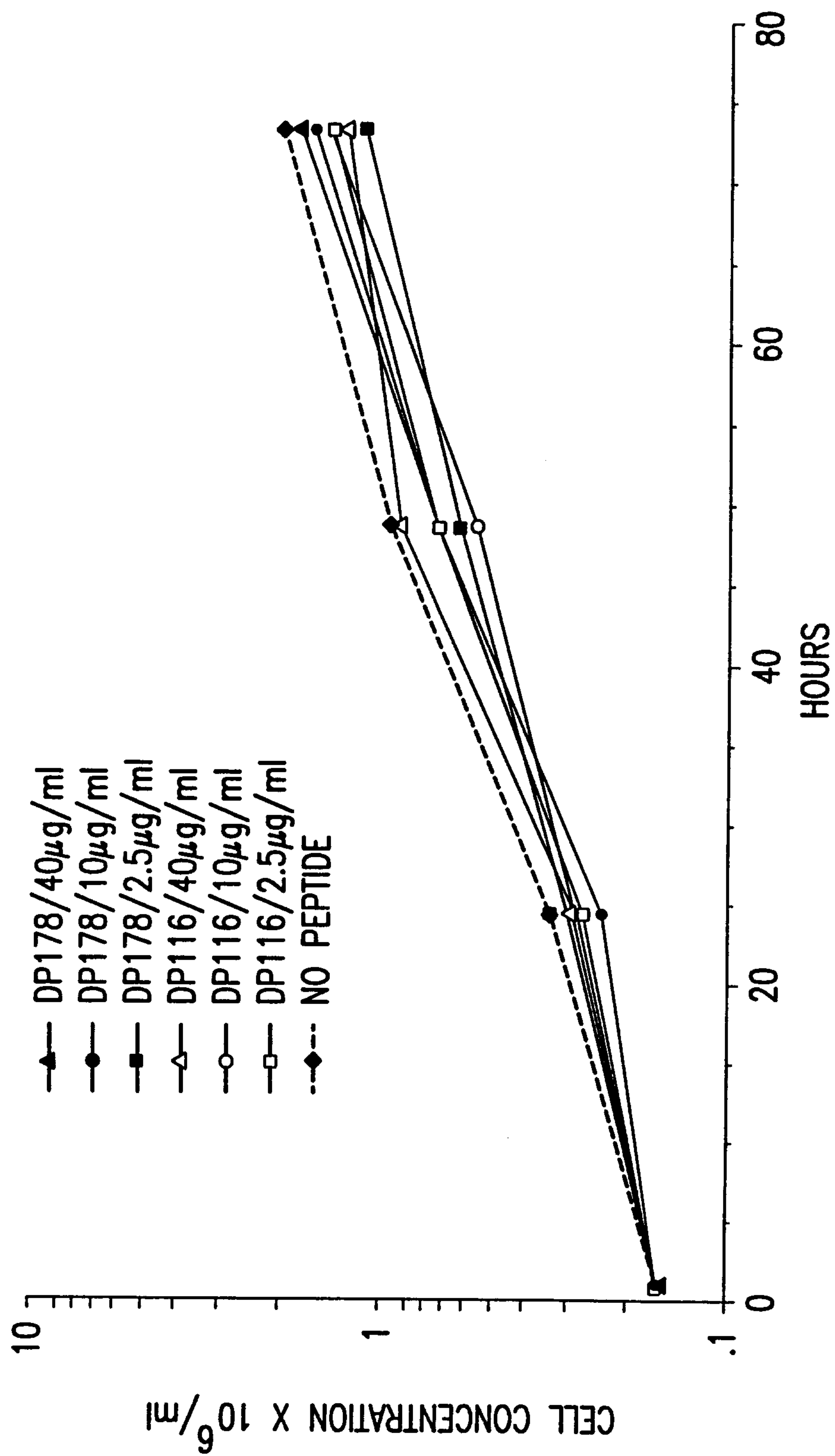


FIG. 6

2164698

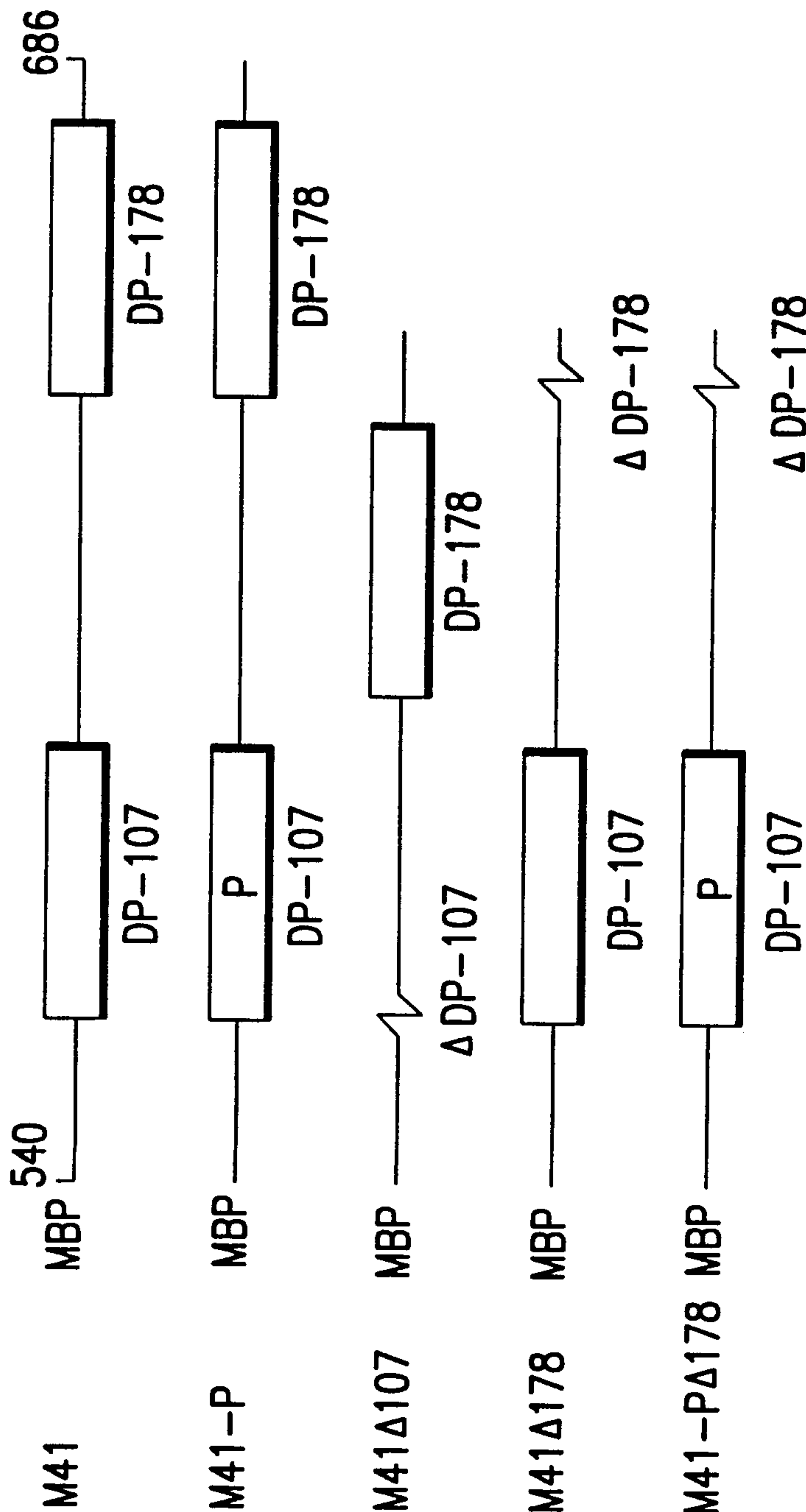
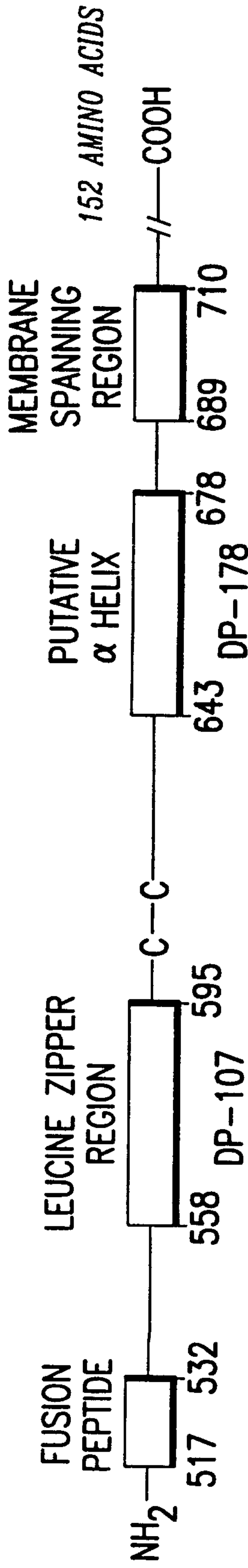


