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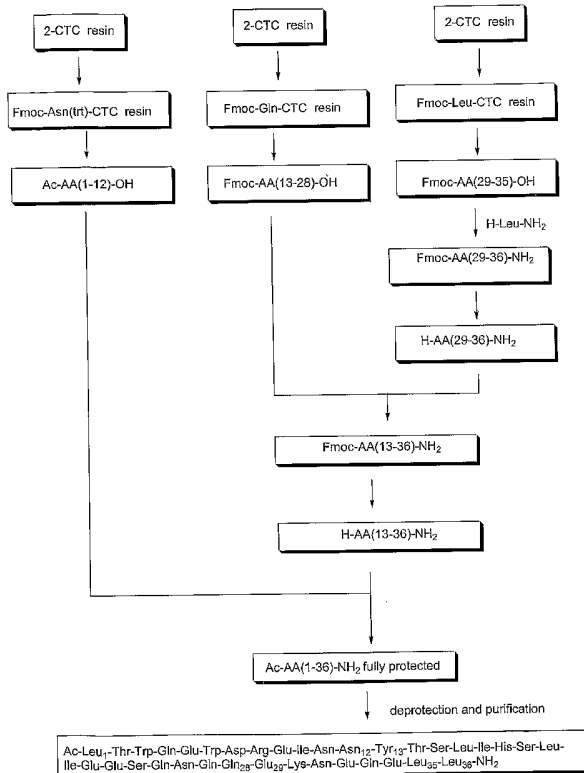
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(54) Title: CONJUGATES COMPRISED OF FATTY ACID AND HIV GP41-DERIVED PEPTIDE

(57) Abstract: Provided is a conjugate comprised of
HIV gp41 -derived peptide operatively linked to fatty
acid, and pharmaceutical compositions and medica-
ments containing the conjugate. Also provided are
methods for producing a conjugate according to the
present invention which include, but are not limited
to, covalently coupling a linker to the HIV gp41 -
derived peptide, wherein the linker is covalently cou-
pled to fatty acid.



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CONJUGATES COMPRISED OF FATTY ACID AND HIV gp41-DERIVED PEPTIDE

FIELD OF INVENTION

The present invention relates to a conjugate comprised of a fatty acid operatively linked to a synthetic peptide derived from Human Immunodeficiency Virus (HIV) gp41. More specifically, the present invention relates to a conjugate, formed by operatively linking one or more fatty acid molecules to an HIV gp41-derived peptide, which demonstrates improved pharmaceutical activity.

BACKGROUND OF THE INVENTION

It is now well known that cells can be infected by HIV through a process by which fusion occurs between the cellular membrane and the viral membrane. The generally accepted model of this process is that the viral envelope glycoprotein complex (gp120/gp41) interacts with cell surface receptors on the membranes of the target cells. Following binding of gp120 to cellular receptors (e.g., CD4 in combination with a chemokine co-receptor such as CCR-5 or CXCR-4), a conformational change is induced in the gp120/gp41 complex that allows gp41 to insert into the membrane of the target cell and mediate membrane fusion.

The amino acid sequence of gp41, and its variation among different strains of HIV, is well known. FIG.1 is a schematic representation of the generally accepted functional domains of gp41 (note the amino acid sequence numbers may vary slightly depending on the HIV strain). Between the fusion peptide and transmembrane anchor are two distinct regions, known as heptad repeat (HR) regions, each region having a plurality of heptads. A heptad is a 7 amino acid residue stretch (the 7 amino acids in each heptad designated "a" through "g"), with a predominance of hydrophobic residues at the first ("a") and fourth ("d") positions, and charged residues frequently at the fifth ("e") and seventh ("g") positions. The amino acid sequence comprising the HR2 region is highly conserved in the HIV-1 envelope protein. The HR2 region has been generally described as comprising amino acid residues of SEQ ID NO:1, or polymorphisms thereof (see, e.g., FIG. 2).

It was discovered that peptides derived from the native sequence of the HR2 region ("HR2 peptides") of HIV gp41 inhibit transmission of HIV to host cells both in *in vitro* assays and in *in vivo* clinical studies. For example, HR2 peptides, as exemplified by DP178 (also known as T20, enfuvirtide, and Fuzeon®; SEQ ID NO:2), T651 (SEQ ID NO:3), T649 (SEQ ID NO:4), blocked infection of target cells with potencies of 0.5 ng/ml (EC50 against HIV-1_{LAI}), 5 ng/ml (IC50; HIV-1 IIIB), and 2 ng/ml (IC50; HIV-1 IIIB), respectively. Efforts have been made to improve the biological activity of HIV gp41-derived peptides, such as by trying to stabilize the helical structure of the peptide.

Use of certain fatty acids in treatment to inhibit HIV has been described previously (see, U.S. Patent No. 5,073,571). More specifically, it is known that HIV depends on N-myristoylation of its gag polypeptide precursor for proper viral assembly. Previously described is the production of fatty acid analogs which exhibit decreased hydrophobicity, yet retain the ability to act as substrate for myristoylating enzymes. Thus, once the fatty acid analog is internalized by an HIV infected cell, and incorporated into the viral gag polyprotein produced within the HIV-infected cell, it is believed that the difference in hydrophobicity of the resultant viral protein incorporating such fatty acid analog upon myristoylation alters the ability of that protein to interact with membranes and other proteins; and hence, assembly of the virus may be inhibited.

Fatty acid conjugation has been used to facilitate the uptake of proteins and peptides into and across cell membranes. Additionally, fatty acids have been coupled directly to a protein or peptide antigen to improve the antigenicity of the antigen. However, difficulties have been encountered in conjugating fatty acids to peptides and proteins, including the lack of solubility of fatty acid-conjugated peptides in aqueous solutions, and the loss of biological activity of peptides and proteins after fatty acid acylation and when bound to serum proteins.

There is a need for improving one or more pharmaceutical activities of an HIV gp41-derived peptide, while maintaining or improving its ability to interfere with the viral fusion process mediated by HIV gp41. The present invention addresses these needs.

SUMMARY OF THE INVENTION

There is provided, in accordance with the present invention, a conjugate comprising fatty acid operatively linked to a synthetic peptide, wherein the synthetic peptide is derived from the HR2 region of gp41. The conjugate offers the advantages of one or more improved pharmaceutical activities, as compared to the synthetic peptide alone, that includes, but not be limited to, increased antiviral activity against HIV strains demonstrating resistance to interference of fusion by an HIV gp41-derived peptide having antiviral activity (e.g., including, but not limited to, any one or more synthetic peptides having a amino acid sequence of SEQ ID NOs: 2, 3, 129); and an improved pharmacokinetic property. With the one or more improved pharmaceutical activities, the conjugate still retains substantial biological activity (e.g., antiviral activity) against HIV-1 strains which have not demonstrated resistance to an HIV-1 derived gp41 peptide having antiviral activity. It is surprising and unexpected that some of the conjugates according to the present invention showed improved antiviral activity, as compared to that of the synthetic peptide alone. Further, this observed improvement in antiviral activity relates to interaction of the bound fatty acid with the HIV-1 gp41-derived peptide, as it cannot be

explained only by an improvement in pharmacokinetics (e.g., by stabilization against metabolism). An improvement in pharmacokinetic properties, such as a reduction in clearance and/ or an increase in the biological half-life of synthetic peptide which is part of the conjugate (e.g., enabling the synthetic peptide to survive longer *in vivo* before being degraded in and/or removed from the bloodstream as compared to synthetic peptide alone), will be apparent to one skilled in the art from the descriptions herein. The conjugate according to the present invention may further comprise a pharmaceutically acceptable carrier.

Another aspect of the present invention is to provide for an HIV gp41-derived peptide having one or more improved pharmaceutical activities, while still retaining substantial biological activity (e.g., antiviral activity), wherein operatively linked to the HIV gp41-derived peptide is a fatty acid (one or more fatty acid molecules).

In another aspect, provided is an antiviral composition against HIV, having one or more improved pharmaceutical activities, comprising an HIV gp41-derived peptide operatively linked to fatty acid.

The present invention also relates to a method of improving one or more pharmaceutical activities of an HIV gp41-derived peptide comprising operatively linking one or more fatty acids to the HIV gp41-derived peptide.

Moreover, the present invention extends to a method of using the conjugate according to the present invention for inhibition of transmission of HIV to a target cell, comprising adding to the virus and the cell an amount of conjugate according to the present invention effective to inhibit infection of the cell by the virus. This method may be used to treat HIV-infected individuals. In a preferred embodiment, inhibiting transmission of HIV to a target cell comprises inhibiting gp41-mediated fusion of HIV-1 to a target cell and/or inhibiting syncytia formation between an HIV-infected cell and a target cell. The present invention also provides for a method of treating HIV infection (preferably, HIV-1 infection) comprising administering to an HIV-infected individual a pharmaceutical composition comprising a composition (e.g., conjugate, antiviral composition) according to the present invention. Preferably, the pharmaceutical composition is in an amount effective to inhibit transmission of HIV to a target cell, and/or in an amount effective to inhibit gp41-mediated fusion of HIV to a target cell. Also provided is a method for inhibition of transmission of HIV to a cell, comprising contacting the virus in the presence of a cell with the conjugate according to the present invention in an amount effective to inhibit infection of the cell by HIV. Also provided is a method for inhibiting HIV fusion (e.g., a process by which HIV gp41 mediates fusion between the viral membrane and cell membrane during infection by HIV of a target cell), comprising contacting the virus in the presence of a cell with an amount of the conjugate according to the present invention

effective to inhibit HIV fusion. These methods may be used to treat HIV-infected individuals.

The present invention also provides the use of a composition according to the present invention, in the manufacture of a medicament for use in therapy of HIV infection (e.g., used in a method of inhibiting transmission of HIV, a method of inhibiting HIV fusion, or a method of treating HIV infection), as described herein. The medicament is preferably in the form of a pharmaceutical composition comprising a conjugate or antiviral composition according to the present invention together with a pharmaceutically acceptable carrier. The medicament may also be used in combination with one or more additional therapeutic agents used in treatment of HIV infection.

The present invention further extends to methods of making the conjugates according to the present invention. One such method disclosed herein comprises the steps of: (a) reacting a reactive functionality of a linker with a reactive functionality of a fatty acid so that the linker and fatty acid are covalently coupled in producing a linker-fatty acid combination; (b) reacting a reactive functionality of the linker, of the linker-fatty acid combination, with a reactive functionality of a synthetic peptide so that the linker-fatty acid combination is covalently coupled to the synthetic peptide, in producing the conjugate according to the present invention. In another embodiment, the linker is covalently coupled to the synthetic peptide, and then the linker, of the synthetic peptide-linker combination, is covalently coupled to a fatty acid. In yet another method, the conjugate is formed by covalently coupling a fatty acid directly to the synthetic peptide (without use of a linker).

The above and other objects, features, and advantages of the present invention will be apparent in the following Detailed Description of the Invention when read in conjunction with accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of HIV-1 gp41 showing the heptad repeat 1 region (HR1) and heptad repeat 2 region (HR2) along with other functional regions of gp41. Exemplary amino acid sequences corresponding to HR1 and HR2, and the amino acid position numbering, are shown for purposes of illustration and in relation to gp160, strain HIV_{IIIB}.

FIG. 2 shows a comparison of the sequences contained within the HR2 region of HIV-1 gp41 for purposes of illustration, and not limitation, as determined from various laboratory strains and clinical isolates, wherein illustrated are some of the variations in amino acid sequence (e.g., polymorphisms), as indicated by the single letter amino acid code.

FIG. 3 is a schematic showing synthesis of a synthetic peptide having an amino acid

sequence of SEQ ID NO:33, using a fragment condensation approach.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "individual", when used herein for purposes of the specification and claims, means a mammal, and preferably a human.

The term "target cell", when used herein for purposes of the specification and claims, means a cell capable of being infected by HIV. Preferably, the cell is a human cell or are human cells; and more preferably, human cells capable of being infected by HIV via a process including membrane fusion.

The term "pharmaceutically acceptable carrier", when used herein for purposes of the specification and claims, means a carrier medium that does not significantly alter the biological activity (e.g., as pertinent to the present invention, antiviral activity) of the active ingredient to which it is added. A pharmaceutically acceptable carrier includes, but is not limited to, one or more of water, buffered water, saline, 0.3% glycine, aqueous alcohols, isotonic aqueous solution; and may further include one or more substances such as glycerol, oils, salts such as sodium, potassium, zinc, magnesium, and ammonium, phosphonates, carbonate esters, fatty acids, saccharides (e.g., mannitol), polymer, polysaccharides, surfactants, excipients, and preservatives and/or stabilizers (to increase shelf-life or as necessary and suitable for manufacture and distribution of the composition). Preferably, the pharmaceutically acceptable carrier is suitable for intravenous, intramuscular, subcutaneous or parenteral administration. As described in more detail in Example 5 herein, according to the present invention a preferred pharmaceutically acceptable carrier comprises a polymer, and more specifically polyethylene glycol ("PEG"), which is a carrier medium that is mixed with the conjugate to improve the solubility of the conjugate in an aqueous solution. The polyethylene glycol, as a component of a pharmaceutically acceptable carrier, preferably has a molecular weight range of no less than about 1,000 daltons and no more than about 10,000 daltons (i.e., not a PEG of a discrete number of units). For example, PEG1500 has been described as having a molecular weight range of about 1430 daltons to about 1570 daltons. In addition to a polymer, a pharmaceutically acceptable carrier may comprise one or more additional components. For example if the pharmaceutically acceptable carrier is a solution, and more preferably an aqueous solution, one preferred additional component is an aqueous alcohol that is in a concentration (v/v) in a range of concentrations of from about 1% to about 15%. Aqueous alcohols are known in the art as any pharmaceutically acceptable water-miscible solvents including, but not limited to, ethanol, isopropanol, n-butanol and other aliphatic mono-hydric alcohols containing at least 2 carbon atoms, and preferably 2 to 4 carbon atoms.

The term "weight percent", as standard in the art and may be used synonymously with weight/volume percent, is used herein for the purposes of the specification and claims to mean milligrams (mg) of an ingredient in the pharmaceutical composition (e.g., polymer) per milliliter(s) (ml) of solution, multiplied by 0.1, as will be more apparent from the following descriptions herein.

The term "solution", as standard in the art in referring to an aqueous fluid into which is dissolved one or more solids, is used herein for the purposes of the specification and claims to mean an aqueous solution containing the conjugate and polymer dissolved therein under realistic use conditions of concentration and temperature as described herein in more detail and as standard in the art for an injectable drug formulation. There are various ways known in the art to distinguish formation of a solution, as opposed to formation of a suspension, such as checking for visual clarity (transparency of a solution versus cloudiness of a suspension), light transmission, centrifugation followed by assaying the contents of the supernatant, and the like.

By the term "amino acid" is meant, for purposes of the specification and claims and in reference to the synthetic peptides used in the present invention, to refer to a molecule that has at least one free amine group and at least one free carboxyl group. The amino acid may have more than one free amine group, or more than one free carboxyl group, or may further comprise one or more free chemical reactive groups other than an amine or a carboxyl group (e.g., a hydroxyl, a sulfhydryl, etc.). The amino acid may be a naturally occurring amino acid (e.g., L-amino acid), a non-naturally occurring amino acid (e.g., D-amino acid), a synthetic amino acid, a modified amino acid, an amino acid derivative, an amino acid precursor, and a conservative substitution. One skilled in the art would know that the choice of amino acids incorporated into a peptide will depend, in part, on the specific physical, chemical or biological characteristics required of the antiviral peptide. Such characteristics are determined, in part, by determination of structure and function (e.g., antiviral activity; as described herein in more detail). For example, the skilled artisan would know from the descriptions herein that amino acids in a synthetic peptide may be comprised of one or more of naturally occurring (L)-amino acid and non-naturally occurring (D)-amino acid. A preferred amino acid may be used to the exclusion of amino acids other than the preferred amino acid.

A "conservative substitution", in relation to amino acid sequence of a synthetic peptide used in the present invention, is a term used hereinafter for the purposes of the specification and claims to mean one or more amino acids substitution in the sequence of the synthetic peptide such that its biological activity is substantially unchanged (e.g., if the peptide inhibits HIV gp41-mediated fusion at a concentration in the nanomolar range before the substitution, after the substitution inhibition of HIV gp41-mediated fusion is still

observed in the nanomolar range). As known in the art "conservative substitution" is defined by aforementioned function, and includes substitutions of amino acids having substantially the same charge, size, hydrophilicity, and/or aromaticity as the amino acid replaced. Such substitutions are known to those of ordinary skill in the art to include, but are not limited to, glycine-alanine-valine; isoleucine-leucine; tryptophan-tyrosine; aspartic acid-glutamic acid; arginine-lysine; methionine-leucine; asparagine-glutamine; and serine-threonine. With particular relevance to the present invention, a conserved substitution is known in the art to also include substituting lysine with ornithine, in providing a free amine group (e.g., epsilon amine). For HIV gp41-derived peptides, such substitutions may also comprise polymorphisms at the various amino acid positions along the HR2 region of gp41 found in any one or more of various clades, laboratory strains, or clinical isolates of HIV, which are readily available from public databases and are well known in the art (see also, FIG. 2, as an illustrative example).

The term "fatty acid" when used herein for purposes of the specification and claims, means a fatty acid comprising no less than 5, and no more than 30, carbon atoms; and is also used to mean a fatty acid analog produced by operatively linking fatty acid to either a linker or an amino acid. For example, as shown in Example 3C, operatively linking a fatty acid to a C-terminal amino acid results in an alkylamide (also referred to herein as "fatty acid"). "A fatty acid" or "fatty acid", when used in reference to operatively linking to an HIV gp41-derived peptide, means one or more fatty acids, unless otherwise specifically indicated herein. "Operatively linking HIV gp41-derived peptide to fatty acid" means that fatty acid may be directly operatively linked to the HIV gp41-derived peptide, or the fatty acid is operatively linked to the HIV gp41-derived peptide through a linker (where the linker serves as the molecular bridge between the fatty acid and the HIV gp41-derived peptide), or a combination thereof (e.g., where more than one fatty acid molecule is operatively linked to synthetic peptide; for example, a fatty acid may be directly coupled to the N-terminal amino acid, and a linker-fatty acid combination may be coupled to an internal amino acid, of the HIV gp41-derived peptide). The more preferred fatty acids useful in this invention are the fatty acids of 12 to 20 carbon atoms. The fatty acid may comprise a monoacid or a diacid. The fatty acid may be saturated (Table 1, "S") or unsaturated (Table 1, "U"). Examples of fatty acids that may be useful in producing a conjugate according to the present invention include, but are not limited to the following illustrated in Table 1.

Table 1

Monoacid	Abbreviation	Chemical name(s)
S	C6	Hexanoic acid
S	C8	(+/-)-2-ethylhexanoic acid; octanoic acid
S	C10	Decanoic acid
S	C12	Dodecanoic acid; lauric acid
S	C14	tetradecanoic acid; myristic acid
S	C16	hexadecanoic acid; palmitic acid
S	C18	octadecanedioic acid; stearic acid
S	C20	eicosanoic acid; arachadic acid
S	C22	Docosanoic acid
S	C24	tetracosanoic acid
S	C28	Octacosanoic acid
S	C30	Triacontanoic acid
U	C10:1	3,7-dimethyl-6-octenoic acid; citronellic acid
U	C12:1	cis-5-dodecenoic acid
U	C14:1	delta 9 cis-tetradecenoic acid; myristoleic acid
U	C16:1	delta 9 cis-hexadecenoic acid; palmitoleic acid
U	C18:1 (cis-9)	delta 11 cis-octadecenoic acid; cis-vaccenic acid
U	C18:1(trans-9)	delta 9 trans-octadecenoic acid; elaidic acid
U	C18:1(cis-11)	delta 11 cis-octadecenoic acid; cis-vaccenic acid
U	C18:2	delta 9 cis 12 cis octadecadienoic acid; linoleic acid
U	C18:3	delta 9 cis 12 cis 15 cis octa-decatrienoic acid; linolenic acid
U	C20:1	delta 11 cis-eicosenoic acid; cis-11-eicosenoic acid
U	C22:1	delta 13 cis-docosenoic acid; erucic acid
U	C24:1	delta 15 cis-tetracosenoic acid; nervonic acid
Diacid	C8 diacid	octanedioic acid; suberic acid
Diacid	C9 diacid	Nonanedioic acid; azelaic acid
Diacid	C10 diacid	Decanedioic acid; sebacic acid
Diacid	C11 diacid	undecanedioic acid
Diacid	C12 diacid	dodecanedioic acid
Diacid	C14 diacid	tetradecanedioic acid; 1,12-dodecanedicarboxylic acid
Diacid	C16 diacid	hexadecanedioic acid
Diacid	C20 diacid	eicosanedioic acid; 1,18-octadecanedicarboxylic acid
Diacid	C22 diacid	docosanedioic acid
Diacid	C26 diacid	hexaxosanedioic acid

A preferred fatty acid may be applied in the present invention to the exclusion of a fatty acid other than the preferred fatty acid.

5 The terms "synthetic peptide" and "HIV gp41-derived peptide" are used synonymously herein, in relation to a peptide employed in the present invention, and for the purposes of the specification and claims, to mean a peptide (a) comprising an amino acid sequence of no less than about 15 amino acids and no more than about 60 amino acid residues in length, and comprises at least a portion of (hence, "derived") the amino

acid sequence (preferably, at least 4 contiguous amino acids) contained in the HR2 region of gp41 of HIV (more preferably of HIV-1); and (b) capable of inhibiting transmission of HIV to a target cell (preferably, by complexing to an HR region of HIV-1 gp41 and inhibiting fusion between HIV-1 and a target cell), as can be determined by assessing antiviral activity *in vitro* and/or *in vivo*, as will be described in more detail herein. More preferably, the synthetic peptide employed in the present invention may comprise a sequence of no less than 28 amino acids and no more than about 55 amino acids in length, and even more preferably no less than about 36 amino acids and no more than about 53 amino acids in length. The term "alone", when used in reference to a synthetic peptide or HIV gp41-derived peptide, means that the peptide itself (not operatively linked to fatty acid); i.e., not part of a conjugate. The term "isolated" when used in reference to a synthetic peptide means that it is substantially free (e.g., no less than 80% pure, and more preferably greater than or equal to 90% pure) of components which have not become part of the integral structure of the peptide itself; e.g., such as substantially free of chemical precursors or other chemicals when chemically synthesized, produced, or modified using biological, biochemical, or chemical processes. The synthetic peptide may comprise, in its amino acid sequence, one or more conservative substitutions and/or one or more polymorphisms found in the sequence of the relevant region of the HIV gp41, or may comprise one or more amino acid substitutions which are added to increase or stabilize helix structure and/or affect oligomerization; provided that it retains substantial antiviral activity against HIV-1 (e.g., an IC₅₀ in the picomolar to nanomolar range). The following are illustrative examples of HIV gp41-derived peptides that can be used to produce a conjugate in accordance with the present invention. However, a preferred synthetic peptide may be used in the present invention to the exclusion of a synthetic peptide other than the preferred synthetic peptide. As apparent to one skilled in the art and from the teachings herein, a lysine in the amino acid sequence of a synthetic peptide may be substituted with another amino acid (naturally occurring or not naturally occurring) having a side chain with a free amino group (e.g., epsilon amine). Ornithine is an illustrative example of such amino acid that may be used to substitute a lysine.

For use according to the present invention, preferably a synthetic peptide comprising sequence derived from the HR2 region of HIV-1 gp41 comprises a contiguous sequence of at least amino acid residues 43 to 46 of SEQ ID NO:1 (e.g., QQEK), or comprising conservative substitutions or polymorphisms therein; as key determinants in this portion of the HR2 region have been found to influence biochemical and antiviral parameters described herein. Illustrative synthetic peptides derived from the HR2 region of HIV gp41 include, but are not limited to, synthetic peptides having the amino acid

sequences shown in SEQ ID NOs: 1-33, and 146; and may further comprise an amino acid sequence having at least 95% identity, and more preferably having at least 90% identity, with any one or more of SEQ ID NOs:1-33, and 146. Note that such synthetic peptides have one or more lysine residues internal to the amino acid sequence of the synthetic peptide and/or at the carboxy terminus (in the case of SEQ ID NOs. 7,16, 31, 32, 146), one or more of which may be chosen to be operatively linked to a fatty acid (directly, or via a linker) in producing a conjugate according to the present invention. The term "internal", when used to refer to an amino acid (e.g., lysine) means that the amino acid is other than the N-terminal amino acid and the C-terminal amino acid (e.g., is located in the amino acid sequence in a position in-between the N-terminal amino acid and the C-terminal amino acid).

More preferably for use according to the present invention, a synthetic peptide derived from the HR2 region of HIV gp41 contains one or more amino acid substitutions (e.g., as compared to a relative portion of the amino acid sequence of SEQ ID NO:1 from which it is derived) which preferably promotes the helicity and/or helix stability of the synthetic peptide ("helix stabilized peptide") in imparting improved biological activity, as disclosed in more detail in co-pending application PCT/US04/42918. Examples of such helix stabilized synthetic peptides are illustrated as having amino acid sequences of SEQ ID NOs:34-124, and may further comprise an amino acid sequence having at least 95% identity, and more preferably having at least 90% identity, with any one or more of SEQ ID NOs:34-124. Other examples of peptides designed for improved helicity and derived from the HR2 region of HIV gp41 may include SEQ ID NOs:125-128. Note that such synthetic peptides have one or more internal lysine residues (and in some cases upwards to 25% of the amino acid sequence of the synthetic peptide), and/or at the carboxy terminus (in the case of SEQ ID NO:124), one or more of which may be chosen to be operatively linked to fatty acid in producing a conjugate according to the present invention.

In another preferred embodiment according to the present invention, the synthetic peptide may comprise a "hybrid" peptide comprising amino acid sequences derived from one or more of HIV-1, HIV-2, and SIV fusion proteins (see, e.g., U.S. Patent No. 6,258,782). Examples of a hybrid synthetic peptide are illustrated as having amino acid sequences of SEQ ID NOs:129-145 and may further comprise an amino acid sequence having at least 95% identity, and more preferably having at least 90% identity, with any one or more of SEQ ID NOs:129-145. Note that such illustrated examples of hybrid synthetic peptides have at least two internal lysine residues, one or more of which may be operatively linked to fatty acid in producing a conjugate according to the present invention.

The term "percent identity", when used herein for purposes of the specification and claims in reference to a sequence used in accordance with the present invention, means that the sequence is compared ("Compared Sequence") to a described or reference sequence ("Reference Sequence"); wherein a percent identity is determined

5 according to the following formula:

$$\text{percent identity} = [1 - (x_C/y_R)] \times 100$$

wherein x_C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Compared Sequence and Reference Sequence wherein (a) each base or amino acid in the Reference Sequence

10 that does not have a corresponding aligned base or amino acid compared to the Compared Sequence, and (b) each gap in the Reference Sequence, and (c) each aligned base or amino acid in the Compared Sequence that is different from an aligned base or amino acid in the Reference Sequence, constitutes a difference; and y_R is the number of bases or amino acids in the Reference Sequence over the length of the Compared

15 Sequence with any gap created in the Reference Sequence as a result of alignment also being counted as a base or amino acid. Methods and software for alignment between two predetermined sequences are well known in the art. Thus, for example, a Reference Sequence may be a synthetic peptide according to any one of SEQ ID NOs:1-146, and a Compared Sequence is an HIV gp41-derived peptide which is compared to the Reference

20 Sequence, in determining an amino acid sequence having at least 95% identity with any one or more of the amino acid sequences of SEQ ID NOs:1-146.

The term "substantially homogeneous", when used herein for purposes of the specification and claims and in reference to a conjugate according to the present invention, means that at least 90%, and more preferably at least 95%, of the resultant

25 conjugate produced contains the synthetic peptide operatively linked to fatty acid, as intended by the method of production (e.g., as a predominant or single species), as described herein in more detail. The conjugate may be purified to be substantially homogeneous using separation technology including, but not limited to, chromatographic techniques known in the art.

30 The term "antiviral activity", when used herein for purposes of the specification and claims, refers to the ability of a composition (e.g., synthetic peptide, or conjugate according to the present invention) to inhibit viral infection of target cells. Preferably, the composition inhibits or prevents HIV-1 entry into, or HIV-1-mediated fusion with, a target cell. Preferably, a conjugate according to the present invention has antiviral activity,

35 against typical strains of HIV-1 and against a resistant HIV-1 isolate, as represented by an IC_{50} of no more than 0.10 $\mu\text{g/ml}$, and more preferably, an IC_{50} of no more than 0.005 $\mu\text{g/ml}$ (see, for example, Example 4, Tables 3-5, and Categories B & A herein). "Antiviral

effective amount" means the amount of the referenced composition in a range needed to effect its antiviral activity.

The term "retaining substantial biological activity", when used herein for purposes of the specification and claims, means that a composition according to the present invention (e.g., conjugate, antiviral composition) demonstrates, as compared to the synthetic peptide alone which is used to make the composition, antiviral activity that is in a range from no less than a two fold reduction in antiviral activity to an increase in antiviral activity.

The term "reactive functionality", when used herein for purposes of the specification and claims, means a chemical group or chemical moiety that is capable of forming a bond with another chemical group or chemical moiety. For example, a reactive functionality of a molecule is reacted with a reactive functionality of another molecule in operatively linking (such as, for example, covalently coupling) the two molecules. In an example of operatively linking fatty acid to the synthetic peptide in forming a conjugate according to the present invention, a reactive functionality of a fatty acid is reacted with a reactive functionality of a synthetic peptide in operatively linking (e.g., by covalently coupling) the fatty acid to the synthetic peptide. In another example of operatively linking fatty acid to the synthetic peptide in forming a conjugate according to the present invention, a first reactive functionality of a linker is reacted with a reactive functionality of a synthetic peptide in operatively linking (e.g., by covalently coupling) the linker to the synthetic peptide, and a second reactive functionality of a linker is reacted with a reactive functionality of a fatty acid in operatively linking the linker to the fatty acid. In another example of operatively linking fatty acid to the synthetic peptide in forming a conjugate according to the present invention, a first reactive functionality of a linker is reacted with a reactive functionality of a fatty acid in operatively linking the linker to the fatty acid, and a second reactive functionality of a linker is reacted with a reactive functionality of a synthetic peptide in operatively linking the linker to the synthetic peptide. With respect to chemical groups, a reactive functionality is known to those skilled in the art to comprise a group that includes, but is not limited to, maleimide, thiol, carboxylic acid, hydrogen, phosphoryl, acyl, hydroxyl, acetyl, hydrophobic, amine, amido, dansyl, sulfo, a succinimide, a thiol-reactive, an amine-reactive, a carboxyl-reactive, and the like. A preferred reactive functionality may be used, in application to the present invention, to the exclusion of a reactive functionality other than the preferred reactive functionality.

Each term "operatively linked" or "operatively linking", when used herein for purposes of the specification and claims, means that the two or more molecules are physically associated by a linking means that does not interfere with the ability of either of the linked molecules to function as described herein. For example, a synthetic peptide,

via its reactive functionality, may be linked through coupling using standard chemistry techniques to a fatty acid through its reactive functionality, in producing a conjugate which retains substantial antiviral activity as compared to the synthetic peptide alone. As known to those skilled in the art, and as will be more apparent by the following embodiments, there are several methods and compositions in which two or more molecules may be operatively linked utilizing reactive functionalities, including chemical means, and by genetic fusion (recombinant expression). Additionally, it is also known in the art that a bond formed by operatively linking two molecules may comprise, but is not limited to, one or more of: covalent, ionic, hydrogen, van der Waals and the like.

The term "linker", when used herein for purposes of the specification and claims, means a compound or moiety that acts as a molecular bridge to operatively link two different molecules (e.g., with respect to the present invention, a first reactive functionality of the linker is covalently coupled to a reactive functionality of the fatty acid, and a second reactive functionality of the linker is covalently coupled to a reactive functionality of the synthetic peptide, in forming the conjugate according to the present invention; wherein a linker comprises two reactive functionalities). The two different molecules (e.g., the synthetic peptide and the fatty acid) may be linked to the linker in a step-wise manner. In general, there is no particular size or content limitations for the linker so long as it can fulfill its purpose as a molecular bridge. Linkers are known to those skilled in the art to include, but are not limited to, chemical chains, chemical compounds (e.g., reagents), amino acids, and the like. The linkers may include, but are not limited to, homobifunctional linkers, heterobifunctional linkers, biostable linkers, and biodegradable linkers, as well known in the art. Preferably, when a linker is used, it is non-planar (e.g., so that the synthetic peptide in the conjugate is not rigidly fixed to the fatty acid in the conjugate but remains flexible in the linkage). Heterobifunctional linkers, well known to those skilled in the art, contain one end having a first reactive functionality to specifically link a first molecule, and an opposite end having a second reactive functionality to specifically link to a second molecule. It will be evident to those skilled in the art that a variety of monofunctional, difunctional, and polyfunctional reagents (such as those described in the catalog of the Pierce Chemical Co., Rockford, Ill.) may be employed as a linker with respect to the present invention. Depending on such factors as the molecules to be linked, and the conditions in which the linking is performed, the linker may vary in length and composition for optimizing such properties as preservation of biological function, stability, resistance to certain chemical and/or temperature parameters, and sufficient stereo-selectivity or size. For example, the linker should not significantly interfere with the ability of the synthetic peptide (to which it is linked) to function as an inhibitor of either or both of HIV fusion and HIV transmission to a target cell. Preferred linkers, as

described herein in more detail, comprise from no less than 2 and no more than 100 units of polyethylene glycol; as may be represented by the formula:

$R_1-(CH_2CH_2O)_n-R_2$; wherein R is a reactive functionality, and n is from 2 to 100, and more preferably, n is from 2 to 30.

One preferred linker, referred to herein as (PEG 13)₂, may be represented by the Formula I, as described in Example I in more detail. Preferred linkers include PEG3; discrete units comprised of PEG3 such as (PEG 3)₂, (PEG 3)₄, (PEG 3)₅, (PEG 3)₉; PEG13; (PEG 13)₂; (PEG 13)₂; PEG25; PEG29; a combination of preferred linkers (e.g., a linker made by combining two or more PEG linkers together; for example, a linker comprised of a PEG13 operatively linked to a PEG3); and amino acid linkers (e.g., comprising combinations of Gly, Ser, and like amino acids; ranging from no less than 3 amino acids to no more than 15 amino acids). A preferred linker may be used, in application to the present invention, to the exclusion of a linker other than the preferred linker.

The term "chemical protecting group" or "CPG", when used herein for purposes of the specification and claims, means a chemical moiety that is used to block a reactive functionality from chemically reacting with another reactive functionality. Chemical protecting groups are well known by those in the art of peptide synthesis to include, but are not limited to, tBu (t-butyl), trt (triphenylmethyl(trityl)), OtBu (tert-butoxy), Boc or t-Boc (tert-butyloxycarbonyl), Fmoc (9-fluorenylmethoxycarbonyl), Aoc (t-amtyloxy-carbonyl), TEOC (β-trimethylethyloxycarbonyl), CLIMOC (2-chloro-1-indanyl methoxyl carbonyl), BIMOC (benz-[f]-indene-3-methoxylcarbonyl), PBF (2,2,4,6,7-pentamethyldihydro-benzofuan-5-sulfonyl), 2-Cl-Z (chlorobenzyl-oxycarbonyl), Alloc (allyloxycarbonyl), Cbz (benzyloxycarbonyl), Adoc (adamantyloxy-carbonyl), Mcb (1-methylcyclobutyloxy-carbonyl), Bpoc (2-(p-biphenyl) propyl-2-oxycarbonyl), Azoc (2-(p-phenylazophenyl) propyl-2-oxycarbonyl), Ddz (2,2 dimethyl-3,5-dimethyloxybenzyl-oxycarbonyl), MTf (4-methoxy-2,3,6-trimethoylbenzenesulfonyl), PMC (2,2,5,7,8-pentamethylchroman-6-sulfonyl), Tos (tosyl), Hmb (2-hydroxyl-4-methoxybenzyl), Poc (2-phenylpropyl-2-oxycarbonyl), Dde (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl), ivDde (1-(4,4-dimethyl-2,6-dioxo-cyclohex-1-ylidene)-3-methylbutyl), benzyl, dansyl, para-nitrobenzyl ester, and the like. A preferred chemical protecting group may be used, in application to the present invention, to the exclusion of a chemical protecting group other than the preferred chemical protecting group.