FIG.7

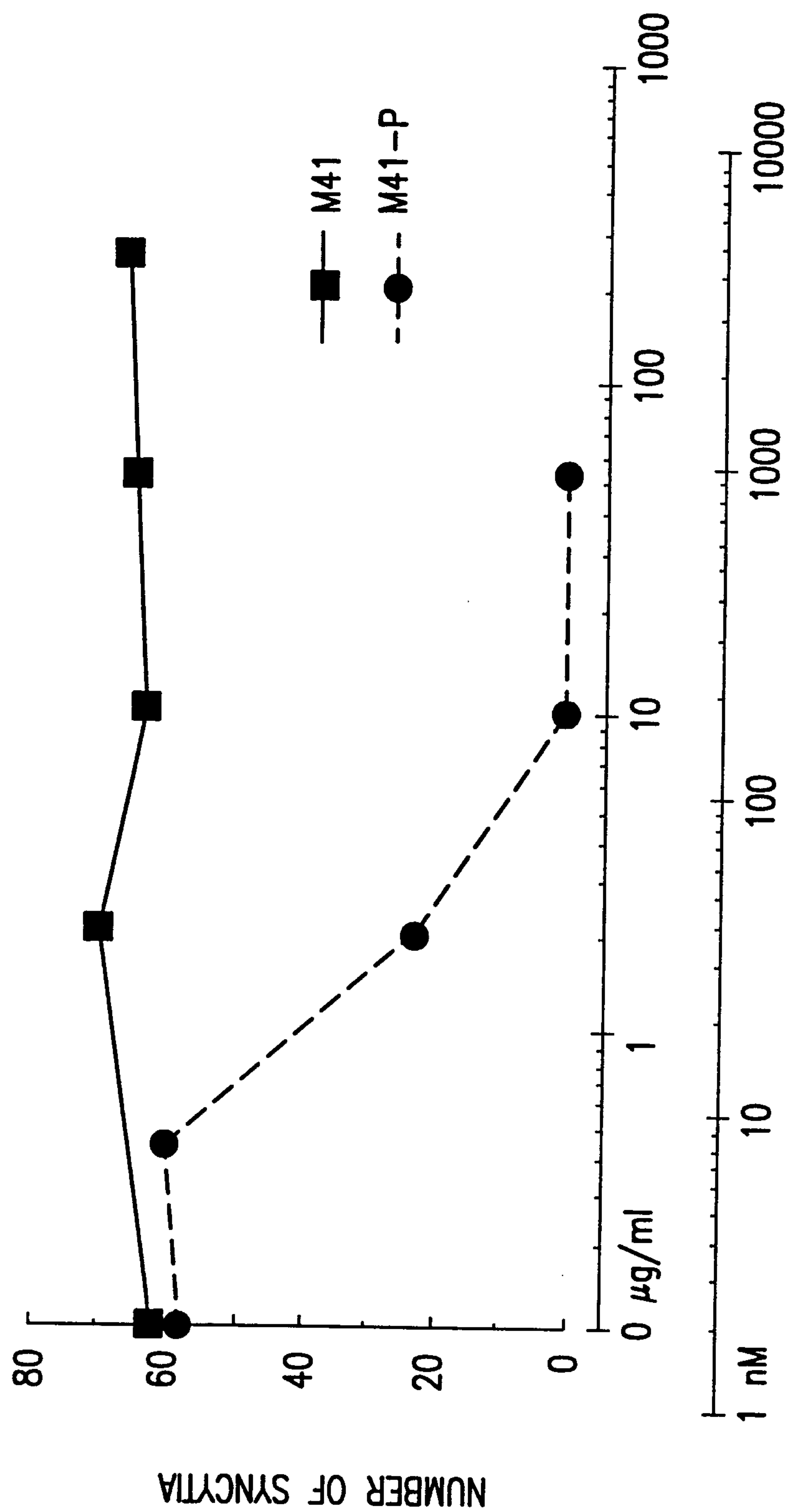


FIG.8

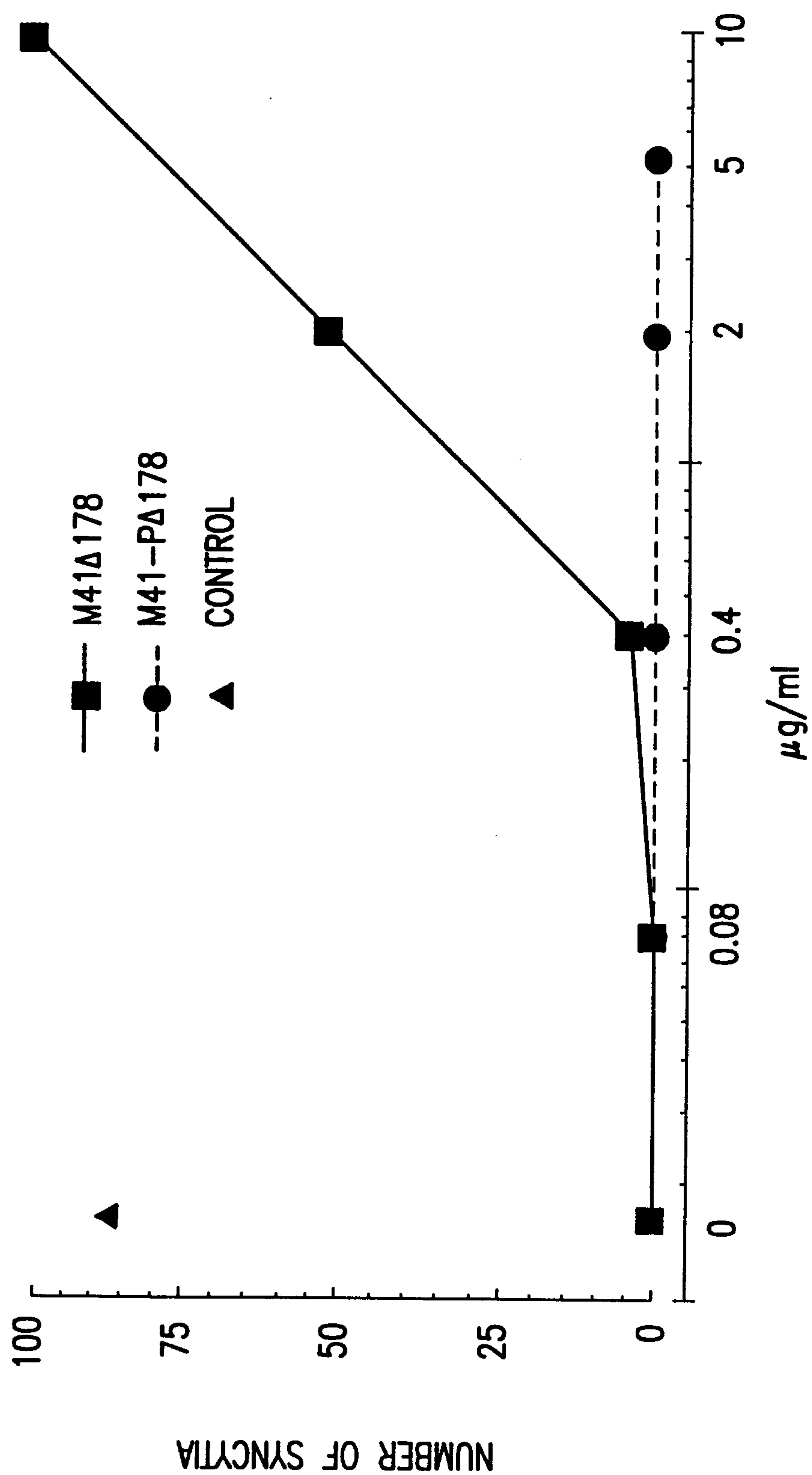


FIG.9

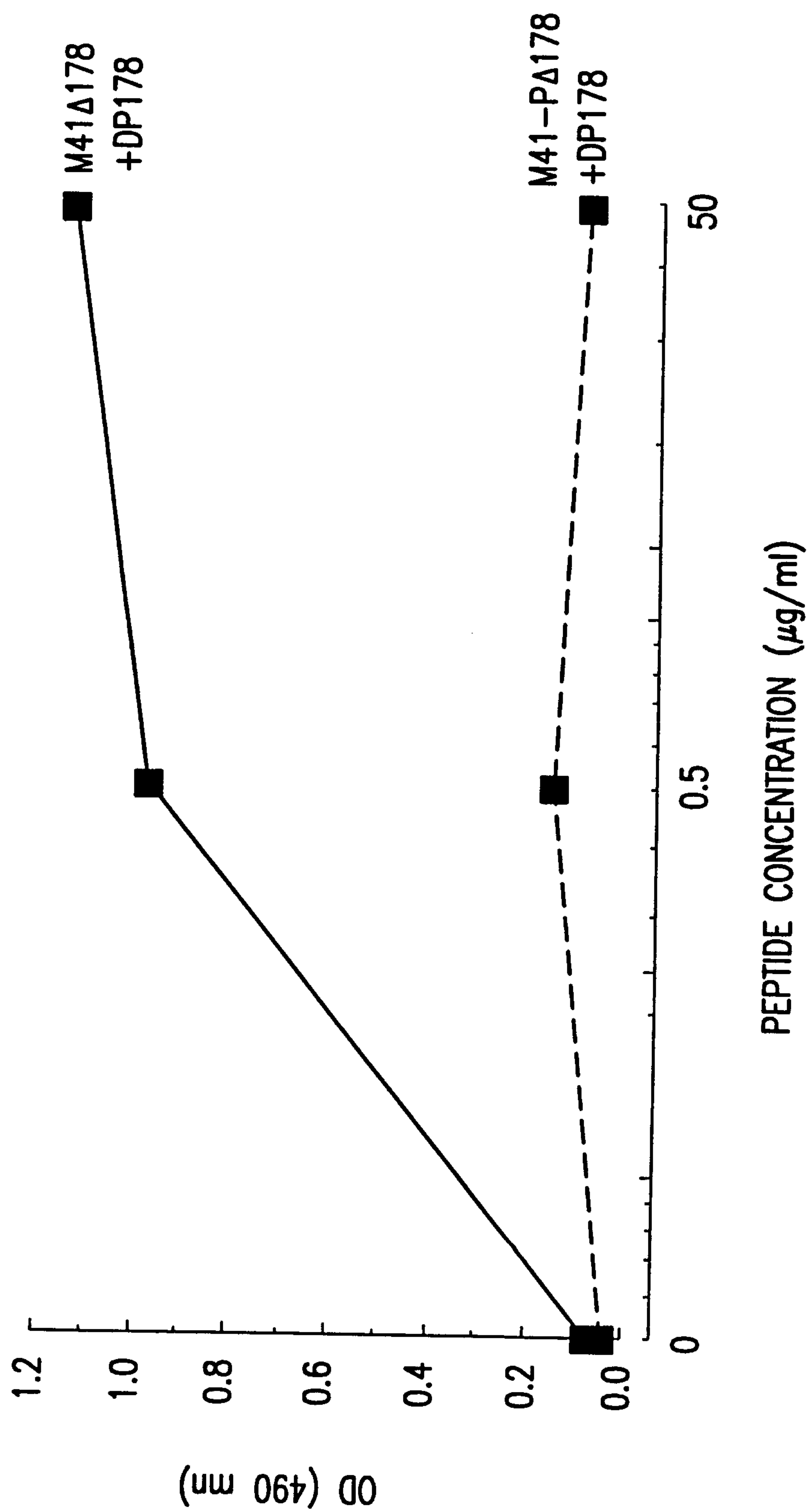


FIG. 10

10/31

SUBSTITUTE SHEET (RULE 26)