The term "deprotection", when used herein for purposes of the specification and claims, is known in the art to mean a process by which chemical protecting group(s) is removed from a molecule containing one or more chemical protecting groups, wherein the molecule comprises an amino acid, peptide fragment, or HIV fusion inhibitor peptide

according to the present invention. Generally, the deprotection process involves reacting the molecule protected by one or more chemical protecting groups with a chemical agent that removes the chemical protecting group. For example, an N-terminal alpha amino group, which is protected by a chemical protecting group, may be reacted with a base to
5 remove base labile chemical protecting groups (e.g., Fmoc, and the like). Chemical protecting groups (e.g., Boc, TEOC, Aoc, Adoc, Bop, Ddz, Cbz, and the like) are removed by acid. Other chemical protecting groups, particularly those derived from carboxylic acids, may be removed by acid or a base.

The terms "first", "second", "third" and the like, may be used herein to: (a) indicate
10 an order; or (b) to distinguish between molecules or reactive functionalities of a molecule; or (c) a combination of (a) and (b). However, the terms "first", "second", "third" and the like, are not otherwise to be construed as limiting the invention.

The term "one or more improved pharmaceutical activities", when used herein for purposes of the specification and claims, means that the composition according to the
15 present invention (e.g., conjugate, antiviral composition) demonstrates, as compared to the synthetic peptide alone which is of the same species as used in forming the composition, an improvement in one or more of: antiviral activity ("increased antiviral potency") against HIV-1 strains which have not developed resistance to HIV fusion inhibitor peptides; antiviral activity ("increased antiviral potency") against HIV strains
20 which have developed resistance to some HIV-1 fusion inhibitor peptides; a broader spectrum of antiviral activity against HIV strains (e.g., active against HIV-1 and HIV-2 versus substantially only HIV-1); a pharmacokinetic property (e.g., either or both of clearance, and biological half-life, as defined herein in more detail).

The term "pharmacokinetic properties", when used herein for purposes of the
25 specification and claims, means the total amount of active ingredient (e.g., synthetic peptide of a conjugate according to the present invention) in a pharmaceutical composition that is systematically available over time. Pharmacokinetic properties may be determined by measuring total systemic concentrations of the conjugate over time after administration, either singularly or in comparison with pharmacokinetic properties after
30 administration of synthetic peptide alone. As an example, pharmacokinetic properties may be expressed in terms of the Area Under the Curve (AUC), biological half-life, and/or clearance. AUC is the integrated measure of systemic active ingredient concentrations over time in units of mass*time/volume. Following the administration of a dose of active ingredient, the AUC from the time of dosing to the time when no active ingredient remains
35 in the body, is a measure of the exposure of the individual to the active ingredient (and/or a metabolite of an active ingredient). A conjugate comprised of synthetic peptide operatively linked to fatty acid has "improved" pharmacokinetic properties when the

conjugate has one or more of (a) a longer biological (terminal elimination) half life ($t_{1/2}$), and (b) a reduction in biological (total body) clearance (Cl), as compared to that of a corresponding synthetic peptide (without fatty acid). In a preferred embodiment, the conjugate typically allows for a reduced clearance, relative to that of a comparable synthetic peptide, such as typically being from about 5 fold reduction to about 40 fold reduction, as will be shown in more detail in the examples herein. In another preferred embodiment, the conjugate typically allows for an increase in ("longer") biological half-life of from about a 20% increase to about a 700% increase relative to a comparable synthetic peptide alone, as will be shown in more detail in the examples herein. The following equations were used to calculate area-under the plasma concentration vs. time curve (AUC), total body clearance (Cl), and terminal elimination half-life ($t_{1/2}$).

$$\text{AUC} = A/a + B/b$$

Where A and B are intercepts and a and b are the rate constants of the exponential equations describing the distribution and elimination phases, respectively. When mono-exponential models were used, the "A" and "a" parameters were eliminated. Further, For non-compartmental analysis, the AUC was determined as the sum of the linear trapazoids measured from the time of dosing until the conjugate was unmeasurable.

$$\text{Cl} = \text{Dose/AUC (expressed in L/K/hr)}$$

$$t_{1/2} = -0.6903/b \text{ (expressed in hr)}$$

[End of formal definition section]

EXAMPLE 1

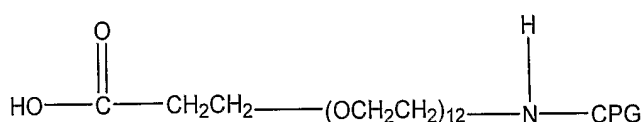
The present invention relates to conjugates comprised of HIV gp41-derived peptide operatively linked to fatty acid either directly or through a linker. In accordance with the present invention, provided are linkers useful for forming a stable linkage between multiple components in forming a conjugate. The linker is of a discrete-length, generally water soluble, as well as soluble in many organic solvents. Preferably, the linker is substantially non-toxic, and substantially non-immunogenic. While described in this Example 1 are preferred linkers of discrete lengths for operatively linking a fatty acid to a synthetic peptide in forming a conjugate according to the present invention, the nature of the individual first component and second components to be linked by use of the linker may be widely varied, as apparent to one skilled in the art, and by using the methods described herein. Accordingly, in one embodiment of the present invention, provided is a linker which can be used to operatively link a first component to a second component in forming a conjugate comprised of the first component linked to the second component.

Such a conjugate may be generally represented by the following structure:

A-linker-B

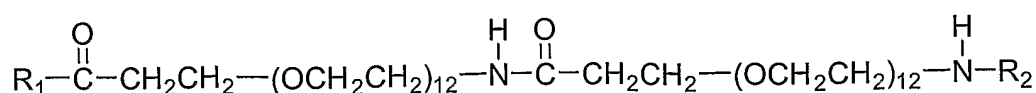
wherein A is a first molecule, B is a second molecule, and wherein the linker is covalently coupled to both A and B. As is described herein in more detail, a first reactive functionality of the linker is chemically reacted with a reactive functionality of the first molecule, and a second reactive functionality of the linker is chemically reacted with a reactive functionality of the second molecule in covalently coupling A to B. Preferably, a reactive functionality is a chemical group selected from the group consisting of a maleimide, thiol, carboxy, hydrogen, phosphoryl, acyl, hydroxyl, acetyl, aldehyde, hydrophobic, amine, amido, dansyl, sulfhydryl, a succinimide (including but not limited to a succinimidyl ester or succinimidyl carbonate), a halogen, a thiol-reactive chemical group, an amine-reactive chemical group, a carboxyl-reactive chemical group, and a hydroxyl-reactive chemical group. A preferred chemical group may be used, in application to the present invention, to the exclusion of a chemical group other than the preferred chemical group.

To illustrate the synthesis of a linker, which is a linker preferred to be used according to the present invention, commercially obtained was starting material known as O-(2-Fmoc-aminoethyl)-O'-(2-carboxyethyl)-undecaethylene glycol, alternatively known as N-Fmoc-amido-dPEG12-acid, and what is referenced herein as "Fmoc-PEG13", generally represented by the structure:



A linker useful with the present invention, referred to herein as (PEG13)₂, is produced by covalently coupling two PEG13 molecules together. The (PEG13)₂ linker may be represented by Formula I, wherein there is a discrete length of the linker with respect to the specific number of ethylene glycol units (i.e., -OCH₂CH₂) in the chemical backbone of the linker (e.g., 26 ethylene glycol units), one or two of which ethylene glycol units may be broken or substituted for in forming a reactive functionality or for linking a first molecule of PEG13 to a second molecule of PEG13 in forming (PEG13)₂.

Formula I:



wherein R₁ and R₂ are each a reactive functionality, and more preferably a reactive functionality selected from the group consisting of a hydrogen, oxygen, a hydroxyl, an

amine-reactive group, a carboxyl-reactive group, and a chemical protecting group ("CPG") used in peptide synthesis to protect a reactive functionality from further chemical reactivity. Generally, it is understood that when R1 and R2 are chosen to be the same reactive functionality or different reactive functionalities having chemical reactivity with the same chemical group, the linker is considered "homobifunctional" or "monofunctional". It is also generally understood that when R1 is chemically reactive with a reactive functionality with which R2 is not chemically reactive, then the linker is considered "heterobifunctional" or "difunctional". As shown by Formula I, the number of intact ethylene glycol units is 24; and hence, there is a discrete length of the (PEG 13)₂ linker based on a backbone structure comprising the ethylene glycol units, to which on each end of the backbone structure is a reactive functionality that does not include intact ethylene glycol units.

The (PEG13)₂ linker may be produced by solid phase synthesis techniques using standard Fmoc protocols. In a preferred embodiment, the production of the linker by solid phase synthesis is carried out on super acid sensitive solid supports which include, but are not limited to, 2-chlorotrityl chloride (2-CTC) resin, and 4-hydroxymethyl-3-methoxyphenoxybutyric acid resin. For example, 2-CTC resin (1.6g, 1.2 mmol/g, 1.92 mmol) was swelled in 10 ml of CH₂Cl₂ twice for 5 minutes each time. PEG13 (Fmoc-PEG13-OH; 0.501 g, 0.6 mmol) was dissolved in 10 ml of CH₂Cl₂ with DIEA (N,N-diisopropylethylamine; 0.401 mL, 2.30 mmol). The solution containing PEG13 was added to the swelled resin, and the resin was agitated for 1 hour. The resin was then washed three times with CH₂Cl₂. After the last wash, added to the resin was 10% DIEA in methanol (10 ml), and the resin was agitated for another 30 minutes. The resin was then washed four times with methanol (10 ml) and twice with CH₂Cl₂ (10 ml). The resin was dried (e.g., overnight), and then swelled with CH₂Cl₂ (10 ml) for 5 minutes. The Fmoc was cleaved by adding 20% piperidine in NMP (N-methylpyrrolidinone; 10 ml) twice for 30 minutes each time. The resin was then washed six times with NMP (10 ml) until a negative chloranil test was achieved. To the resin was added PEG13 (Fmoc-PEG13-OH; 0.507 g, 0.60 mmol) dissolved in 10 ml of DMF (dimethylformamide) and TCTU (tetramethyluronium tetrafluoroborate; 0.237g, 0.66 mmol) and DIEA (0.418 mL, 2.40 mmol), and the resin was agitated until a negative Kaiser test (approximately 2 hours). The resin was then washed three times with NMP. The product (PEG13)₂ was cleaved from the resin using 2% TFA in CH₂Cl₂ (15 ml) for 15 minutes. The solvent was removed under reduced pressure to afford a preparation of (PEG13)₂, which then was purified to high purity using HPLC.

Typically, (PEG13)₂ is produced with a first reactive functionality consisting of a carboxylic acid at one end of the linker, and a second reactive functionality comprising an

amine group at an opposite end of the linker. As known in the art, the carboxylic acid can be activated to react with a free amino group of a molecule (e.g., peptide) to which it is to be covalently coupled. Typically, the carboxylic acid of a PEG molecule can be converted to an aldehyde group, and the aldehyde group can be covalently linked to a molecule bearing an amine group using the method of reductive amination. As an example, the carboxylic acid of (PEG13)₂ is activated to form an active ester (e.g., by reaction with N,N'-disuccinimidyl oxalate in the presence of pyridine or N,N'-dimethylaminopyridine) which will then react specifically with a free amino group of the molecule to be coupled to (PEG13)₂. Alternatively, the (PEG13)₂ active ester may be reacted with another reagent (e.g., ω,ω aminoalkane, N-carboxyalkylmaleimide, or aminoalkanoic acids) and then be coupled to a reactive functionality (e.g., amino or thiol group) of the molecule to which it is to be coupled. In another variation, the carboxyl group of (PEG13)₂ is esterified with a suitable carboxyl-activating agent (e.g., using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), or bromoacetyl N-hydroxysuccinimide, or 1,1-carbonyldiimidazole) to form the active ester of (PEG13)₂, which is then coupled to an amino groups of the molecule to be covalently coupled to (PEG13)₂.

In another example, the hydroxyl group of a PEG molecule can be converted to an aldehyde group, and the aldehyde group can be covalently linked to a molecule bearing an amine group using a method of reductive amination. An amine group of a PEG molecule may be coupled to an amine group of a molecule, to be chemically coupled to the linker, such as through the use of a chemical spacer (e.g., urea).

In another embodiment, a preferred linker used in producing a conjugate according to the present invention comprises (PEG3)_n, wherein n = 1-10. To illustrate the synthesis of the linker comprising (PEG3)_n (where n > 1), commercially obtained was starting material known as 8-amino-3,6 dioxaoctanoic acid, and what is referenced herein as "PEG3", generally represented by the structure:



wherein R₁ and R₂ are each a reactive functionality, and more preferably a reactive functionality selected from the group consisting of a hydrogen, oxygen, a hydroxyl, an amine-reactive group, a carboxyl-reactive group, and a chemical protecting group ("CPG") used in peptide synthesis to protect a reactive functionality from further chemical reactivity. Generally, it is understood that when R₁ and R₂ are chosen to be the same reactive functionality or different reactive functionalities having chemical reactivity with the same chemical group, the linker is considered "homobifunctional" or "monofunctional". It is also generally understood that when R₁ is chemically reactive with a reactive

functionality with which R2 is not chemically reactive, then the linker is considered "heterobifunctional".

Various discrete length linkers, having PEG3 as a subunit, were produced by covalently coupling two or more PEG3 molecules together to get the desired discrete length of the linker. Thus, for example, to produce (PEG3)₂, two PEG3 units were covalently coupled; to produce (PEG3)₃, a PEG3 unit was covalently coupled to a (PEG3)₂; and successive PEG3 units may be covalently coupled to achieve a (PEG3)_n where n = 4-10. More preferably, the specific number of ethylene glycol units (i.e., -OCH₂CH₂) in the chemical backbone of the linker ranges from 3 to 30 ethylene glycol units, depending on the value of n, one or two of which ethylene glycol units may be broken or substituted for in forming a reactive functionality or for linking a molecule of PEG3 to a another molecule of PEG3 in forming (PEG3)_n. Methods by which a reactive functionality of a (PEG3)_n linker may be operatively linked to a reactive functionality of a molecule are essentially the same as those described herein for the (PEG13)₂ linker.

In an illustrative example, a (PEG3)₂ linker is produced by solid phase synthesis similar in chemistry and methodology to that described above for making a (PEG13)₂ linker. For example, 2-CTC resin (15g, 1.61 mmol/g, 24.15 mmol) was swelled in 150 ml of CH₂Cl₂ twice for 5 minutes each time. PEG3 (Fmoc-PEG3-OH; 3.32g, 9 mmol) was dissolved in 150 ml of CH₂Cl₂ with DIEA (N,N-diisopropylethylamine; 2.90 mL, 18 mmol). The solution containing PEG3 was added to the swelled resin, and the resin was agitated for 2 hours. The resin was then washed three times with CH₂Cl₂. After the last wash, added to the resin was 10% DIEA in methanol (50 ml), and the resin was agitated for another 45 minutes. The resin was then washed four times with methanol (100 ml) and twice with CH₂Cl₂ (100 ml). The resin was dried (e.g., overnight). Three grams of the dried resin was then swelled with CH₂Cl₂ (30 ml) for 5 minutes. The Fmoc was cleaved by adding 20% piperidine in NMP (N-methylpyrrolidinone; 30 ml) twice for 30 minutes each time. The resin was then washed six times with NMP (30 ml) until a negative chloranil test was achieved. To the resin was added PEG3 (Fmoc-PEG3-OH; 0.73 g, 1.9 mmol) dissolved in 30 ml of DMF (dimethylformamide) with TCTU (tetramethyluronium tetrafluoroborate; 0.37g, 1.9 mmol) and DIEA (0.64 mL, 3.8 mmol), and the resin was agitated until a negative Kaiser test (approximately 2 hours). The resin was then washed three times with NMP. The product (PEG3)₂ was cleaved from the resin using 2% TFA in CH₂Cl₂ (15 ml) for 15 minutes. The solvent was removed under reduced pressure to afford a preparation of (PEG3)₂, which then was purified to high purity using HPLC. Using the same methodologies outlined above, a linker may be produced which comprises a combination of PEG13 and/or PEG3 (e.g., (PEG13)₂, (PEG3)_n), and/or PEG25.

In another embodiment, a preferred linker used in producing a conjugate according to the present invention comprises either PEG25, or PEG29. For example, a PEG25 linker may be modified or produced to comprise various functional groups, as described above for the (PEG13)₂ linkers, and (PEG3)_n linkers. For example, useful PEG24 linkers may include, but are not limited to N-Fmoc-amido-dPEG₂₄, and N-Amino-dPEG₂₄-t-butyl ester.

In this embodiment, a conjugate according to the present invention is produced by operatively linking HIV gp41-derived peptide to fatty acid through a linker. In this example, illustrated is a method by which fatty acid is operatively linked to a linker. There are a variety of ways by which a fatty acid can be operatively linked to a linker, whereby a reactive functionality of the fatty acid is chemically reacted with a reactive functionality of the linker in forming a linker-fatty acid combination. A preferred method involves production of the linker-fatty acid combination by solid phase synthesis techniques using standard Fmoc (or CPG) protocols. For example, as described above, (PEG13)₂ was produced by solid phase techniques. While (PEG13)₂ remained coupled to the resin (in the form of resin-(PEG13)₂-Fmoc), the resin was swelled in 15 ml of CH₂Cl₂ for 5 minutes. Fmoc was cleaved from the linker by adding 20% piperidine in NMP (10 ml) twice for 20 minutes each time. The resin was then washed six times with NMP (15 ml) until a negative chloranil test was achieved. To the resin was added the fatty acid to be coupled to the linker (in this illustration, C18, also known as octadecanoic acid or stearic acid; 0.25 g, 0.9 mmol) dissolved in 10 ml of DMF and TCTU (0.31g, 0.9 mmol) and DIEA (0.3 mL, 1.8 mmol), and the resin was agitated until a negative Kaiser test (approximately 1.5 hours). The resin was then washed six times with CH₂Cl₂ (15 ml) for 15 minutes each, and then once with methanol (15 ml). The solvent was removed under reduced pressure to afford a preparation of linker-fatty acid combination, which then was purified as a salt for high purity using liquid chromatography with mass spectrometric detection. Thus, this method can be accommodated using any of the aforementioned preferred linkers, and using any size fatty acid (preferably comprising no less than 5, and no more than 30, carbon atoms; and more preferably a fatty acid having a number of carbon atoms in the range of from 12 to 20 carbon atoms).

EXAMPLE 2

Synthetic peptides, used to make a conjugate according to the present invention, were synthesized by one of two methods. A first method is by linear synthesis; e.g., using standard solid-phase synthesis techniques manually or on a peptide synthesizer and using standard Fmoc peptide chemistry or other standard peptide chemistry (using CPGs). A preferred method for synthesis of a synthetic peptide used in the present

invention is by a fragment condensation approach. Briefly, 2 or more fragments are synthesized, each fragment containing a respective portion of the complete amino acid sequence of the synthetic peptide to be synthesized. In the synthesis of a fragment, if desired, incorporated may be an amino acid having its free amine (e.g., side chain amine) chemically protected by a chemical protecting group. The fragments are then assembled (covalently coupled together in a manner and order) such that the synthetic peptide is produced (with the proper amino acid sequence). For example, for purposes of illustrative conjugates, as described in Example 4 herein, T20 (SEQ ID NO:3) and T1249 (SEQ ID NO:129) were made linearly on a peptide synthesizer; however, they may also be synthesized by fragment condensation approach, as previously described in more detail (see, e.g., U.S. Patent Nos. 6,015,881, and 6,258,782, respectively). Based on the teachings herein, it is apparent to one skilled in the art that a fragment assembly approach can be used to produce a synthetic peptide for use in making a conjugate according to the present invention. Depending on the length of, the amino acid sequence of, and the number and location of amino acids with side chain amines in the amino acid sequence of, a particular synthetic peptide, anywhere from 2 to 4 fragments have been synthesized, and then assembled to complete the synthesis of that particular synthetic peptide.

In another example of this embodiment, the synthetic peptide has an amino acid sequence of SEQ ID NO:33. A fragment condensation approach involving assembly of either 2 or 3 fragments was most often used to synthesize a synthetic peptide having the amino acid sequence of SEQ ID NO:33 for producing conjugates according to the present invention. For the fragment condensation approach involving 3 fragments, a fragment comprising the amino acids 1-12 of SEQ ID NO:33, a fragment comprising amino acids 13-28 of SEQ ID NO:33, and a fragment comprising amino acids 29-36, were each individually synthesized, and then assembled to produce a synthetic peptide having the amino acid sequence of SEQ ID NO:33. In a two fragment approach, assembled were a fragment comprising amino acids 21-36 of SEQ ID NO:33, and a fragment comprising amino acids 1-20 of SEQ ID NO:33. For the fragment condensation approach involving 3 fragments, a peptide fragment comprising the first 12 amino acids of SEQ ID NO:33 (see FIG. 3, "AA(1-12)", was synthesized by standard solid phase synthesis (using a super acid sensitive resin; e.g., 4-hydroxymethyl-3-methoxyphenoxy-butyric acid resin, or 2-chlorotrityl chloride resin- "CTC", FIG. 3), with acetylation of ("Ac", as a chemical group) the N-terminus, while having a hydroxyl group (-OH) at the C-terminus (see, FIG. 3, "Ac-AA(1-12)-OH"). A peptide fragment comprising amino acids 13-28 of SEQ ID NO:33 (see, FIG. 3, "AA(13-28)"), was synthesized by standard solid phase synthesis with Fmoc at the N-terminus (as a chemical protecting group), and -OH at the C-terminus

(see, FIG. 3, "Fmoc-AA(13-28)-OH"). A peptide fragment comprising amino acids 29-35 of SEQ ID NO:33 (see, FIG. 3, "AA(29-35)"), was synthesized by standard solid phase synthesis with Fmoc at the N-terminus (as a chemical protecting group), and -OH at the C-terminus (see, FIG. 3, "Fmoc-AA(29-35)-OH"). Each peptide fragment was cleaved from the resin used for its solid phase synthesis by cleavage reagents, solvents, and techniques well known to those skilled in the art. Each peptide fragment was then isolated by removing the majority of above mentioned solvents by distillation and precipitating the peptide fragment by the addition of water with or without an alcohol containing-cosolvent. Each resulting solid was isolated by filtration, washed, reslurried in water or alcohol/water, refiltered, and dried in a vacuum oven

As shown in FIG. 3, peptide fragment Fmoc-AA(29-35)-OH of SEQ ID NO:33 was chemically coupled in solution phase to a leucine amide (amino acid 36 of SEQ ID NO:33 which was amidated as a chemical group), to result in a peptide fragment having comprising amino acids 29-36 of SEQ ID NO:33 with amidation of the C-terminus ("Fmoc-AA(29-36)-NH₂"). Peptide fragment Fmoc-AA(29-35)-NH₂ (12.51 g, 6.4 mmol, 1 eq), H-Leu-NH₂*HCl (1.059 g, 6.4 mmol, 1 eq), and HOAT (1.30 g, 9.5 mmol, 1.5 eq) were dissolved in DMF (50 ml, 4 vol) treated with DIEA (5.39 ml, 31.8 mmol, 5 eq) and stirred at room temperature until dissolved (about 5 minutes). Then the solution was cooled using an ice bath, and TBTU (2.45 g, 7.6 mmol, 1.2 eq) was added. The reaction was stirred for approximately 5 minutes at 0 °C, then at room temperature for 2.5 hours. Analysis by HPLC showed no more starting fragment. The reaction was stirred overnight. Then the reaction mixture was poured into 400 ml of water, and the solids formed were collected via filtration and dried. The dry solid was suspended in 400 ml 3:1 MTBE:hexanes and stirred for 4 hours, then collected and dried. Then the solids were suspended in 400 mL 1:1 ethanol:water and stirred for 1 hour. The solids were collected and dried. Piperidine (1.5 ml, 15.2 mmol, 2.4 eq) was added to remove the Fmoc protecting group. The Fmoc chemical protecting group of the peptide fragment Fmoc-AA(29-36)-NH₂ may also be removed using a base such as potassium carbonate in organic solvents such as DMF, NMP, methyl t-butyl ether (MTBE), hexane, or mixtures thereof. As shown in FIG. 3, the result is a substantially pure preparation of peptide fragment H-AA(29-36)-NH₂ of SEQ ID NO:33.

As illustrated in FIG. 3, a solution phase reaction was then performed in which peptide fragment H-AA(29-36)-NH₂ of SEQ ID NO:33 is combined with peptide fragment Fmoc-AA(13-28)-OH of SEQ ID NO:33 to yield a peptide fragment Fmoc-AA(13-36)-NH₂ of SEQ ID NO:33. Peptide fragment H-AA(29-36)-NH₂ (8.74 g, 4.7 mmol, 1 eq), peptide fragment Fmoc-AA(13-28)-OH (16.32 g, 4.7 mmol, 1 eq), and HOAT (0.96 g, 7.1 mmol, 1.5 eq) were dissolved in DMF (100 ml, 20 vol), cooled with an ice bath, and treated with

DIEA (4.0 ml, 23.5 mmol, 5 eq). Added to the reaction was TBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyltetrafluoro-borate; 1.81 g, 5.6 mmol, 1.2 eq), and the reaction was stirred for about 5 minutes at 0°C, then 2 hours at room temperature. Analysis by HPLC showed no more starting material. Then piperidine (2 ml, 20 mmol, 4 eq) was added to
5 remove the Fmoc, and the reaction stirred for 3 hours. The reaction was poured into 450 ml water and the solids filtered off and dried. Then the solids were suspended in 450 ml 3:1 MTBE:hexanes and stirred for about 3 hours. The solids were collected and dried, then suspended in 450 ml 1:1 ethanol:water and stirred for about 1 hour. The solids were filtered off and dried affording an amino acid sequence of H-AA(13-36)-NH₂ as a
10 substantially pure white solid as determined by high performance liquid chromatography (HPLC) analysis for purity.

As illustrated in FIG. 3, peptide fragment H-AA(13-36)-NH₂ of SEQ ID NO:33 was then assembled in a solution phase reaction with peptide fragment Ac-(1-12)-OH of SEQ ID NO:33 to yield a synthetic peptide having the amino acid sequence of SEQ ID NO:33
15 (see, e.g., FIG. 3, Ac-(1-36)-NH₂). Peptide fragment H-AA(13-36)-NH₂ (11.18 g, 2.20 mmol, 1 eq), peptide fragment Ac-AA(1-12)-OH (6.69 g, 2.20 mmol, 1 eq), and HOAT (0.450 g, 3.31 mmol, 1.5 eq) were dissolved in DMF (120 ml, 10 vol), cooled with an ice bath, and treated with DIEA (2.0 ml, 11.5 mmol, 5 eq). Added to the reaction was TBTU (0.847 g, 2.64 mmol, 1.2 eq). After stirring 5 minutes at 0°C, the reaction was stirred at
20 room temperature for 2 hours after which HPLC showed the reaction was complete. Then hydrazine (10 mL, 321 mmol, 146 eq) was added and the reaction (to remove the ivdde CPG on Lys30), and stirred for about 20 minutes. Analysis by HPLC showed no more protected fragment, so the reaction was poured into 500 ml ice-water. The solids formed were collected and dried. As shown in FIG. 3, the side chain chemical protecting
25 groups of synthetic peptide Ac-AA(1-36)-NH₂ may be removed by acidolysis or any other method known to those skilled in the art for deprotecting a peptide by removing side chain chemical protecting groups. In this example, synthetic peptide Ac-AA(1-36)-NH₂ (16.98 g, 2.09 mmol) was treated with TFA (trifluoroacetic acid):DTT(dithiothreitol):water (95:5:5; 200 ml) and stirred at room temperature for 4 hours. MTBE (approximately 500 ml) was
30 added, and a precipitate was collected by filtration. The dried powder was then dissolved in 400 ml of 3:1% water/acetonitrile containing 1% HOAc (acetic acid), and was reacted for 20 hours; followed by purification (by HPLC). The result was a substantially pure preparation of synthetic peptide having the amino acid sequence of SEQ ID NO:33, as determined by HPLC analysis for purity (95% yield).

35 The above-described methods and chemistry used to synthesize a synthetic peptide having the amino acid sequence of SEQ ID NO:33, may also be used to synthesize other synthetic peptides for use in producing conjugates according to the

present invention. Thus, for example, a fragment condensation approach involving assembly of 3 fragments was most often used to synthesize a synthetic peptide having the amino acid sequence of SEQ ID NO:37 for producing conjugates according to the present invention. A fragment comprising the first 12 amino acids of SEQ ID NO:37 was synthesized by standard solid phase synthesis (using a super acid sensitive resin), with acetylation ("Ac") of the N-terminus while having a hydroxyl group (-OH) at the C-terminus. A fragment comprising amino acids 13-26 of SEQ ID NO:37 was synthesized by standard solid phase synthesis with Fmoc at the N-terminus, and -OH at the C-terminus. A fragment comprising amino acids 27-37 of SEQ ID NO:37 was synthesized by standard solid phase synthesis with Fmoc at the N-terminus, and -OH at the C-terminus. The fragment comprising amino acids 27-37 of SEQ ID NO:37 was chemically coupled to amino acid 38 in solution phase to result in a fragment comprising amino acids 27-38 with amidation of the C-terminus. The fragment of amino acids 13-26 of SEQ ID NO:37 was then chemically coupled with the fragment of amino acids 27-38 of SEQ ID NO:37 (after removal of Fmoc from the N-terminal amino acid 27). The resulting amino acid sequence, having amino acids 13-38 of SEQ ID NO:37, was chemically coupled with the fragment comprising amino acids 1-12 of SEQ ID NO:37 (after removal of Fmoc from the N-terminal amino acid 13) in forming a synthetic peptide comprising the amino acid sequence of SEQ ID NO:37. The synthetic peptide was deprotected/ decarboxylated (to remove tBU, trt, and Boc used in the synthesis of each fragment) with a deprotection step using a cocktail of trifluoroacetic acid/ dithiothreitol/water (volume percent:90/5/5) at 30°C, for 5 to 6 hours with stirring; and then purified using reverse-phase high performance liquid chromatography. Peptide identity was confirmed with electrospray mass spectrometry.

In another example of this embodiment, the synthetic peptide has an amino acid sequence of SEQ ID NO:37, except that there is an additional amino acid at the C-terminus, a lysine. To produce this synthetic peptide, having an amino acid sequence of SEQ ID NO:124, used were a fragment condensation approach along with a method of site-specific chemical modification. This is an example in which the synthetic peptide, used to make a conjugate according to the present invention, has two or more lysine residues (e.g., one or more internal lysines and a C-terminal lysine, or two or more internal lysines), each having an epsilon amine to which a linker-fatty acid combination could be covalently coupled. A method of site-specific chemical modification is utilized to block a selected epsilon amine(s) from further reactivity, so that in a subsequent reaction in which the synthetic peptide is operatively linked to fatty acid, the fatty acid to be operatively linked to one or more specific amino acids which are unblocked in the synthetic peptide. The method of site-specific chemical modification has been described

in more detail in co-pending application PCT/US2005/07486.

As an illustrative example, a fragment comprising the first 12 amino acids of SEQ ID NO:124 was synthesized with acetylation ("Ac") of the N-terminus while having a hydroxyl group (-OH) at the C-terminus. A fragment comprising amino acids 13-26 of SEQ ID NO:124 was synthesized by standard solid phase synthesis with Fmoc at the N-terminus, and -OH at the C-terminus. A fragment comprising amino acids 27-37 of SEQ ID NO:124 was synthesized by standard solid phase synthesis with Fmoc at the N-terminus, -OH at the C-terminus, and using Fmoc-Lys(ivDde) as amino acid residue 30 ("K30"), so that the chemical protecting group ivDde blocks the epsilon amine group of K30 from reacting subsequently with an amine-reactive, reactive functionality. Amino acids 38 and 39 of SEQ ID NO:124 were covalently coupled with H- at the N-terminus and an amidated C-terminus. The fragment comprising amino acids 38 and 39 was coupled with the fragment comprising amino acids 27-37 of SEQ ID NO:124 in solution phase to result in a fragment comprising amino acids 27-39 with amidation of the C-terminus. The fragment of amino acids 13-26 of SEQ ID NO:124 was chemically coupled with the fragment of amino acids 27-39 of SEQ ID NO:124 (after removal of Fmoc from the N-terminal amino acid 27). The resulting amino acid sequence having amino acids 13-39 of SEQ ID NO:124 was chemically coupled with the fragment comprising amino acids 1-12 of SEQ ID NO:124 (after removal of Fmoc from the N-terminal amino acid 13) in forming a synthetic peptide comprising the amino acid sequence of SEQ ID NO:124 having K30 with an ivDde protected side chain, and K39 with a free epsilon amine. The synthetic peptide was deprotected/ decarboxylated (to remove tBU, trt, and Boc used in the synthesis of each fragment) with a deprotection step using a cocktail of trifluoroacetic acid/dithiothreitol/water (volume percent:90/5/5) at 30°C, for 5 to 6 hours with stirring. Then the product was solidified using MTBE and collected. The solid was decarboxylated in 1:1 water:acetonitrile at about pH 5 for 24 hours, and then purified using reverse-phase high performance liquid chromatography. This deprotection step did not remove the ivDde from the side chain of K30. Thus, the isolated synthetic peptide was then operatively linked (e.g., covalently coupled) to a linker-fatty acid combination site-specifically to the free (unblocked) epsilon amine of K39 of SEQ ID NO:124. Following the coupling of synthetic peptide to linker-fatty acid, the ivDde may be removed by treating the conjugate with hydrazine (3% (v/v)) in a deprotection reaction. In the instances where the free N-terminal amine is the desired reactive functionality of a synthetic peptide to which is operably linked fatty acid, linker, or linker-fatty acid combination (see, e.g., Table 3, compounds having reference numbers 32-37), the internal lysine may be protected using the method of site-specific chemical modification. The N-terminal amino acid is protected by Fmoc or similar chemical protecting group so

that upon deprotection, the free (unblocked) N-terminal amine was then operatively linked to a linker-fatty acid combination. The epsilon amine of the internal lysine was then deprotected in a subsequent deprotection reaction.

EXAMPLE 3

The present invention relates to conjugates comprised of HIV gp41-derived peptide operatively linked to fatty acid either directly to the synthetic peptide, or through a linker which operatively links synthetic peptide and fatty acid. In this example and in Example 4, illustrated are several HIV gp41-derived peptides used to produce a conjugate according to the present invention. However, it is understood HIV gp41-derived peptides, other than those illustrated herein, may be employed in the conjugate according to the present invention, particularly because this class of synthetic peptides shares structural, biochemical, and functional features. More particularly, this class of synthetic peptides all comprise coiled coil heptad repeats which comprise structural features that contribute to the antiviral activity of the synthetic peptides. From the present invention, it is shown that having fatty acid operatively linked thereto can result in an interaction with these structural features that enhances functional activity; i.e., results in one or more improved pharmaceutical activities, as described herein in more detail. Other shared structural, biochemical and functional features of the class of HIV gp41-derived peptides include, but may not be limited to, an amino acid sequence containing one or more leucine zipper-like motifs, a propensity for coiled coil structure, a propensity for oligomerization, and ability to inhibit transmission of HIV to a target cell.

In a method of producing a conjugate according to the present invention, there are several ways to operatively link fatty acid to the synthetic peptide. As illustrated in Example 3A herein, in one embodiment of this method, the linker was first operatively linked to the synthetic peptide (via a first reactive functionality of the linker), and then the fatty acid was operatively linked to the linker (via a second reactive functionality of the linker). As illustrated in Example 3B herein, in another embodiment of this method, the linker was first operatively linked (via a first reactive functionality of the linker) to fatty acid in producing a linker-fatty acid combination, and then the linker-fatty acid combination was operatively linked to the synthetic peptide (via a second reactive functionality of the linker). As illustrated in Example 3C herein, in another embodiment of this method, the fatty acid was operatively linked to synthetic peptide by chemically coupling a reactive functionality of the fatty acid with a reactive functionality of the synthetic peptide. Examples 3A through 3E also illustrate various embodiments of a conjugate according to the present invention.