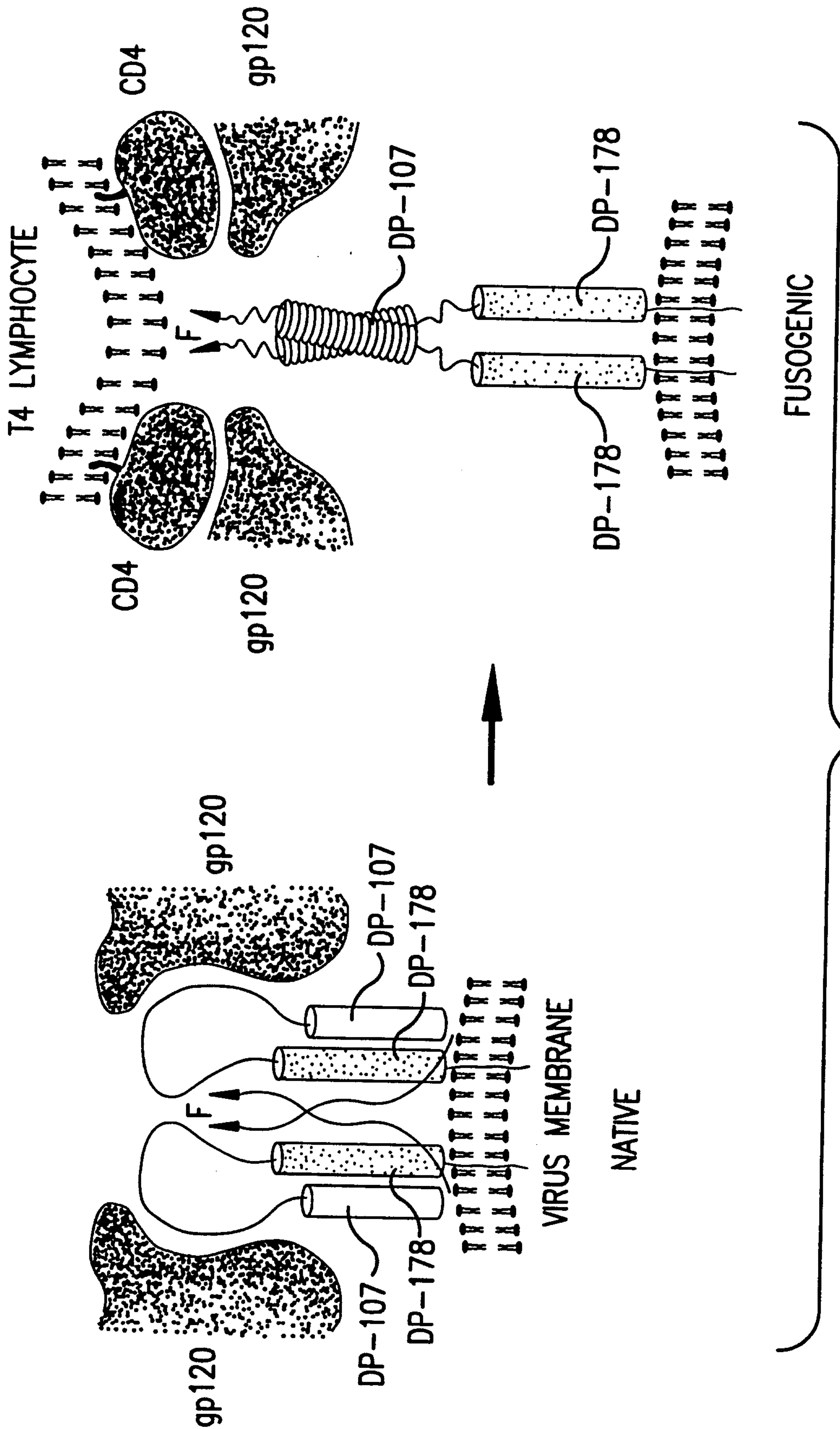
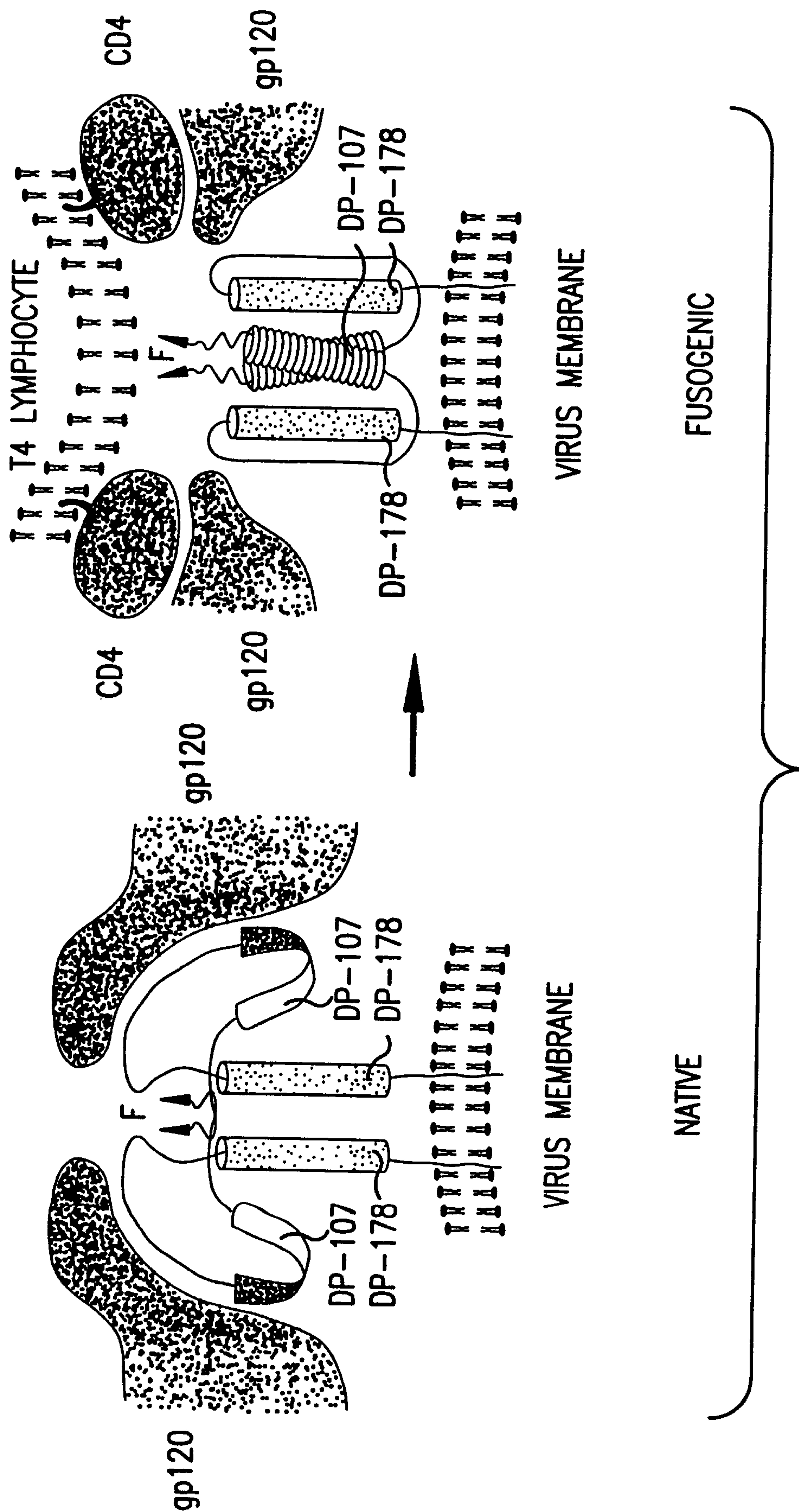