Example 3A

Illustrated is a method for producing a conjugate according to the present invention comprising operatively linking synthetic peptide to fatty acid by first operatively linking the linker to the synthetic peptide, followed by operatively linking the fatty acid to the linker of the synthetic peptide-linker combination. In this example, the N-terminal amine was protected, and the epsilon amine of the internal lysine (amino acid 30 of SEQ ID NO:37) was free for reacting with an amine-reacting reactive functionality of the linker. However, if it was desired to operatively link the linker to the N-terminal amino acid of the synthetic peptide, the N-terminal amine would be free for reactivity, and the epsilon amine of the internal lysine would be protected during the operatively linking of the linker to synthetic peptide. A synthetic peptide having the amino acid sequence of SEQ ID NO:37 (0.250 g, 34.5 nmol) and a linker (Fmoc-PEG(13)₂-OH; 0.051 g, 35.4 nmol) were dissolved in 2.5 ml DMF, and added were HOAT (0.014 g, 0.10 mmol), DIEA (0.030 ml, 0.17 mmol), and TBTU (0.014 g, 43.6 nmol). The reaction was stirred for about 4 hours. Analysis by HPLC showed no more starting material present, so the reaction was poured into 20 ml H₂O. The solids formed were collected and dried (0.261 g, 30.1 nmol, 87% yield). This process formed a synthetic peptide-linker combination (synthetic peptide covalently coupled to linker; "SEQ ID NO:37-PEG(13)₂-Fmoc"). The synthetic peptide-linker combination may then be covalently coupled to fatty acid.

Where the linker of the synthetic peptide-linker combination has a second reactive functionality protected by a chemical protecting group (so as to not react with synthetic peptide in the process of operatively linking the linker through its first reactive functionality to the synthetic peptide), the chemical protecting group is removed (in a "deprotection" step) to make that reactive functionality available for chemically coupling with a reactive functionality of the fatty acid, in making a conjugate comprised of synthetic peptide conjugated to fatty acid through use of a linker. For example of a deprotection step, the synthetic peptide-linker combination (SEQ ID NO:37-PEG(13)₂-Fmoc; 0.261 g, 30.1 nmol) was dissolved in 1.5 ml DMF, piperidine (0.012 ml, 0.12 mmol) was added, and then the reaction was stirred for 1.5 hours. Analysis of the reaction by HPLC showed 3 peaks, so an additional amount of piperidine (0.010 ml, 0.10 mmol) was added. The reaction was stirred for an additional 1 hour. The HPLC trace was unchanged, so the reaction was poured into 20 ml H₂O. The solids formed were collected and dried. The solids were then suspended in 20 ml 3:1 hexanes:MTBE and stirred for about 5 hours. The solids were filtered off and dried. Then the solids were re-suspended in 20 ml 1:1 H₂O:Ethanol and stirred for 1.5 hours. The solids were collected and dried (0.204 g, 24.2 nmol).

In an example of operatively linking the synthetic peptide-linker combination to a fatty acid the synthetic peptide-linker combination (SEQ ID NO:37-PEG(13)₂-OH; 0.204,

24.2 nmol) was dissolved in 2 ml DMF. In a separate flask, hexadecanedioic acid (C16 diacid, 0.021, 73.3 nmol) was dissolved in 1 ml DMF; DIEA (0.030 ml, 0.17 mmol), HOAT (1-Hydroxy-7-azabenzotriazole; 0.016 g, 0.12 mmol), and TBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyltetrafluoro-borate; 0.023 g, 71.6 nmol) were added, and the acid pre-activated for about 5 minutes. Then the pre-activated acid solution was added to a solution containing the synthetic peptide-linker combination, and the reaction stirred for about 2.5 hours. Analysis by HPLC showed no more starting material (synthetic peptide-linker combination) present, so the conjugate was poured into 20 ml of H₂O. The solids formed were collected and dried (0.206 g, 23.6 nmol, 98% yield). The conjugate may then be globally deprotected. For example, the conjugate (0.377 g, 43.3 nmol) was dissolved in 10 ml degassed 85:5:5:5 TFA:H₂O:DTT:Phenol, and stirred for 4 hours. Then the reaction was transferred to a 125 ml Ehrlenmeyer flask, and 75 ml MTBE was added slowly. The solids formed were collected and dried. The solids were then suspended in 40 ml 25% CH₃CN in H₂O, and dilute NH₄OH was added drop-wise until the peptide dissolved (~pH 8.5). Then the pH was adjusted to about pH 5 with acetic acid, and the white suspension was stirred overnight. Then the solvent was then taken off on a lyophilizer. The conjugate was further purified using HPLC to yield a substantially homogenous conjugate comprising a synthetic peptide (having an amino acid sequence of SEQ ID NO:37) operatively linked to fatty acid (a C16 fatty acid) through a linker, and where the linker is covalently coupled to the epsilon amine of the internal lysine (amino acid in position 30) of SEQ ID NO:37.

Example 3B

In another embodiment, a method for producing a conjugate according to the present invention involves first operatively linking linker to fatty acid in forming a linker-fatty acid combination, followed by operatively linking the linker of a linker-fatty acid combination to a synthetic peptide, in forming a conjugate comprised of synthetic peptide operatively linked to fatty acid. For example, a linker-fatty acid combination is made, such as by using the methods of operatively linking linker to fatty acid, as described above. A synthetic peptide having the amino acid sequence of SEQ ID NO:33 was operatively linked to a linker-fatty acid combination comprising PEG(13)₂ covalently coupled to C18 ("C18-PEG(13)₂-OH"). The C18-PEG(13)₂-OH was operatively linked to the epsilon amine of an internal lysine (in amino acid position 30) of a synthetic peptide having the amino acid sequence of SEQ ID NO:33. The synthetic peptide (0.60 g, 0.13 mmol) was dissolved in 10 ml DMF, and DIEA (0.116 ml, 0.37 mmol) was added. In a separate flask, the C18-PEG(13)₂-OH (0.296 g, 0.20 mmol) was dissolved in 10 ml DMF and 5 ml CH₂Cl₂. Then DIEA (0.232 ml, 1.33 mmol), HOAT (0.038 g, 0.28 mmol), and

TBTU (0.064 g, 0.20 mmol) were added, and the acid pre-activated for about 5 minutes. The activated acid solution containing the linker-fatty acid combination was then added to the solution containing the synthetic peptide, and the reaction was stirred for about 3 hours. Analysis by HPLC showed no more starting synthetic peptide, so the reaction was poured slowly into 125 ml chilled MTBE, and diluted to approximately 200 ml with MTBE. The solids were collected and dried, yielding conjugate comprising synthetic peptide-linker-fatty acid, wherein the linker was covalently coupled to the epsilon amine of an internal amino acid (K30) of the synthetic peptide. The conjugate was deprotected, and further purified to high purity using HPLC.

Example 3C

In another embodiment, fatty acid was operatively linked to synthetic peptide directly by reacting a reactive functionality of the fatty acid with a reactive functionality of the synthetic peptide. Synthetic peptide may be operatively linked to fatty acid directly in at least two different ways. In one embodiment, the synthetic peptide is first synthesized, and then fatty acid is operatively linked directly to the synthetic peptide, in a method of producing a conjugate according to the present invention. In another embodiment, the fatty acid is operatively linked directly to a peptide fragment, and such fragment is then used in the fragment condensation approach to synthesizing the synthetic peptide, in a method of producing a conjugate according to the present invention. This embodiment illustrates (a) operatively linking fatty acid to synthetic peptide in a method of producing a conjugate according to the present invention; and (b) a conjugate according to the present invention, wherein fatty acid is operatively linked to a terminal amino acid of the synthetic peptide. For purposes of illustration, presented are two examples. The first example illustrates operatively linking fatty acid to the C-terminus of the synthetic peptide by operatively linking the fatty acid to a peptide fragment used in the synthesis of synthetic peptide. In this example, the fatty acid was covalently coupled to the amino acid to be incorporated into the peptide fragment as the terminal amino acid. The second example illustrates operatively linking fatty acid to the N-terminus of the synthetic peptide, in forming a conjugate. In this second example, the synthetic peptide was first synthesized, and then fatty acid was operatively linked to the synthetic peptide at the N-terminus.

In this first example, produced is CPG-Leu-C18 to be incorporated as the C-terminal amino acid of a peptide fragment comprising amino acids 21-36 of a synthetic peptide having the amino acid sequence of SEQ ID NO:33 ("AA(21-36)"). CPG-Leu-OH (1.05 g, 3.96 mmol, 1.0 eq) was dissolved in DMF (10 mL, 10 vol) and DCM (20 mL, 20 vol), and HOAT (1.076 g, 7.91 mmol, 2 eq) and DIEA (2.75 mL, 15.8 mmol, 4 eq) were added. The resulting yellow solution was cooled to 0 ± 5 °C, and then TBTU (1.525 g,

4.75 mmol, 1.2 eq) and octadecylamine (C18 fatty acid; 1.274 g, 4.73 mmol, 1.2 eq) were added, and the reaction was stirred at $0 \pm 5^\circ\text{C}$ for 5 minutes. The reaction had thickened, so it was allowed to heat to $25 \pm 5^\circ\text{C}$ and additional DCM (10 mL, 10 vol) was added. The reaction was stirred at $25 \pm 5^\circ\text{C}$ for 2 hours, after which HPLC showed no more starting material present. The dichloromethane was taken off under reduced pressure, and the resulting oily solid was suspended in 100 mL water. The oily solid was collected by filtration and dried over night. The result is CPG-Leu-NH(CH₂)₁₇CH₃ ("CPG-Leu-C18"). A deprotection reaction was then performed to remove the chemical protecting group (CPG). CPG-Leu-C18 (1.002 g, 1.93 mmol, 1 eq) was dissolved in DCM (10 mL, 10 vol), and methanol (MeOH, 10 mL, 10 vol) and Pd/C (palladium on carbon; 0.257 g) was added. The reaction was placed under a hydrogen (H₂) atmosphere and stirred over night. HPLC showed no more starting material, so the reaction was filtered through celite, and the solvent taken off in vacuo, affording H-Leu-C18 as a solid in 94% yield (0.697 g, 1.82 mmol).

To produce peptide fragment Fmoc-AA(21-36)-NHC18 using a peptide fragment Fmoc-AA(21-35)-OH combined with H-Leu-C18 in a solution phase process, the peptide fragment Fmoc-AA(21-37)-OH (2.01 g, 0.508 mmol, 1.0 eq), H-Leu-C18 (0.234 g, 0.611 mmol, 1.2 eq), HOAT (1.04 g, 0.764 mmol, 1.5 eq) and DIEA (0.354 mL, 2.03 mmol, 4 eq) were dissolved in DMF (20 mL, 10 vol) and DCM (5 mL, 2.5 vol), and the reaction was cooled to $0 \pm 5^\circ\text{C}$. TBTU (0.197 g, 0.613 mmol, 1.2 eq) was added, stirred for 5 minutes at $0 \pm 5^\circ\text{C}$, and then allowed to react at $25 \pm 5^\circ\text{C}$ for 2 hours or until the reaction was shown complete by HPLC. The Fmoc chemical protecting group of the peptide fragment Fmoc-AA(21-36)-NHC18 was then removed prior to isolation of the fragment H-AA(21-36)-NHC18. In this deprotection reaction, piperidine (0.301 mL, 3.04 mmol, 6 eq) was added, and the solution was stirred for 1 hour at $25 \pm 5^\circ\text{C}$ or until analysis by HPLC showed that substantially all the Fmoc was removed from the peptide fragment. DCM was taken off in vacuo, and the remaining aqueous solution poured into 50 mL ice water. The gummy solid was isolated by filtration and dried over night. The peptide fragment was then reslurried in 1:1 EtOH/water (20 mL, 10 vol) for 2 hours. The solids were collected and dried. Then the peptide fragment was slurried in 3:1 hexanes:MTBE (20 mL, 10 vol) over night and then isolated by filtration and redried. The result is a substantially pure preparation of peptide fragment having fatty acid operatively linked thereto ("H-AA(21-36)-NHC18").

To produce a conjugate comprised of a synthetic peptide, having an amino acid sequence of SEQ ID NO:33 operatively linked to fatty acid ("Ac-AA(1-36)-NHC18"), peptide fragment H-AA(21-36)-NHC18 (1.501 g, 0.366 mmol, 1 eq), peptide fragment comprising amino acids 1-20 of SEQ ID NO:33 ("Ac-AA(1-20)-OH"; 1.628 g, 0.368 mmol,

1.0 eq), and HOAT (0.075 g, 0.551 mmol, 1.5 eq) and DIEA (0.255 ml, 0.146 mmol, 4 eq) were dissolved in DMF (15 ml, 10 vol), and the reaction was cooled to $0 \pm 5^\circ\text{C}$. Added to the reaction was TBTU (0.141 g, 0.439 mmol, 1.2 eq). The reaction was stirred for 5 minutes at $0 \pm 5^\circ\text{C}$, and then at $25 \pm 5^\circ\text{C}$ for 3 hours or until the reaction was shown to be complete by HPLC. The reactor was cooled, and water (50 mL, 33 vol) was slowly added. The gummy solid was isolated by filtration and washed with additional water. The collected solid dried in a vacuum oven at $35 \pm 5^\circ\text{C}$. The conjugate Ac-AA(1-36)-NHC18 was then deprotected (by removing the side chain chemical protecting groups) and decarboxylated (at the tryptophan residues) by using the methods described herein, or any other method known to those skilled in the art, for deprotection and decarboxylation, with subsequent purification. The result was a substantially pure preparation (deprotected and decarboxylated) of a conjugate comprising a synthetic peptide operatively linked to a fatty acid, wherein the fatty acid is operatively linked to the C-terminus of the synthetic peptide.

The following example illustrates (a) operatively linking fatty acid to synthetic peptide in a method of producing a conjugate according to the present invention; and (b) a conjugate according to the present invention, wherein fatty acid is operatively linked to a terminal amino acid of the synthetic peptide. The previous example illustrated operatively linking fatty acid to the C-terminus of the synthetic peptide, while this example illustrates operatively linking fatty acid to the N-terminus of the synthetic peptide, in forming a conjugate according to the present invention. Briefly, a synthetic peptide having an amino acid sequence of SEQ ID NO:33 was synthesized by a fragment condensation approach using a peptide fragment comprising amino acids 1-20 of SEQ ID NO:33 ("AA(1-20)"), and a peptide fragment comprising amino acids 21-36 of SEQ ID NO:33 ("AA(21-36)"). Fatty acid was then operatively linked to the N-terminus of the synthetic peptide in forming the conjugate.

Fmoc-AA(1-20)-OH (500mg, 1eq), H-AA(21-36)NH₂ (417mg, 1eq), 6-Cl-HOBt (36.5mg, 2eq), DIEA (56.4ul, 3eq) and DMF (10ml, 20vol) were charged into a 50-ml flask. The resulted solution was cooled to 0°C by ice bath. TBTU (45mg, 1.3eq) was added into the solution, and stirred for 1 hour with ice bath, and then 3 hours at room temperature. Piperidine (64ul, 6eq) was added, and the reaction was stirred for 1 hour. The reaction mixture was cooled again by ice bath to 0°C , and pre-cooled water was added in slowly. The precipitated peptide was vacuum filtered, washed by 10% EtOH-water, and dried in the oven overnight. The solid was re-slurried in MTBE twice and dried in the oven again. A synthetic peptide having an amino acid sequence of SEQ ID NO:33 ("H-AA(1-36)-NH₂") was obtained as white solid. H-AA(1-36)-NH₂ (400mg, 1eq), fatty acid (C18-OPFP; 33mg, 1.5eq), and DIEA (42ul, 5eq) were dissolved in DMF (5ml, 10vol)

and stirred for 2 hours at room temperature. The reaction mixture was cooled by ice bath and pre-cooled water was added in slowly. The precipitated conjugate ("C18-AA(1-36)-NH₂") was vacuum filtered, washed by 10% EtOH-water, and dried in the oven overnight. The preparation of conjugate was then deprotected, and purified. C18-AA(1-36)-NH₂ (180mg) was dissolved in 15vol of TFA/DTT/water (90/5/5) and stirred at room temperature for 4.5 hours. Pre-cooled MTBE was added into the reactor slowly to maintain the temperature at less than 10°C. The resulting slurry was stirred at room temperature for 30 minutes before vacuum filtration. After washing with MTBE, the solid was reslurried in acetonitrile with pH adjusted to 4 by AcOH and DIEA overnight. The suspension was diluted by 50mM NH₄OAc and the solution was filtered and directly loaded onto HPLC for purification, followed by lyophilization. The result is a substantially homogeneous (>90% purity by HPLC) conjugate comprised of fatty acid operatively linked to the N-terminus of synthetic peptide having an amino acid sequence of SEQ ID NO:33.

Example 3D

This example illustrates several embodiments of a conjugate according to the present invention: (a) a conjugate having more than one fatty acid operatively linked to synthetic peptide; (b) operatively linking fatty acid, through a linker, to the terminal amino acid of the synthetic peptide; and (c) operatively linking fatty acid, through a linker, to an internal amino acid of the synthetic peptide. In this example, a linker-fatty acid combination was operatively linked to the N-terminus, and a linker-fatty acid combination was operatively linked to lysine in amino acid position 30, of a synthetic peptide having an amino acid sequence of SEQ ID NO:33. However, using the methods described herein, or methods well known in the art, more than one fatty acid can be operatively linked to the same position in the amino acid sequence of the synthetic peptide, in producing a conjugate having more than one fatty acid molecule operatively linked to synthetic peptide. For example, using methods known in the art, and using the teachings herein, operatively linked to an amino acid of the synthetic peptide is a fatty acid comprising: (a) a branched fatty acid operatively linked to a linker, with the linker operatively linked to the synthetic peptide; (b) a branched linker, having operatively linked thereto at each of more than one branch of the linker, a fatty acid; and (c) a branched linker (or linker having multiple reactive functionalities, such as amino acid) having operatively linked thereto, at each of more than one branch of the linker, a linker-fatty acid combination. In one such example, a linker (e.g., comprised of amino acid lysine) was used to link to multiple linker-fatty acid combinations (e.g., PEG3-C18 and PEG3-C18) together, and then the resultant linker-fatty acid combination containing more than one fatty acid molecule was operatively

linked to synthetic peptide (to the epsilon amine of an internal amino acid comprising lysine). See Table 5 (Ref # 33-15) for an illustrative example of a conjugate comprised of more than one fatty acid molecule operatively linked to a single amino acid position of synthetic peptide.

5 To produce a conjugate comprising more than one fatty acid, a synthetic peptide having an amino acid sequence of SEQ ID NO:33 was synthesized by a fragment condensation approach using a peptide fragment comprising amino acids 1-20 of SEQ ID NO:33 ("AA(1-20)") having operatively linked at the N-terminus a linker-fatty acid combination ("C18-PEG3"), and a peptide fragment comprising amino acids 21-36 of
10 SEQ ID NO:33 having a linker-fatty acid combination operatively linked to an internal lysine ("K30" of SEQ ID NO:33) of the peptide fragment ("AA(21-30(PEG3-C18)-36)"). One gram of Fmoc-AA(1-20) was put into peptide reactor, which was temperature-controlled at 30°C with circulating bath. 10 vol of DCM was charged into the reactor, and the reaction was agitated with nitrogen for 15 minutes, and then drained. Fmoc protection
15 group was removed by the treatment of 20% piperidine in NMP ((N-methyl pyrrolidinone; 10 vol) for 20 minutes, twice. NMP was used to wash the resin until a negative chloranil test was obtained. The reactor with resin was charged with pre-activated C18-PEG3-OH/6-Cl-HOBt/TBTU/DIEA (2/2/2/2.3eq) in DMF/DCM(1:1, 8vol) and the mixture was agitated by nitrogen until a negative ninhydrine test was obtained. The resin was washed
20 with NMP (10 volx4) and DCM (10 volx4). The reactor was charged with 1%TFA in DCM (10vol) and the suspension was agitated by nitrogen for 2 minutes at 0°C, then DCM solution was drained into a flask with pyridine (1.26vol relative to TFA). The resin was washed by DCM twice and IPA twice. The solvent in the pooled solution was removed under reduced pressure. The residue was dissolved in EtOH, and precipitated with water.
25 The solid was collected by vacuum filtration, washed by 10% EtOH-water and dried in the vacuum oven overnight. The resulting white solid is a preparation of C18-PEG3-AA(1-20)-OH.

C18-PEG3-AA(1-20)-OH (113mg, 1eq), AA(21-30(PEG3-C18)-36)-NH₂ (100mg, 1eq), 6-Cl-HOBt (8.2mg, 2eq), DIEA (12.5ul, 3eq) and DMF (2ml, 20 vol) were charged
30 into a 25-ml flask. The resulted solution was cooled to 0°C by ice bath. TBTU (10mg, 1.3eq) was added, and solution was stirred for 1 hour with ice bath, and 3 hours at room temperature. The reaction mixture was cooled again by ice bath to 0°C and pre-cooled water was added in slowly. The precipitated peptide was vacuum filtered, washed by 10% EtOH-water, and then dried in the oven overnight. The preparation of conjugate ("C18-
35 PEG3-AA(1-30-(PEG3-C18)-36)-NH₂ ") was then deprotected, and purified. C18-PEG3-AA(1-30-(PEG3-C18)-36)-NH₂ (180mg) was dissolved in 15 vol of TFA/DTT/water (90/5/5) and stirred at room temperature for 4.5 hours. Pre-cooled MTBE was added into

the reactor slowly to maintain the temperature at less than 10°C. The resulting slurry was stirred at room temperature for 30 minutes before vacuum filtration. After being washed with MTBE, the solid was reslurried in acetonitrile with the pH adjusted to 4 by AcOH, and remained in DIEA overnight. The suspension was diluted by 50mM NH₄OAc and the solution was filtered and directly loaded onto an HPLC for purification. The result is a substantially homogeneous conjugate comprised of fatty acid operatively linked (through a linker) to the N-terminus, and fatty acid operatively linked (through a linker) to an internal amino acid (lysine in amino acid position 30), of a synthetic peptide having an amino acid sequence of SEQ ID NO:33.

Example 3E

This example illustrates a conjugate comprising a synthetic peptide operatively linked to fatty acid, through a linker, to an internal amino acid of the synthetic peptide. In this example, a linker-fatty acid combination was operatively linked to lysine in amino acid position 30, of a synthetic peptide having an amino acid sequence of SEQ ID NO:33. The fragments Ac-AA(1-20)-OH (amino acids 1-20 of SEQ ID NO:33) and Fmoc-AA(21-35)-OH (amino acids 21-35 of SEQ ID NO:33) were synthesized by standard solid phase peptide synthesis, and then cleaved from the resin with dilute TFA in DCM. A 25 L reactor was charged with DMF (10 vol, 16 L) followed by Fmoc-AA(21-35)-OH (1600 g, 404.2 mmol), H-Leu-NH₂ (free base, 63.15 g, 1.2 eq, 485.0 mmol), 6 Cl-HOBt (82.26 g, 1.2 eq, 485.0 mmol), and DIEA (105.6 mL, 1.5 eq, 606.3 mmol). The mixture was stirred to dissolve all solids and then cooled to 0-5°C. TBTU (155.7 g, 1.2 eq, 485.0 mmol) was added and the reaction was stirred at 0-5°C for 10 minutes. The solution was heated to 25±5°C and stirred for 30 minutes, and the reaction was complete as determined by HPLC analysis of a sample of that reaction. Piperidine (160 mL, 4.0 eq, 1.62 mol) was added, and the reaction was stirred at 25±5°C for 30 minutes. The deprotection was monitored by HPLC, and deemed completed after 2 hours. The solution was cooled to 0-5°C, and precooled water (≤5°C, 10 vol) was added slowly over 20 minutes while maintaining a temperature below 10°C. The suspension was stirred at ≤10°C for 30 minutes, and then filtered over 40 minutes. The solid was washed with 50% EtOH/H₂O (2 × 4 vol) and dried. The damp solid was reslurried in 50% EtOH/H₂O (10 vol) at 20±5°C for 3 hours, filtered, washed with 50% EtOH/H₂O (5 vol), and dried. The damp solids were reslurried in MTBE:Heptane, 2:1 (8 vol) at 20±5 °C for over 4 hours. The solids were collected by filtration, washed with MTBE:Heptane, 2:1 (5 vol) , and dried to yield H-AA(21-36)-NH₂.

To produce a synthetic peptide having the amino acid sequence of SEQ ID NO:33, a 25 L reactor was charged with DMF (20 vol, 16.4 L) followed by Ac-AA(1-20)-

OH (820 g, 185 mmol), H-AA(21-36)-NH₂ (719 g, 1.01 eq, 187 mmol), 6 Cl-HOBt (40.8 g, 1.3 eq, 240.5 mmol), and DIEA (74.1 mL, 2.3 eq, 425.5 mmol). The mixture was stirred to dissolve all solids and then cooled to 0-5°C. TBTU (77.2 g, 1.3 eq, 240.5 mmol) was added and the reaction was stirred at 0-5°C for 15 minutes. The solution was heated to 25±5°C and stirred for 30 minutes. HPLC analysis indicated that the reaction was complete after 70 minutes. The solution was cooled to 0-5°C and precooled water (≤5°C, 15 vol) was added slowly over 30 minutes while maintaining a temperature below 10°C. The suspension was stirred at ≤10°C for 1 hour, and then filtered over 50 minutes. The solid was washed with H₂O (2 × 10 vol) and dried to yield Ac-AA(1-36)-NH₂ (a synthetic peptide having the amino acid sequence of SEQ ID NO:33).

In a deprotection reaction, a solution of TFA (90%, 6.3 L), H₂O (5%, 350 mL), and DTT (5%, 350 g) was prepared in a 25 L reactor and cooled to 0-10°C. Ac-AA(1-36)-NH₂ (700 g) was added over 13 minutes while maintaining a temperature below 5°C. The mixture was heated to 20±2°C and the resulting solution was stirred for 5 hours. During this time MTBE (25 vol, 17.5 L) was cooled to -5±5°C. The reaction solution was cooled to 0±5°C, and the MTBE was added so that the temperature remained below 10°C. The slurry was heated to 10-15°C and stirred for 30 minutes. The solids were collected by filtration and washed with MTBE (3 × 5 vol). The product was dried on the filter overnight and then returned to the reactor which had previously been charged with ACN (10 vol), HOAc (0.1 vol) and DIEA (0.1 vol). The pH was in the desired range (4-5) so no adjustment was required. The slurry was warmed to 25±5°C and stirred until HPLC showed no change in the product profile. The solids were isolated by filtration, washed with ACN (3 times, 5 vol) and dried, followed by purification (by HPLC). The result was a substantially pure preparation of synthetic peptide having the amino acid sequence of SEQ ID NO:33, as determined by HPLC analysis for purity.

A linker-fatty acid combination comprising C18-PEG3-OPFP (PFP is pentafluorophenol) was produced as follows. C18-PEG3-OH (25.08g, 1eq) and pentafluorophenol (PFP, 10.74g, 1eq) were mixed in DCM (500ml). The resulting suspension was cooled to 0°C, then 1,3-diisopropylcarbodiimide (DIC, 9.14ml, 1eq) was added. The reaction mixture was stirred at 0°C for 30 minutes, and then for another 5 hours at room temperature, or until the reaction was shown to be completed. DCM was partially removed by evaporation under reduced pressure. The precipitate was removed by vacuum filtration and residual DCM was further removed by evaporation under reduced pressure. The solid was dried under high vacuum at room temperature overnight or until a constant weight. Following purification, a purified preparation of C18-PEG3-OPFP was obtained as white solid.

To produce a conjugate, the synthetic peptide having the amino acid sequence of SEQ ID NO:33 (77.73 g, 17.27 mmol, 1 eq) was dissolved in DMF (777 mL, 10 vol) with DIEA (30.1 mL, 10 eq, 172.7 mmol) to a clear yellow solution. C18-PEG3-OPFP (12.34 g, 1.2 eq, 20.7 mmol) was added and the solution was stirred for 3 hours at 20-25°C. The reaction was monitored by HPLC until it was determined that the reaction was complete. The reaction solution was added to MTBE (50 vol, 3.89 L) to precipitate the product, and the resulting suspension was stirred for 1 hour at 20-25°C. The solids were isolated by filtration, and washed with MTBE (25 vol, 1.94 L, followed by an additional 1 L MTBE). The solids were dried until they could easily be returned to the reaction vessel where they were reslurried in DCM (10 vol, 777 mL) for 2 hours at 20-25°C. The solids were again collected by filtration, washed with DCM (5 vol, 388 mL), and dried to yield a substantially homogeneous (92.7% purity) conjugate comprised of a synthetic peptide operatively linked to fatty acid through a linker, wherein the synthetic peptide has the amino acid sequence of SEQ ID NO:33, wherein operatively linked to an internal amino acid (lysine at amino acid position 30) of the synthetic peptide is a linker-fatty acid combination, and wherein the linker-fatty acid combination is PEG3-C18.

EXAMPLE 4

As described in more detail herein, a conjugate comprised of synthetic peptide operatively linked to fatty acid according to the present invention was compared, for biological activity (i.e., antiviral potency against HIV-1) and pharmacokinetic properties, to a corresponding synthetic peptide alone. For determining biological activity, an HIV-1 infection assay was used to determine the respective antiviral potencies for the comparison. In using an *in vitro* assay for demonstrating antiviral potency, it is important to note that antiviral effect of synthetic peptide demonstrated in the *in vitro* assay has been correlated with the antiviral effect of the synthetic peptide *in vivo*. In determining antiviral activity (e.g., one measure being the ability to inhibit transmission of HIV to a target cell) of the conjugates produced according to the present invention, used is an *in vitro* assay which has been shown, by data generated using synthetic peptides derived from either of the HR regions of HIV gp41, to be predictive of antiviral activity observed *in vivo*. More particularly, antiviral activity observed using an *in vitro* infectivity assay ("Magi-CCR5 infectivity assay"; see, e.g., U.S. Patent No. 6,258,782) has been shown to reasonably correlate to antiviral activity observed *in vivo* for the same HIV gp41-derived peptides. To further emphasize this point, T20 (SEQ ID NO:2) and T1249 (SEQ. ID NO:129) each have demonstrated potent antiviral activity against HIV in both the *in vitro* infectivity assay and human clinical trials.

The infectivity assays score for reduction of infectious virus titer employing the

indicator cell lines MAGI or the CCR5 expressing derivative cMAGI. Both cell lines exploit the ability of HIV-1 tat to transactivate the expression of a β -galactosidase reporter gene driven by the HIV-LTR. The β -gal reporter has been modified to localize in the nucleus and can be detected with the X-gal substrate as intense nuclear staining within a few days of infection. The number of stained nuclei can thus be interpreted as equal to the number of infectious virions in the challenge inoculum if there is only one round of infection prior to staining. Infected cells are enumerated using a CCD-imager and both primary and laboratory adapted isolates show a linear relationship between virus input and the number of infected cells visualized by the imager. In the MAGI and cMAGI assays, a 50% reduction in infectious titer ($V_n/V_o = 0.5$) is significant, and provides the primary cutoff value for assessing antiviral activity ("IC₅₀" is defined as the dilution resulting in a 50% reduction in infectious virus titer).

The conjugates and combinations tested for antiviral activity were diluted into various concentrations, and tested in duplicate or triplicate against an HIV inoculum adjusted to yield approximately 1500-2000 infected cells/well of a 48 well microtiter plate. The conjugate (in the respective dilution) was added to the cMAGI or MAGI cells, followed by the virus inocula; and 24 hours later, an inhibitor of infection and cell-cell fusion (e.g., T20) is added to prevent secondary rounds of HIV infection and cell-cell virus spread. HIV gp41-derived peptides have been found to bind to human serum albumin ("HSA"). In testing and comparing *in vitro* antiviral activity of a conjugate according to the invention and HIV gp41-derived peptides alone, HSA was included in the assay so as to more mimic the conditions that the synthetic peptide and conjugate would encounter *in vivo*. Thus, the assay was performed in the absence of human serum or HSA, and also was performed in the presence of HSA (45 mg/ml of HSA fraction V containing fatty acids). The cells were cultured for 2 more days, and then fixed and stained with the X-gal substrate to detect HIV-infected cells. The number of infected cells for each control and substantially homogeneous conjugate dilution was determined with the CCD-imager, and then the IC₅₀ is calculated (typically expressed in μ g/ml). The HIV strains used in the antiviral assays were HIV_{IIIB} (Tables 3-5, "IIIB"), and an HIV isolate which has demonstrated resistance to the antiviral activity of HIV gp41-derived peptides, such as those having an amino acid sequence of any one of SEQ ID NOs:2-4. This resistant isolate (Tables 4 & 5, "RI"), representative of resistant isolates that may be demonstrated in HIV-infected individuals treated with T20 (SEQ ID NO:2), was included in the antiviral assays to determine what, if any effect, operatively linking fatty acid to synthetic peptide would have relative to potency against resistant isolates. For comparison purposes, the compositions were placed into one of five categories, depending on their calculated IC₅₀.

"Category A" is used herein to designate compositions with a calculated IC₅₀ value less than or equal to 0.005 µg/ml. "Category B" is used herein to designate compositions with a calculated IC₅₀ value less than or equal to 0.10 µg/ml, but greater than 0.005 µg/ml. "Category C" is used herein to designate compositions with a calculated IC₅₀ value less than or equal to 1.0 µg/ml, but greater than 0.10 µg/ml. "Category D" is used herein to designate compositions with a calculated IC₅₀ value greater than 1.0 µg/ml but less than or equal to 10 µg/ml. "Category E" is used herein to designate compositions with a calculated IC₅₀ value greater than 10 µg/ml.

For determining pharmacokinetic activity, synthetic peptides or conjugates comprising synthetic peptide operatively linked to fatty acid were dosed intravenously in cynomolgus monkeys (*Macaca fascicularis*). At various times post-dose, blood samples were drawn and plasma isolated by centrifugation. Plasma samples were stored frozen until analysis by LC-MS (liquid chromatography/mass spectrometry) in the electrospray, positive-ion mode. Synthetic peptides or conjugates were eluted from a reversed-phase HPLC column with a gradient of acetonitrile in a buffer of 10 mM ammonium acetate, pH 6.8. At the time of analysis, plasma samples were deproteinated with either two or three volumes of acetonitrile containing 0.5% formic acid. Calibration standards in cynomolgus plasma samples were prepared at the same time as the samples and analyzed before and after the samples containing either synthetic peptide or conjugate comprising synthetic peptide and fatty acid. Pharmacokinetic properties were calculated from the plasma concentration-time data using either mono-exponential or bi-exponential or non-compartmental mathematical models. Models were derived by non-linear least squares optimization.

To illustrate the present invention, and using the methodologies described herein, various conjugates were produced. As shown in Tables 3, 4, and 5, these conjugates and combinations (identified by reference number, "Ref #") varied by the type of fatty acid used ("FA"; see also Table 1), type of linker compound used ("Linker"), and to what amino acid (e.g., one or more of internal lysine, N-terminal amino acid, C-terminal amino acid, epsilon amino acid of C-terminal lysine) of the synthetic peptide was operatively linked linker and/or fatty acid ("Linkage"). Lysine is represented by the one letter code: "K". As shown in Tables 3, and 5, compared was antiviral activity in the absence of HSA (IC₅₀), antiviral activity in the presence of HSA with fatty acids (IC₅₀ + HSA FrV), and pharmacokinetic properties of total body clearance (CL), and terminal elimination half-life (t_{1/2}).

Illustrated in Table 3 are various conjugates produced using a synthetic peptide having an amino acid sequence of any one of SEQ ID NO:2, SEQ ID N:129, SEQ ID NO:37, and SEQ ID N:124.