FIG.11A



Sequence	Positions												Motifs																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
	A	D	A	D	A	D	A	D	A	D	A	D	A	D																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	L	S	K	N	Y	H	L	E	N	E	V	A	R	L	K	K	L																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									

FIG.12

Sequence	Positions												Motifs																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
	A	D	A	D	A	D	A	D	A	D	A	D																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
DP-107 (env_hv1bru)L1=D	N	N	L	R	A	I	E	A	Q	Q	H	L	L	Q	A	R	I																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						

FIG.13

Sequence	A	D	E	K	V	E	L	S	K	N	A	D	T	V	W	G	I	K	Q	L	A	D	A	D			
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	S	K	N	Y	H	L	E	N	E	V	A	R	L	K	K	L
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	Q	A	R	I

Sequence	Parent Motif	Hybrid Motif
GCN4 (gcn4 yeast)	[LMNV] {CFGIMPSTW}	
DP-107 (env_hv1bru)L1=D	[ILQT] {CFIMPSTY}	[ILMNQTV] {CFIMPT}
DP-107 (env_hv1bru)L1=D	[ILQTV] {CDFIMPST}	[ILMNQTV] {CFIMPT}
DP-107 (env_hv1bru)L1=D	[ILQTV] {CDFIMPST}	[ILMNQTV] {CFIMPT}
DP-107 (env_hv1bru)L2=D	[EKLNQV] {CDFKMPSVY}	[EKLMNQV] {CFMP}
DP-107 (env_hv1bru)L2=D	[EKLNQV] {CFKMPS}	[EKLMNQV] {CFMP}
DP-107 (env_hv1bru)L2=D	[EKLNQV] {CFKMPS}	[EKLMNQV] {CFMP}

FIG. 14

Sequence	A	D	A	D	A	D	A	D	A	D	A	D	A	D	Positions				Parent Motif	Hybrid Motif																	
DP-107 (env_hv1bru)L1=D	N	N	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	A	V	E	R	Y	L	K	D	Q						
DP-107 (env_hv1bru)L2=D	N	N	L	R	A	I	E	A	Q	H	L	L	Q	L	T	V	W	G	I	K	Q	L	A	V	E	R	Y	L	K	D	Q						
DP-178 (env_hv1bru)Y1=A	Y	T	S	L	I	H	S	L	I	E	E	S	Q	N	Q	E	K	N	E	Q	E	L	L	E	L	D	K	W	A	S	L	W	N	W	F		
DP-178 (env_hv1bru)Y1=D			Y	T	S	L	I	H	S	L	I	E	E	S	Q	N	Q	E	K	N	E	Q	E	L	L	E	L	D	K	W	A	S	L	W	N	W	F
FLU LOOP 36	I	E	K	T	N	E	K	F	H	Q	I	E	K	E	F	S	E	V	E	G	R	I	Q	D	L	E	K	Y									

FIG. 16

Sequence	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	Parent Motif	Hybrid Motif	
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	L	S	K	N	Y	H	L	[LMNV] {CFGIMPTW}
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	I	E	A	Q	H	L	L	Q	L	[ILQTV] {CDFIMPST}
DP-178 (env_hv1bru)Y1=A	Y	T	S	L	I	M	S	L	I	E	S	Q	N	Q	Q	E	K	N	[EFKLQWY] {CFGMPRVY}
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	L	S	K	N	Y	H	L	[LMNV] {CFGIMPTW}
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	I	E	A	Q	H	L	L	Q	L	[ILQTV] {CDFIMPST}
DP-178 (env_hv1bru)Y1=D		Y	T	S	L	I	H	S	L	I	E	S	Q	N	Q	Q	E	K	[EFILNQSWY] {CFGMPRVY}
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	L	S	K	N	Y	H	L	[LMNV] {CFGIMPTW}
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	I	E	A	Q	H	L	L	Q	L	[EKLNV] {CFKMP}
DP-178 (env_hv1bru)Y1=A	Y	T	S	L	I	H	S	L	I	E	S	Q	N	Q	Q	E	K	N	[EFKLQWY] {CFGMPRVY}
GCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	L	S	K	N	Y	H	L	[LMNV] {CFGIMPTW}
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	I	E	A	Q	H	L	L	Q	L	[EKLNV] {CFKMP}
DP-178 (env_hv1bru)Y1=D		Y	T	S	L	I	H	S	L	I	E	S	Q	N	Q	Q	E	K	[EFILNQSWY] {CFGMPRVY}