Table 3

Ref. #	FA	Linker	Linkage	IC50 IIIB	IC50 IIIB+ HSA FrV	CL L/K/h	t1/2 (hr)
SEQ ID NO:2	None	None	None	A	B	0.04	1.6
2-1	C18	PEG3	K21	B	B	0.002	24
SEQ ID NO:129	None	None	None	A	B	0.012	5.9
129-1	C18	PEG3	K21	B	B	0.002	17.1
SEQ ID NO:37	None	None	None	B	B	0.045	5.3
37-1	C16	PEG13	K30	B	B	0.006	28
37-2	C16	PEG3	K30	B	B	0.005	22
37-3	C16	(PEG3) ₂	K30	B	B	0.007	10
37-4	C8	(PEG13) ₂	K30	B	B	0.20	0.4
37-5	C16	(PEG3) ₉	K30	B	A	0.008	6.3
37-6	C18	(PEG3) ₉	K30	B	A	0.006	8.4
37-7	C18	PEG3	K30	B	B	0.005	10.2
37-8	C18	(PEG3) ₂	K30	B	B	0.006	10.7
37-9	C22	(PEG13) ₂	K30	B	A	0.004	9.6
37-10	C18	PEG13	K30	B	B	0.008	8.2
37-11	C12	(PEG13) ₂	K30	B	A	0.015	2.7
37-12	C16	(PEG13) ₂	K30	A	A	0.007	8.9
37-13	C20	(PEG13) ₂	K30	A	A	0.007	18.3
37-14	C18	(PEG13) ₂	K30	A	B	0.006	13.2
37-15	C16 diacid	PEG13	K30	B	C	0.005	16.6
37-16	C16	(PEG3) ₂	K30	B	B	0.006	10
37-17	C16 diacid	(PEG13) ₂	K30	B	B	0.005	8.1
37-18	C16 diacid	(GGS) ₃	K30	B	C	0.004	20.4
37-19	C16 diacid	(PEG3) ₄	K30	B	C	0.006	11.3
37-20	C16 diacid	(PEG3) ₅	K30	B	C	0.004	8.5
37-21	C16 diacid	GGS	K30	C	C	0.005	19.2
37-22	C16	None	K30	C	C	0.004	10.3
37-23	C18	None	K30	C	C	0.003	13.5
37-24	C16 diacid	None	K30	B	C	0.005	18.2
37-25	Acetyl	(PEG13) ₂	K30	B	B	0.082	1.3
37-26	C18	(PEG13) ₂	N-terminus	B	A	0.008	4.9
37-27	C16 diacid	(PEG3) ₂	N-terminus	B	C	0.005	14.5
37-28	C16 diacid	(PEG13) ₂	N-terminus	B	B	0.01	9.0
37-29	C16 diacid	(PEG3) ₄	N-terminus	C	C	0.007	19.4
37-30	C16 diacid	(PEG3) ₅	N-terminus	C	C	0.008	15.7
37-31	C16 diacid	GGS	N-terminus	B	C	0.004	23.1
37-32	C12 diacid	None	N-terminus	B	B	0.01	15.0
37-33	C12	None	N-terminus	B	B	0.003	13.7
37-34	C16 diacid	None	N-terminus	B	B	0.01	15.7
37-35	C12	None	C-terminus	C	C	0.006	15.7
37-36	C12amide	None	C-terminus	B	B	0.014	12.0
SEQ ID NO:124	Acetyl	None	K39	B	B	0.058	5.7
124-1	C18	(PEG13) ₂	K39	A	B	0.007	7.1

124-2	C16 diacid	GGG	K39	B	C	0.004	21.2
124-3	C16 diacid	(PEG13) ₂	K39	B	C	0.008	11.2
124-4	C16 diacid	(PEG3) ₂	K39	B	B	0.01	9.3
124-5	C16	None	K39	B	C	0.005	18
124-6	None	(PEG13) ₂	K39	B	B	0.02	13

With respect to the results shown in Table 3, it is important to point out that antiviral activity encompassed by Category A is the most desirable biological activity for using a synthetic peptide, or conjugate according to the invention, for use as a pharmaceutical composition to inhibit HIV infection. Thus, achieving Category A antiviral activity is a significant advantage of the synthetic peptide, or conjugate comprising synthetic peptide. In summarizing the results shown in Table 3, it is quite unexpected that many of the conjugates comprised of synthetic peptide-linker-fatty acid according to the present invention retained substantially the same antiviral activity as compared to synthetic peptide alone (i.e., had antiviral activity represented by Category B), in addition to showing improvement in one or more pharmacokinetic properties of total body clearance (CL), and terminal elimination half-life ($t_{1/2}$). See, for example, Table 3, Ref #s 37-1, 37-2, 37-3, 37-7, 37-8, 37-10, 37-16, 37-17, 37-32, 37-33, 37-34, and 37-36.

Unexpected and surprisingly, in many cases the conjugate showed improved antiviral activity (Category A) as compared to synthetic peptide alone. For example, several conjugates according to the present invention (Table 3, Ref. #s 37-5, 37-6, 37-9, 37-11, 37-12, 37-13, 37-14, 37-26, and 124-1) showed better antiviral activity (Category A, in either or both of IIB or IIB in presence of HSA) than the antiviral activity (Category B) of the synthetic peptide alone, in addition to showing significant improvement in one or more pharmacokinetic properties of total body clearance (CL), and terminal elimination half-life ($t_{1/2}$). For example, a conjugate comprised of synthetic peptide operatively linked to fatty acid showed the unexpected improvement in antiviral activity, and significant improvements in total body clearance (e.g., as much as a 30 fold decrease) and terminal elimination half life (up to about a seven fold increase). Referring to Table 3, it is interesting to note that the conjugates showing the unexpected improvement in antiviral activity, as compared to synthetic peptide alone, varied in fatty acid content (e.g., C12, C16, C18, C20, C22), and linkers (e.g., (PEG13)₂, (PEG3)₉), and linkage (internal lysine (K30), C-terminal lysine (K39), and N-terminal amino acid).

A representative sample of conjugates illustrated in Table 3 were also assessed for antiviral potency against a representative resistant isolate ("RI"), as described above. The results are shown in Table 4.

Table 4

Ref. #	FA	Linker	Linkage	IC50 IIIB	IC50 R1
SEQ ID NO:2	None	None	None	A	E
SEQ ID NO:129	None	None	None	A	D
SEQ ID NO:37	None	None	None	B	B
37-6	C18	(PEG3) ₉	K30	B	A
37-13	C20	(PEG13) ₂	K30	A	A
37-14	C18	(PEG13) ₂	K30	A	A

As shown in Table 4, a conjugate according to the present invention shows unexpected improvement in antiviral activity against a representative resistant isolate, as compared to synthetic peptide alone (SEQ ID NO:37). The conjugates demonstrated a 7 to 20-fold increase in antiviral potency against the resistant isolate than the activity of the synthetic peptide alone. It was surprising and unexpected to observe that the conjugates, as compared to HIV gp41-derived peptides alone, typically demonstrate one or more improved pharmaceutical activities while retaining substantial biological activity.

In another illustration of the present invention, and using the methodologies described herein, various conjugates and combinations were produced based on a synthetic peptide having the amino acid sequence of:

LTWQEWDREINNYTSLIHSLIEESQNQQEKNEQELL (SEQ ID NO:33).

A conjugate comprised of a synthetic peptide having the amino acid sequence of SEQ ID NO:33 operatively linked to fatty acid is a preferred conjugate according to the present invention; and may be represented by the following formula, with B being operatively linked to the N-terminus, Z being operatively linked to the C-terminus, and J being operatively linked to an internal amino acid, lysine,

B-LTWQEWDREINNYTSLIHSLIEESQNQQEKNEQELL-Z

I
J

wherein B, Z, and J may each be a member selected from the group consisting of a fatty acid, a linker- fatty acid combination, and a chemical group. A fatty acid may comprise one or more fatty acid molecules. A linker-fatty acid combination may comprise one or more linkers and one or more fatty acid molecules. A chemical group may comprise, but is not limited to, one or more of: a reactive functionality, or a chemical protecting group. As shown herein (see, e.g., Table 3, conjugate with Ref # 33-13), an internal amino acid other than at position 30 of SEQ ID NO:33 may be substituted and/or operatively linked to fatty acid. Techniques useful for introducing a chemical group at the N-terminus of a peptide fragment, or the C-terminus of a peptide fragment, or an internal amino acid, or a combination thereof, are well known in the art; some of which are described in detail herein.

Table 5 shows a comparison of various conjugates and synthetic peptide alone. Compared is antiviral activity in the absence of HSA (IC₅₀), antiviral activity in the presence of HSA with fatty acids (IC₅₀ + HSA FrV), and pharmacokinetic properties of total body clearance (CL), and terminal elimination half-life (t_{1/2}).

5

Table 5

Ref#	FA	Linker	Linkage	IC ₅₀ IIIB	IC ₅₀ + HSA FrV	IC ₅₀ R1	CL L/K/h	t _{1/2} (hr)
SEQ ID NO:33	None	None	none	B	B	E	0.30	0.6
33-1	C18	PEG25	K30	A	A		0.01	6.3
33-2	C18	PEG3	K30	A	A	C	0.007	8.9
33-3	C18	PEG13	K30	B	B		0.01	7.7
33-4	C18	(PEG13) ₂	K30	A	A	C	0.009	7.28
33-5	C18	None	K30	B	B		0.005	10.8
33-6	C12 diacid	None	N-terminus	B	C		0.004	21.2
33-7	Acetyl	(PEG13) ₂	K30	B	B		0.29	1.0
33-8	C12	PEG3	K30	A	B	C	0.02	5.4
33-9	C16	PEG3	K30	A	B	C	0.004	15.4
33-10	C20	PEG3	K30	A	A	B		
33-11	C18	None	C-terminus	A	A	C		
33-12	C18	PEG3	C-terminus	A	A	B	0.004	10.6
33-13	C18	PEG3	K29*	B	B	C	0.004	7.0
33-14	2- C18**	2-PEG3**	K30	A	A	B	0.001	≥25
33-15	C18	PEG3	on each of K30, & N- terminus	B	B	D		
33-16	C18	None	N-terminus	B	B	D	0.001	31.0
33-17	C18	PEG3	N-terminus	B	B	D	0.001	27.1
33-18 ***	C18	PEG3	K30	A	B		0.007	3.9
33-19 ****	C18	PEG3	K30	A	B		0.001	27.8

*- Glu, in amino acid position 29 of SEQ ID NO:33, was substituted by a Lys.

10 **- A PEG3-C18 was operatively linked to another PEG3-C18 by a linker, all of which is operatively linked to K30 in a synthetic peptide having an amino acid sequence of SEQ ID NO:33.

***- Many of the conjugates illustrated herein, are acetylated at the N-terminus, and amidated at the C-terminus. 33-18 is a conjugate with an amine at the N-terminus and amide at the C-terminus.

15 ****- Similarly, 33-19 has a free amine at the N-terminus; however, it is not amidated at the C-terminus.

With respect to the results shown in Table 5, and as previously summarized herein, antiviral activity encompassed by Category A is the most desirable biological activity with respect to use as a pharmaceutical composition to inhibit HIV infection. In summarizing the results shown in Table 5, it is quite unexpected and surprising that all of these conjugates not only retained at least substantially the same antiviral activity as compared to synthetic peptide alone (Category B), but most showed improved antiviral activity (Category A) (increased antiviral potency), even in the presence of HSA. For example, several conjugates according to the present invention (Table 5, Ref. #s 33-1, 33-2, 33-4, 33-8, 33-9, 33-10, 33-11, 33-12, 33-14, 33-18, 33-19) showed better antiviral activity (Category A) than the antiviral activity (Category B) of the synthetic peptide alone, in addition to showing significant improvement in both total body clearance (CL), and terminal elimination half-life ($t_{1/2}$) as compared to synthetic peptide alone.

Referring to Table 5, it is interesting to note that the conjugates showing the unexpected improvement increasing antiviral potency, as compared to synthetic peptide alone, varied in linkers (e.g., (PEG13)₂, PEG3, and PEG25). Further, the unexpected improvement in antiviral activity and improvement in pharmacokinetic properties were not observed for a synthetic peptide having a linker which is acetylated rather than being coupled to a fatty acid (see, Table 5, Ref. # 33-7). Thus, only a conjugate comprised of synthetic peptide operatively linked to fatty acid showed the unexpected improvement in antiviral activity, and significant improvements in one or more pharmacokinetic properties (e.g., total body clearance (e.g., a log decrease or more) and terminal elimination half life (greater than a ten fold increase). Also shown in Table 5 (by several representative examples; e.g., Ref. #s 33-2, 33-4, 33-8, 33-9, 33-10, 33-11, 33-12, 33-13, 33-14, 33-15, 33-16, and 33-17), a conjugate according to the present invention shows unexpected improvement in antiviral activity against a representative resistant isolate, as compared to synthetic peptide alone. The conjugates demonstrated a 20 to 100-fold increase in antiviral potency against the representative resistant isolate as compared to the antiviral activity of the synthetic peptide alone.

A preferred conjugate, as an antiviral composition, according to the present invention is represented by the sequence of:

B-LTWQEWDREINNYTSLIHSLIEESQNQQEKNEQELL-Z

I
J

wherein B and Z each comprise a chemical group, and J is a linker-fatty acid combination comprising PEG3-C18 which is operatively linked (via a reactive functionality of the PEG3 linker) to a synthetic peptide having the amino acid sequence of SEQ ID NO:33 via the lysine at amino acid position 30 of SEQ ID NO:33 (See also, Table 5, Ref#

33-2). When the antiviral activity of this preferred conjugate was compared with the antiviral activity of synthetic peptide alone (SEQ ID NO:33) against a panel of several clinical isolates of HIV-1, this preferred conjugate consistently demonstrated an increased antiviral potency, typically ranging from 2 fold more potent (e.g., 2 fold decrease in IC₅₀ value) to 50 fold more potent. Additionally, this preferred conjugate was compared to synthetic peptide alone (SEQ ID NO:33) for activity against a representative HIV-2 strain (HIV2_{NIH2}). This preferred conjugate showed significant antiviral activity against HIV-2 (Category B) as compared to the synthetic peptide alone (Category C), and further demonstrated about a 10 fold increase in antiviral potency against HIV-2. It was surprising and unexpected to observe that the conjugates, as compared to HIV gp41-derived peptides alone, typically demonstrate one or more improved pharmaceutical activities while retaining substantial biological activity.

EXAMPLE 5

The present invention provides for conjugates, comprised of an HIV gp41-derived peptide operatively linked to fatty acid. Antiviral activity of such conjugates can be utilized in a method for inhibiting transmission of HIV to a target cell, comprising adding to the virus and target cell an amount of conjugate according to the present invention effective to inhibit infection of the cell by HIV, and more preferably, to inhibit HIV-mediated fusion between the virus and the target cell. This method may be used to treat HIV-infected individuals (therapeutically) or to treat individuals newly exposed to or at high risk of exposure (e.g., through drug usage or high risk sexual behavior) to HIV (prophylactically). Thus, for example, in the case of an HIV-1 infected individual, an effective amount of conjugate would be a dose sufficient (by itself and/or in conjunction with a regimen of doses) to reduce HIV viral load in the individual being treated. As known to those skilled in the art, there are several standard methods for measuring HIV viral load which include, but are not limited to, one or more of quantitative cultures of peripheral blood mononuclear cells, and plasma HIV RNA measurements. The conjugates of the invention can be administered in a single administration, intermittently, periodically, or continuously, as can be determined by a medical practitioner, such as by monitoring viral load.

Depending on the formulation containing the conjugate, and such factors as the compositions of the fatty acid and synthetic peptide used in producing the conjugate, and whether or not further comprising a pharmaceutically acceptable carrier and the nature of the pharmaceutically acceptable carrier, the conjugate according to the present invention may be administered with a periodicity ranging from days to weeks, or possibly a longer time interval. Further, a conjugate according to the present invention may be used, in

antiviral therapy as an antiviral composition or a medicament, when used in combination or in a therapeutic regimen (e.g., when used simultaneously, or in a cycling on with one drug and cycling off with another) with other antiviral agents used for treatment of HIV.

For example, in one preferred embodiment, one or more antiviral agents may be combined in therapy with conjugate according to the present invention, thus increasing the efficacy of the therapy, and lessening the ability of the virus to become resistant to the antiviral drugs. Such combinations may be prepared from effective amounts of antiviral agents (useful in treating of HIV infection) currently approved or approved in the future, but are not limited to, one or more additional therapeutic agents selected from the following: reverse transcriptase inhibitor, including but not limited to, abacavir, AZT (zidovudine), ddC (zalcitabine), nevirapine, ddI (didanosine), FTC (emtricitabine), (+) and (-) FTC, reverset, 3TC (lamivudine), GS 840, GW-1592, GW-8248, GW-5634, HBY097, delaviridine, efavirenz, d4T (stavudine), FLT, TMC125, adefovir, tenofovir, and alovudine; protease inhibitor, including but not limited to, amprenivir, CGP-73547, CGP-61755, DMP-450, indinavir, nelfinavir, PNU-140690, ritonavir, saquinavir, telinavir, tipranavir, atazanavir, lopinavir; viral entry inhibitor, including but not limited to, fusion inhibitor (enfuvirtide, other fusion inhibitor peptides, and small molecules), chemokine receptor antagonist (e.g., CCR5 antagonist, such as ONO-4128, GW-873140, AMD-887, CMPD-167; CXCR4 antagonist, such as AMD-070), an agent which affects viral binding interactions (e.g., affects gp120 and CD4 receptor interactions, such as BMS806, BMS-488043; and/or PRO 542, PRO140; or lipid and/or cholesterol interactions, such as procaine hydrochloride (SP-01 and SP-01A)); integrase inhibitor, including but not limited to, L-870, and 810; RNaseH inhibitor; inhibitor of rev or REV; inhibitor of vif (e.g., vif-derived proline-enriched peptide, HIV-1 protease N-terminal-derived peptide); viral processing inhibitor, including but not limited to betulin, and dihydrobetulin derivatives (e.g., PA-457); and immunomodulator, including but not limited to, AS-101, granulocyte macrophage colony stimulating factor, IL-2, valproic acid, and thymopentin. Effective dosages of these illustrative antiviral agents, which may be used in combinations with conjugate according to the present invention, are known in the art. Such combinations may include a number of antiviral agents that can be administered by one or more routes, sequentially or simultaneously, depending on the route of administration and desired pharmacological effect, as is apparent to one skilled in the art.

Effective dosages of a conjugate of the invention for administration to an HIV-infected individual may be determined through procedures well known to those in the art; e.g., by determining potency, biological half-life, bioavailability, and toxicity. In a preferred embodiment, an effective conjugate dosage range is determined by one skilled in the art using data from routine *in vitro* and *in vivo* studies well known to those skilled in the art.

For example, *in vitro* infectivity assays of antiviral activity, such as described herein, enables one skilled in the art to determine the mean inhibitory concentration (IC) of the synthetic peptide-linker-fatty acid conjugate necessary to block some amount of viral infectivity (e.g., 50% inhibition, IC₅₀; or 90% inhibition, IC₉₀). Appropriate doses can then
5 be selected by one skilled in the art using pharmacokinetic data from one or more standard animal models, so that a minimum plasma concentration (C_{min}) of the conjugate is obtained which is equal to or exceeds a predetermined IC value. While dosage ranges typically depend on the route of administration chosen and the formulation of the dosage, an exemplary dosage range of the conjugate according to the present
10 invention (daily or periodic dosing) may range from no less than 0.1 µg/kg body weight and no more than 10 mg/kg body weight; preferably a dosage range of from about 10µg/kg body weight to 1.4 mg/kg body weight; and more preferably, a dosage of between from about 10 mg to about 250 mg of conjugate.