FIG.17

2164698

Sequence	Positions																Parent Motif	Hybrid Motif
	A	D	D	A	D	A	D	A	D	A	D	A	D	A	D	A		
CCN4 (gcn4 yeast)	M	K	Q	L	E	D	K	V	E	E	L	S	K	N	Y	H	[LMNV] {CFGIMPTW}	
DP-107 (env_hv1bru)L1=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	[ILQTV] {CDFIMPST}	
DP-107 (env_hv1bru)L2=D	N	N	L	L	R	A	I	E	A	Q	H	L	L	Q	L	T	[EKLNV] {CFKMPST}	
DP-178 (env_hv1bru)Y1=A	Y	T	S	L	I	H	S	L	I	E	S	Q	N	Q	E	K	[EFKLQWY] {CFGMPRVY}	
DP-178 (env_hv1bru)Y1=D				Y	T	S	L	I	H	S	L	I	E	S	Q	N	[EFILNQSWY] {CFGMPRVY}	
C-FOS (fos_human)	T	D	T	L	Q	A	E	T	D	Q	L	E	D	E	K	S	[IKLT] {CFGHIMPRVWY}	
C-JUN (tap1_human)	I	A	R	L	E	E	K	V	K	T	L	K	A	Q	N	S	[AILNV] {CDFGHILPVWY}	
C-MYC (myo_human)	E	Q	K	L	I	S	E	E	D	L	L	E	K	R	R	E	[ELR] {ACFGMPVWY}	
FLU LOOP 36	I	E	K	T	N	E	K	F	H	Q	I	E	K	E	F	S	[FILTV] {ACFLMPTVW}	[AEFIKLMNQIRSTVWY] {CFP}
																	= {CDGHP} {CFP}	

FIG.18

2164698

$P-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(1)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(2)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(3)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(4)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(5)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(7)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(8)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(9)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-\{P\}(10)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-X(1,12)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$
 $P-X(13,23)-[LIV]-\{P\}(6)-[LIV]-\{P\}(6)-[LIV]$

FIG.19

Fusion ♥ALLMOTI5♥
 Peptide ♣107x178x4♣
 ♥.....ELGFELG A AGSTMGARSM TLT VQARQ ♣LLSGIVQQQ DP107-NNL

LRAIEAQOHL LOLT V WGIKO LOARIL AVER YLKDO-DP107 QLLG♥♥ I WGC

♥ALLMOTI5♥ ♣107x178x4♣
 LVS Coiled-Coil
 SGKLICT TAVP ♥WNASWS NKSLEQIWNN MTWM *E ♣WDRELNN DP178-

YTSLIHSL IEESONOOEK NEOELLELDK* WASLWNWF-DP178 NI

♦Transmembrane Region♦
 TNWLWYIK♣ ♦IFIMIVGGLVGLRIVEAVLSIV NRVRQGYS♥ PL

♣P23LZIPC♣
 SFQTHLPTPR GPDR ♣PEGIEE EGGERDRDRS IRLVNGSLAL IWDDLRLSL♣ CL

♥ALLMOTI5♥ ♣107x178x4♣
 F ♥SYHRLRDLL LIVTRIVELL GRRGW ♣EALKY WWNLLOQYWSQ

ELKNSAVSLLNAT♣ AIAVAEG TDRVIEVVQG A♥ CRAIRHIPR

RIRQGLERIL L

FIG. 20

21/31

SUBSTITUTE SHEET (RULE 26)

2164698

Fusion ♥ ALLMOTI5 ♥
 Peptide ♠ 107x178x4 ♠
 ♥ ELGFL LGVGSALAS GVA ♠ VSKVLHLEGEVNKIKSA

 ♣ P1&12LZIPC ♣
LLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ ♥ LL ♣ PIVNKQ

 ♠ 107x178x4 ♠
 SC ♠ SISNIETVI ♣ EEQOKNNRLLLEITREESVNAG ♠ VTPVSTMLTNSELLSL

 ♣ P1&12LZIPC ♣
 ♥ ALLMOTI5 ♥
 INDM ♣ PI ♥ TNDQKKLMSNNVQI V ♣ RQQSYSI ♣ MSIIKEEVLAYV

 VQ ♥ LPLYGVIDTPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS

 FFPQAETCKV QSNRVFCDTM NSLTLPSEIN LCNVDIFNPK

 YDCKIMTSKT DVSSSVITSL GAIVSCYGKT KCTASNKNRG

 IIKTFSNGCDYVSNKGMDTV SVGNTLYYVN KQEGKSLYVK G

 ♣ P7, 12, & 23LZIPC ♣
 ♠ 107x178x4 ♠ ♥ ALLMOTI5 ♥
 EPIINFYDPLVF ♣ PSDE ♠ EDASISQVNEKINQSLAF ♥ I ♣ RKSDELL ♣

 ♦ Transmembrane Region ♦
HNVNA ♠ GKSTTN ♦ IMTTIIIVIIIVILLSLIAVGLLLY ♥ C ♦

 KARSTPVTLS KDQLSGINNI AFSN

FIG. 21

22 / 31

Fusion
 Peptide ♡ALLMOTI5♡ ♠107x178x4♠
ELGELG ♡AAGTA MGAAA ♠TALTYQSQHLLAGILOQOKNLLAAV

♠107x178x4♠
EAQ♠ QQM ♠LKLTIWGVKNLNARVTALEKYLEDQARLN♠ AWG♡ CA

LVS Coiled-Coil
 ♡ALLMOTI5♡ ♠107x178x4♠
 WKQVCHTTVP WQWNNRTPDW ♡NNMT *WLE ♠WEROISYLEGNIT

♠107x178x4♠
TOLEEARAQEEKNLD♠ AYOKLSS* WSDFWSW♡ FDF ♠SKWLN ♦ILK

♦Transmembrane Region♦
IGELDVLGIIGLRLLYTV♦ XS♠ CIARVRQGYS PLSPQIHHP WKGQPDNAEG

PGEGGDKRKN SSEPWQKESG TAEWKSNEWCK RLTNWCSISS IWL YNS

♡ALLMOTI5♡
 ♡CLTL LVHLRSAFQY IQYGLGELKA AAQEAVVALA RLAQNAGYQIWL♡

ACRSAYRA IINSPRRVRQ GLEGILN

FIG. 22

Fusion
 Peptide ♡ALLMOTI5♡
EAG ♡VVL AGVALGVATA AQITAGIALHQ ♡*SNLNAQAIQ

♡107x178x4♡
 LVS Coiled-Coil

SLRTSLEQSNKAIEEIREATOETVIA* VOGVQDY♡ VNNEL♡ VP

♡ALLMOTI5♡
 ♡107x178x4♡
 ♡P6 & 12LZIPC♡
 AMQHMSCELVGQRLGLRLLRYYTELLSIFGPSLRD ♡PISA ♡♡EISIQALIVAL

GGEIHKILEKLGYSGD♡ MIAILES_{RG}IKTKI♡ THVDLP_{GF} ILSISY

♡P1 & 12LZIPC♡
 ♡PTLSEVKGVIVHRLEAV♡ SYNIGSQEWYTTVPRYIATNGYLISNFD_{ESS}CVFVS

ESAICSQNSL YPMSPLLQQC IRGDTSSCAR TLVSGTMGNK FILSKGNIVA

NCASILCKCY STSTINQSP DKLLTFLASD TCPLVEIDGA TIQVGGRQYP

LVS Coiled-Coil
 ♡ALLMOTI5♡
 ♡P12 & 23LZIPC♡
 DMVYEGKVAL G ♡PAISLD ♡RL*DYGTNLGNALKKLDDAKVLI♡

♡Transmembrane Region♦
DSS♡ NQILETVRRS♡* SFN ♦EGSLLSVPI_LSCTALALLLLIYCC♦

K RRYQQTLKQH TKVDPAFKPD LTGTSKSYVR SL

FIG. 23

24/31

SUBSTITUTE SHEET (RULE 26)

Fusion ♥ALLMOTI5♥
 Peptide
 ♥.....FIGAI IGSVALGVA TAAQITAASA LIQANQNAAN ♠107x178x4♠
 ♠ILRLKESITA

TIEAVHEVTDGLSQLAVA♠ VG KM♥ QQFVNDQFNNTAQELDCIKITQQV

♥ALLMOTI5♥
 GVELNLYLTELT TV FGPQITSPAL ♥TQLTIQALYNAGGNMDYLLTKLGVG

♣P1 & 12LZIPC♣
 NNQLSSLIGSGLIT GN♥ ♣PILYDSQT QLLGIQVTLP SVGNLNNMRATYLET

LSVST TKGFASALVP KVVVTQVGSVI EELDTSYCIE TDLDLYCTRI VTFPMSPGIY

SCLNGNTSAC MYSKTEGALT TPYMTLKGSV IANCKMTTCR CADPPGIISQ

♥ALLMOTI5♥
 ♠107x178x4♠
 NYGEAVSLID RHSCN ♠♥VLSLD GITLRLSGEF DATYQKNISI LDSQVIVTG

LVS Coiled-Coil
 *NLDISTELGNY NNSISNALDK LEESNSKLDK VNVKLTSTSA ♦Trans-
 ♦LIT* YIA

membrane Region♦
LTAISLVCGILSLV♥♠ LACYLMY♦ KQKAQQKTLLWLGNNTLGQMRATTKM

FIG. 24

25 / 31

2164698

Fusion ♥ALLMOTI5♥
 Peptide ♠107x178x4♠ *LVS Coiled-Coil*
FFGGV ♠IG ♥TIALG *VATSAQITAAVALVEAKQARSDIEKLKE

AIRDTNKAVQSVQSSIGNLIVAISVQ* DYVNKE♥♠ IVPSIARLGCEAAG

♥ALLMOTI5♥
 ♠107x178x4♠
 LQLGIALTQH ♠♥YSELTNIEGDNIGSLOEKGIKLOGIASLYRTNITE♥♠

♣P5 & 12LZIPC♣
 IFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRL ♣PLLTRLNTQIYR

VDSISYNI♣ QNREWI♣ PLPSHIMTKGAFLGGADVKECIEAFSSYIC

PSDPGFVLNHEMESCLSGNISQCPRTVVKSDIVPRYAFVNGGVVANCITT

TCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTP

♥ALLMOTI5♥
 ♠107x178x4♠
 ♣P6 & 23LZIPC♣
 NDITLNNVALD ♣PIDI ♠SIELN ♥KAKSDLEESKEWI♣ RRSNQKL♣

♦Transmembrane Region♦
DSIGNWHQSSTT ♦IIIV♠ LIM IIILEIINVT II♦ IIHVKYV♥ R

IQKRNRVDQN DKPYVLTNK

FIG. 25

26 / 31

2164698

Fusion
Peptide
.....GLEGAI AGFIENGWEGMIDGWYGFRHQNSEGTG

♠107x178x4♠
♥ALLMOTI5♥
LVS Coiled-Coil
*Q ♥AADLKST ♠QA AIDQINGKLN RVIEKTNEKFHQIEKEEVEGRIQ
DLEKYVEDTKIDL* WSYNAELLYALENQHTI♠ DLT♥ DSEMKNKLFETR
RQLRENAEEMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALNNRFQIKG
VELKSGYKDWILWISFAISCFLLCVVLLGFIMWACQQRGNIRCNICI

FIG. 26

AV	CD	RSV F2	
+	+ / ++	T-142	YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST
++	+ / +++	T-143	YTSVITIELSNIKENKCNGTDAKVKLIKQELDKYK
+	+ / ++	T-144	TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKN
-	+ / +	T-145	SVITIELSNIKENKCNGTDAKVKLIKQELDKYKNA
-	+ / -	T-146	VITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
-	-	T-147	ITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
-	-	T-148	TIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTE
-	+ / -	T-149	IELSNIKENKCNGTDAKVKLIKQELDKYKNAVTEL
-	-	T-150	ELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQ
-	+ / +	T-151	LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQ
-	+ / ++	T-152	SNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLL
-	+ / +	T-153	NIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLM
-	+ / ++	T-154	IKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQ
-	+ / +	T-155	KENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQS
-	-		ENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQST

FIG.27

AV	CD	RSV	
+++	+/-	T-67	DEFDASISQVNEKINQSLAF IRKSDELL
		F1-178	GEPIINFYDPLVFPSDEFDASISQVNEKINQSLAF IRKSDELLHNVNACKSTT
+/-		T-104	IINFYDPLVFPSDEFDASISQVNEKINQSLAF IRK
+/-		T-105	INFYDPLVFPSDEFDASISQVNEKINQSLAF IRKS
+/-		T-106	NFYDPLVFPSDEFDASISQVNEKINQSLAF IRKSD
+		T-107	FYDPLVFPSDEFDASISQVNEKINQSLAF IRKSDE
++		T-108	YDPLVFPSDEFDASISQVNEKINQSLAF IRKSDEL
++		T-109	DPLVFPSDEFDASISQVNEKINQSLAF IRKSDELL
+		T-110	PLVFPSDEFDASISQVNEKINQSLAF IRKSDELLH
++		T-111	LVFPSDEFDASISQVNEKINQSLAF IRKSDELLHN
++	+/-	T-112	VFPSDEFDASISQVNEKINQSLAF IRKSDELLHNV
++	+/-	T-113	FPSDEFDASISQVNEKINQSLAF IRKSDELLHNVN
++	+/-	T-114	PSDEFDASISQVNEKINQSLAF IRKSDELLHNVNA
++	+/-	T-115	SDEFDASISQVNEKINQSLAF IRKSDELLHNVNAG
++	+/-	T-116	DEFDASISQVNEKINQSLAF IRKSDELLHNVNACK
++	+/-	T-117	EFDASISQVNEKINQSLAF IRKSDELLHNVNACKS
++	+/-	T-118	FDASISQVNEKINQSLAF IRKSDELLHNVNACKST
++	+/-	T-119	DASISQVNEKINQSLAF IRKSDELLHNVNACKSTT

(T-67 LIKE)

FIG.28

2164698

AV	CD	HPF3 178	YTPNDITLNNNSVALDPIDISIELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQSSTT
-	-	189	YTPNDITLNNNSVALDPIDISIELNKA	SDLEESKE	
-	-	190	TPNDITLNNNSVALDPIDISIELNKA	SDLEESKEW	
-	-	191	PNDITLNNNSVALDPIDISIELNKA	SDLEESKEWI	
-	-	192	NDITLNNNSVALDPIDISIELNKA	SDLEESKEWIR	
-	+/-	193	DITLNNNSVALDPIDISIELNKA	SDLEESKEWIRR	
+/-	+/-	194	ITLNNNSVALDPIDISIELNKA	SDLEESKEWIRRS	
+/-	+/+ +	195	TLNNNSVALDPIDISIELNKA	SDLEESKEWIRRSN	
+	+/+	196	LNNNSVALDPIDISIELNKA	SDLEESKEWIRRSNQ	
+	+/+	197	NNNSVALDPIDISIELNKA	SDLEESKEWIRRSNQK	
+++	+/+	198	NSVALDPIDISIELNKA	SDLEESKEWIRRSNQKL	
++	+/+	199	SVALDPIDISIELNKA	SDLEESKEWIRRSNQKLD	
-		200	VALDPIDISIELNKA	SDLEESKEWIRRSNQKLD	
+++		201	ALDPIDISIELNKA	SDLEESKEWIRRSNQKLD	SIGN
+++		202	LDPIDISIELNKA	SDLEESKEWIRRSNQKLD	SIGN
+++		203	DPIDISIELNKA	SDLEESKEWIRRSNQKLD	SIGN
++		204	PIDISIELNKA	SDLEESKEWIRRSNQKLD	SIGNW
+++		205	IDISIELNKA	SDLEESKEWIRRSNQKLD	SIGNWH
+		206	DISIELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQ
+		207	ISIELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQS
+		208	SIELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQSS
++		209	IELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQSST
++		210	ELNKA	SDLEESKEWIRRSNQKLD	SIGNWHQSSTT

FIG.29

CD	HPF3 107	GTIALGVATSAQITA AVALVEAKQARSDIEKLKEAIRDTNKAVQSVQSSIGNLIVA I KSVQDYVNKE IVP
+/+	157	ALGVATSAQITA AVALVEAKQARSDIEKLKEAIRD
+/+	158	LGVATSAQITA AVALVEAKQARSDIEKLKEAIRDT
+/-	159	GVATSAQITA AVALVEAKQARSDIEKLKEAIRDTN
+/+	160	VATSAQITA AVALVEAKQARSDIEKLKEAIRDTNK
+/+	161	ATSAQITA AVALVEAKQARSDIEKLKEAIRDTNKA
+/-	162	TSAQITA AVALVEAKQARSDIEKLKEAIRDTNKAV
+/+	163	SAQITA AVALVEAKQARSDIEKLKEAIRDTNKAVQ
+ /+++	164	AQITA AVALVEAKQARSDIEKLKEAIRDTNKAVQS
+/+	165	QITA AVALVEAKQARSDIEKLKEAIRDTNKAVQSV
+/-	166	ITA AVALVEAKQARSDIEKLKEAIRDTNKAVQSVQ
+/-	167	TAAVALVEAKQARSDIEKLKEAIRDTNKAVQSVQS
+/-	168	AAVALVEAKQARSDIEKLKEAIRDTNKAVQSVQSS
+/-	169	AVALVEAKQARSDIEKLKEAIRDTNKAVQSVQSSI
+/-	170	VALVEAKQARSDIEKLKEAIRDTNKAVQSVQSSIG
+/-	171	ALVEAKQARSDIEKLKEAIRDTNKAVQSVQSSIGN
+/-	172	LVEAKQARSDIEKLKEAIRDTNKAVQSVQSSIGNL
+/-	173	VEAKQARSDIEKLKEAIRDTNKAVQSVQSSIGNL I
+ /++	174	EAKQARSDIEKLKEAIRDTNKAVQSVQSSIGNL IV
	T-40	AKQARSDIEKLKEAIRDTNKAVQSVQSSIGNL IVA
+ /++	175	KQARSDIEKLKEAIRDTNKAVQSVQSSIGNL IVAI
+ /+++	176	QARSDIEKLKEAIRDTNKAVQSVQSSIGNL IVAIK
+/-	177	ARSDIEKLKEAIRDTNKAVQSVQSSIGNL IVAIKS
+/-	178	RSDIEKLKEAIRDTNKAVQSVQSSIGNL IVAIKSV
-	179	SDIEKLKEAIRDTNKAVQSVQSSIGNL IVAIKSVQ
-	180	DIEKLKEAIRDTNKAVQSVQSSIGNL IVAIKSVQD
-	181	IEKLKEAIRDTNKAVQSVQSSIGNL IVAIKSVQDY
-	182	EKLKEAIRDTNKAVQSVQSSIGNL IVAIKSVQDYV
+ /++	183	KLKEAIRDTNKAVQSVQSSIGNL IVAIKSVQDYVN
+ /+++	184	LKEAIRDTNKAVQSVQSSIGNL IVAIKSVQDYVNK
-	185	KEAIRDTNKAVQSVQSSIGNL IVAIKSVQDYVNKE
-	186	EAIRDTNKAVQSVQSSIGNL IVAIKSVQDYVNKE I
-	187	AIRDTNKAVQSVQSSIGNL IVAIKSVQDYVNKE IV
-	188	IRDTNKAVQSVQSSIGNL IVAIKSVQDYVNKE IVP

FIG.30

HIV1LAI (DP-178; SEQ ID:1)	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF
HIV1SF2 (DP-185; SEQ ID:3)	YTNTIYNLLEESQNQQEKNEQELLELDKWASLWNWF
HIV1RF (SEQ ID:4)	YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF
HIV1MN (SEQ ID:5)	YTSLIYSLLEKSQTQQEKNEQELLELDKWASLWNWF
HIV2ROD (SEQ ID:6)	LEANISKSLEQAQIQQEKMYELQKLNSWDIFGNWF
HIV2NIHZ (SEQ ID:7)	LEANISQSLEQAQIQQEKMYELQKLNSWDVFTNWL
DP180 (SEQ ID:2)	SSESFTLLEQWNNWKLQLAEQWLEQINEKHYLEDIS
DP118 (SEQ ID:10)	QQLLDVVKRQQEMLRLTVWGTKNLQARVTAIEKYLKDQ
DP125 (SEQ ID:8)	CGGNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQ
DP116 (SEQ ID:9)	LQARILAVERYLKDQQQ