A conjugate of the present invention may be administered to an individual by any
15 means that enables the active agent to reach the target cells (cells that can be infected by HIV). Thus, the conjugates of this invention may be administered by any suitable technique, including oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, or subcutaneous injection or infusion, intradermal, or implant), nasal, pulmonary, vaginal, rectal, sublingual, or topical routes of administration, and can be formulated in dosage
20 forms appropriate for each route of administration. The specific route of administration will depend, e.g., on the medical history of the individual, including any perceived or anticipated side effects from such administration, and the formulation of conjugate being administered (e.g., the nature of the fatty acid, linker, and synthetic peptide of which the conjugate comprises). Most preferably, the administration is by injection (using, e.g.,
25 intravenous or subcutaneous means), but could also be by continuous infusion (using, e.g., slow-release devices or minipumps such as osmotic pumps, and the like). A conjugate according to the present invention may further comprise a pharmaceutically acceptable carrier; and may further depend on the formulation desired, site of delivery, the method of administration, the scheduling of administration, and other factors known to
30 medical practitioners. Such carriers may include, but are not limited to, gels, implants, and slow-release complexes, as known to those skilled in the art.

One embodiment of a pharmaceutical composition according to the present invention comprises a conjugate comprised of fatty acid operatively linked to synthetic peptide, and a pharmaceutically acceptable carrier comprising a polymer. In this
35 embodiment, the polymer is a component of the pharmaceutically acceptable carrier added to produce the pharmaceutical composition, and is not covalently coupled to the

conjugate. The pharmaceutical composition may be in solid (e.g., powder or cake form), or the pharmaceutically acceptable carrier may comprise one or more additional components so that the pharmaceutical composition is a solution, and more preferably an aqueous solution. For example, one preferred additional component is an aqueous alcohol that is in a concentration (v/v) in a range of concentrations of from about 1% to about 15%. In one embodiment of the pharmaceutical composition according to the present invention, the conjugate is in a concentration in the pharmaceutical composition of no less than 200 mg/ml, and no more than 750 mg/ml; and the polymer is in a concentration of no less than 10 weight percent and no more than 75 weight percent of the pharmaceutical composition. In another embodiment of the pharmaceutical composition according to the present invention, the conjugate is in a concentration of no less than 30 weight percent, and no more than 70 weight percent, and the polymer is in a concentration of no less than 30 weight percent and no more than 70 weight percent, of the pharmaceutical composition.

In illustrating this embodiment, first described are experiments determining the aqueous solubility of a conjugate according to the invention (Ref# 33-2; See Table 5). To determine the aqueous solubility of this conjugate at pH 8, a volume of water was held at pH 8.0 by addition of 1 N NaOH while the conjugate (in powder form) was added until a two phase slurry was obtained. The supernatant (upper solution of the slurry) was sampled and analyzed by HPLC for the concentration of the conjugate. By this method, the concentration of conjugate in solution was 263 mg/ml. In another method for assessing solubility in aqueous solution, and effects of pH relative to physiological conditions on such solubility, water was added to vial containing conjugate (in lyophilized powder form). The pH was adjusted to various pH values, and at those values, an aliquot of the supernatant was withdrawn, and concentration of conjugate was determined by the Edelhoch method, as known in the art. By this method, over a pH range of 6.8 to 7.8, the apparent aqueous solubility of the conjugate ranged from about 150 mg/ml to about 200 mg/ml.

Illustrated in this example is a pharmaceutical composition comprising: a conjugate comprised of synthetic peptide operatively linked to fatty acid; and a pharmaceutically acceptable carrier comprising a polymer. A pharmaceutical composition comprised of 50 weight percent conjugate (See Table 5 Ref# 33-2) and of 50 weight percent polymer (PEG 1500) was prepared as follows. Polyethylene Glycol 1500 (PEG 1500) was added to pharmaceutical grade water, conjugate was added to this solution, and then the solution was stirred at ambient conditions for fifteen minutes. With the addition of 1 N NaOH, the conjugate was dissolved and solution pH was adjusted to 7.0. Concentration was adjusted to 25 mg/mL of conjugate and 25 mg/ml of PEG 1500 with

pharmaceutical grade water. This solution was filtered through a 0.2 um nylon filter, and lyophilization vials were filled with 2 mL of the solution containing 25 mg/mL conjugate and 25 mg/mL PEG 1500. The vial content was then lyophilized to obtain a pharmaceutical composition that contains 50 mg/vial conjugate and 50 mg/vial PEG

5 1500.

To assess the effects of polymer on solubility of the conjugate in the pharmaceutical composition, the pharmaceutical composition comprising lyophilized conjugate and polymer were reconstituted with 0.1 mL, 0.12 mL and 0.2 mL of either pharmaceutical grade water, or 10% ethanol in pharmaceutical grade water, respectively, to achieve conjugate at a final concentration of 500 mg/mL, 416 mg/mL and 250 mg/mL. When reconstituted with 0.2 mL of pharmaceutical grade water, or 10% ethanol in pharmaceutical grade water, the resulting solution has conjugate in a concentration of approximately 250 mg/mL, and this solution was physically stable for more than 48 hours. When reconstituted with 0.12 mL pharmaceutical grade water, or 10% ethanol in pharmaceutical grade water, the resulting solution has conjugate in a concentration of approximately 416 mg/mL, and this solution was physically stable for approximately 12 hours. When reconstituted with 0.1 mL pharmaceutical grade water, or 10% ethanol in pharmaceutical grade water, the concentration of conjugate is approximately 500 mg/mL, and the solution is physically stable for approximately 4 hours. In each case, the solutions became a clear, colorless solution. Thus, as opposed to a maximum solubility of about 260 mg/ml of conjugate in pharmaceutical grade water, the addition of a polymer as a pharmaceutically acceptable carrier significantly increased the solubility of the conjugate to concentrations of about 500 mg/ml or greater.

The foregoing examples and specific embodiments of the present invention have been described in detail for purposes of illustration. In view of the descriptions and illustrations, others skilled in the art can, by applying current knowledge, readily modify and/or adapt the present invention for various applications without departing from the basic concept; and thus, such modifications and/or adaptations are intended to be within the meaning and scope of the appended claims.

What is claimed is:

1. A conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid, wherein the conjugate has antiviral activity against HIV.
2. A conjugate according to claim 1, wherein the HIV gp41-derived peptide is operatively
5 linked to fatty acid directly or through a linker.
3. A conjugate according to claim 1, wherein fatty acid comprises one fatty acid molecule.
4. A conjugate according to claim 1, wherein fatty acid comprises more than one fatty
10 acid molecule.
5. A conjugate according to claim 2, wherein fatty acid or linker is operatively linked to an amino group of the HIV gp41-derived peptide.
- 15 6. A conjugate according to claim 2, wherein fatty acid directly or through a linker is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.
- 20 7. A conjugate according to claim 2, wherein a linker operatively links fatty acid to the HIV gp41-derived peptide, and wherein the linker comprises a polyethylene glycol comprising between 2 to 30 ethylene glycol units.
8. A conjugate according to claim 2, wherein a linker operatively links fatty acid to the
25 HIV gp41-derived peptide, wherein the linker is selected from the group consisting of PEG3, (PEG3)_n, (PEG13)_x, PEG25, PEG29, and a combination thereof, and wherein n is a number from 1 to 10, and x is 1 or 2.
9. A pharmaceutical composition comprising a conjugate and a pharmaceutically
30 acceptable carrier, wherein the conjugate comprises HIV gp41-derived peptide operatively linked to fatty acid directly or through a linker, and wherein the conjugate has antiviral activity against HIV.
10. A pharmaceutical composition according to claim 9, wherein fatty acid comprises one
35 fatty acid molecule.
11. A pharmaceutical composition according to claim 9, wherein fatty acid comprises

more than one fatty acid molecule.

12. A pharmaceutical composition according to claim 9, wherein the pharmaceutically acceptable carrier comprises polyethylene glycol.

5

13. A pharmaceutical composition according to claim 9, wherein the pharmaceutically acceptable carrier comprises: polyethylene glycol; and aqueous alcohol in a concentration ranging from about 1% to about 15% volume/volume of the pharmaceutical composition.

10

14. An HIV gp41-derived peptide having one or more improved pharmaceutical activities resulting from the HIV gp41-derived peptide having fatty acid directly or through a linker operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.

15

15. An HIV gp41-derived peptide according to claim 14, wherein fatty acid comprises one fatty acid molecule.

20

16. An HIV gp41-derived peptide according to claim 14, wherein fatty acid comprises more than one fatty acid molecule.

25

17. An HIV gp41-derived peptide according to claim 14, wherein the one or more improved pharmaceutical activities is selected from the group consisting of increased antiviral potency against HIV-1, increased antiviral potency against HIV-1 isolates resistant to antiviral activity of a synthetic peptide having the amino acid sequence of SEQ ID NO:2, increased antiviral potency against HIV-2, an improvement in clearance, an improvement in biological half life, and a combination thereof.

30

18. An HIV gp41-derived peptide according to claim 14, wherein the HIV gp41-derived peptide has an amino acid sequence of SEQ ID NO:33.

35

19. An antiviral composition comprising a conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid directly or through a linker, wherein fatty acid or linker is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof, and wherein the conjugate has antiviral activity.

20. A conjugate comprising HIV gp41-derived peptide operatively linked to fatty acid, wherein the HIV gp41-derived peptide has an amino acid sequence of SEQ ID. NO:33, wherein the fatty acid is operatively linked to the HIV gp41-derived peptide through a linker, wherein the fatty acid comprises C18, wherein the linker comprises PEG3, and
5 wherein the linker is operatively linked to an internal amino acid comprising lysine of the HIV gp41-derived peptide.

21. A conjugate according to claim 20, wherein fatty acid comprises one fatty acid molecule operatively linked to the internal amino acid.

10 22. A conjugate according to claim 20, wherein fatty acid comprises more than one fatty acid molecule operatively linked to the internal amino acid.

23. A pharmaceutical composition comprising a conjugate and a pharmaceutically acceptable carrier, wherein the conjugate comprises HIV gp41-derived peptide
15 operatively linked to fatty acid, wherein the HIV gp41-derived peptide has an amino acid sequence of SEQ ID. NO:33, wherein the fatty acid is operatively linked to the HIV gp41-derived peptide through a linker, wherein the fatty acid comprises 18 carbon atoms, wherein the linker comprises PEG3, and wherein the linker is operatively linked to an
20 internal amino acid comprising lysine of the HIV gp41-derived peptide.

24. A pharmaceutical composition according to claim 23, wherein fatty acid comprises one fatty acid molecule.

25 25. A pharmaceutical composition according to claim 23, wherein fatty acid comprises more than one fatty acid molecule.

26. A pharmaceutical composition according to claim 23, wherein the pharmaceutically acceptable carrier comprises polyethylene glycol.

30 27. A pharmaceutical composition according to claim 23, wherein the conjugate is in a concentration in the pharmaceutical composition of no less than 200 mg/ml, and no more than 750 mg/ml; and wherein the polyethylene glycol is in a concentration of no less than 10 weight percent and no more than 75 weight percent of the pharmaceutical
35 composition.

28. A pharmaceutical composition according to claim 23, wherein the conjugate is in a concentration of no less than 30 weight percent and no more than 70 weight percent of

the pharmaceutical composition, and the polymer is in a concentration of no less than 30 weight percent and no more than 70 weight percent of the pharmaceutical composition.

29. A pharmaceutical composition according to any of claims 23, 24, 25, 26, 27, or 28,
5 wherein the pharmaceutically acceptable carrier comprises: polyethylene glycol; and aqueous alcohol in a concentration ranging from about 1% to about 15% volume/volume of the pharmaceutical composition.

30. Use of a conjugate according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 20, 21, or 22,
10 in combination with one more additional therapeutic agents, in an amount effective for treatment of HIV infection.

31. Use of a conjugate according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 20, 21, or 22,
15 in the manufacture of a medicament, wherein the medicament is used in the treatment of HIV infection.

32. A method of increasing antiviral activity of an HIV gp41-derived peptide against one or more of HIV-1 and HIV-2, the method comprising operatively linking one or more fatty acids directly or through a linker to the HIV gp41-derived peptide.

33. The method according to claim 32, wherein the one or more fatty acids or linker is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.

34. A method for producing a conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid, the method comprising:
(a) operatively linking a linker to fatty acid in forming a linker-fatty acid combination; and
(b) operatively linking the linker-fatty acid combination, through a reactive functionality of
30 the linker of the linker-fatty acid combination, to the HIV gp41-derived peptide.

35. The method according to claim 34, wherein the linker is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.

36. The method according to claim 34, wherein the linker-fatty acid combination

comprises more than one fatty acid molecule.

37. A method for producing a conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid, the method comprising the steps in the order of:

5 (a) operatively linking a linker to the HIV gp41-derived peptide; and
(b) operatively linking a reactive functionality of the linker to fatty acid;
in forming a conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid.

10 38. The method according to claim 37, wherein the linker is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.

15 39. The method according to claim 37, wherein more than one fatty acid molecule is operatively linked to the HIV gp41-derived peptide.

40. A method for producing a conjugate comprised of HIV gp41-derived peptide operatively linked to fatty acid, the method comprising operatively linking fatty acid
20 directly to the HIV gp41-derived peptide, wherein fatty acid is operatively linked to an amino acid of the HIV gp41-derived peptide selected from the group consisting of an N-terminal amino acid, an internal amino acid, a C-terminal amino acid, and a combination thereof.

25 41. The method according to claim 40, wherein more than one fatty acid molecule is operatively linked to the HIV gp41-derived peptide.

42. A method for treating an individual infected with HIV comprising administering to the individual an antiviral effective amount of a conjugate according to any one of claims 1, 2,
30 3, 4, 5, 6, 7, 8, 20, 21, or 22.

43. The method of claim 42, wherein the conjugate is administered in combination with an antiviral effective amount of one or more additional therapeutic agents for treatment of HIV infection.

35 44. A method for treating an individual infected with HIV comprising administering to the individual an antiviral effective amount of a pharmaceutical composition according to any

one of claims 9, 10, 11, 12, 13, 23, 24, 25, 26, 27, 28, or 29.

45. The method of claim 44, wherein the pharmaceutical composition is administered in combination with an antiviral effective amount of one or more additional therapeutic

5 agents for treatment of HIV infection.

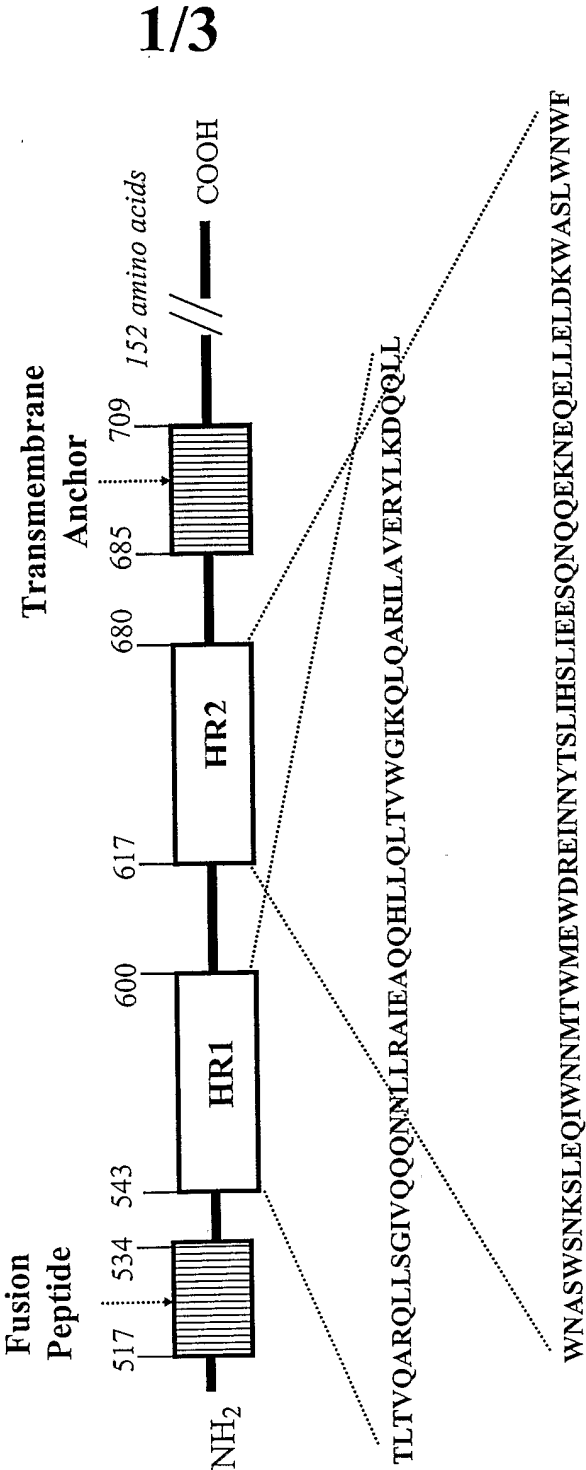
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FIG. 1



2/3

FIG. 2

MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	
-----+-----+-----+-----	
10 20 30	
-----+-----+-----	
	<u>Isolate</u>
624 MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	NL4-3
631 MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	LAV1a
626 TTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	IIIB
626 TTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	HXB2
619 MTWMQWEKEINNYTGLIYNLIEESQNQQEKNEQELL	DH12
MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	BRU
TTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	HXB2
MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELL	pNL4-3
625 MTWLQWDKEISNYTNIIYDLIEEAQNQQEKNEQDLL	Ug273-A
623 MTWMEWEREIDNYTNTIYTLLEESQLQQEKNEQELL	Us2-B
619 MTWMQWDREISNYTGTIYRLLEDSONQQEKNEKDLL	Ug268-C
MTWMEWEREIDNYTGLIYSLIEESQTQQEKNEQELL	Se365-D
620 MTWIEWEREISNYTNQIYEILTESQNQQDRNEKDLL	CM240-E
612 MTWMEWEKEISNYSYEIYRLIEQSONQQEKNEQELL	Bz126-F
613 MTWIQWDREISNYTQQIYSLIEESQNQQEKNEQDLL	HH8793-G

3/3

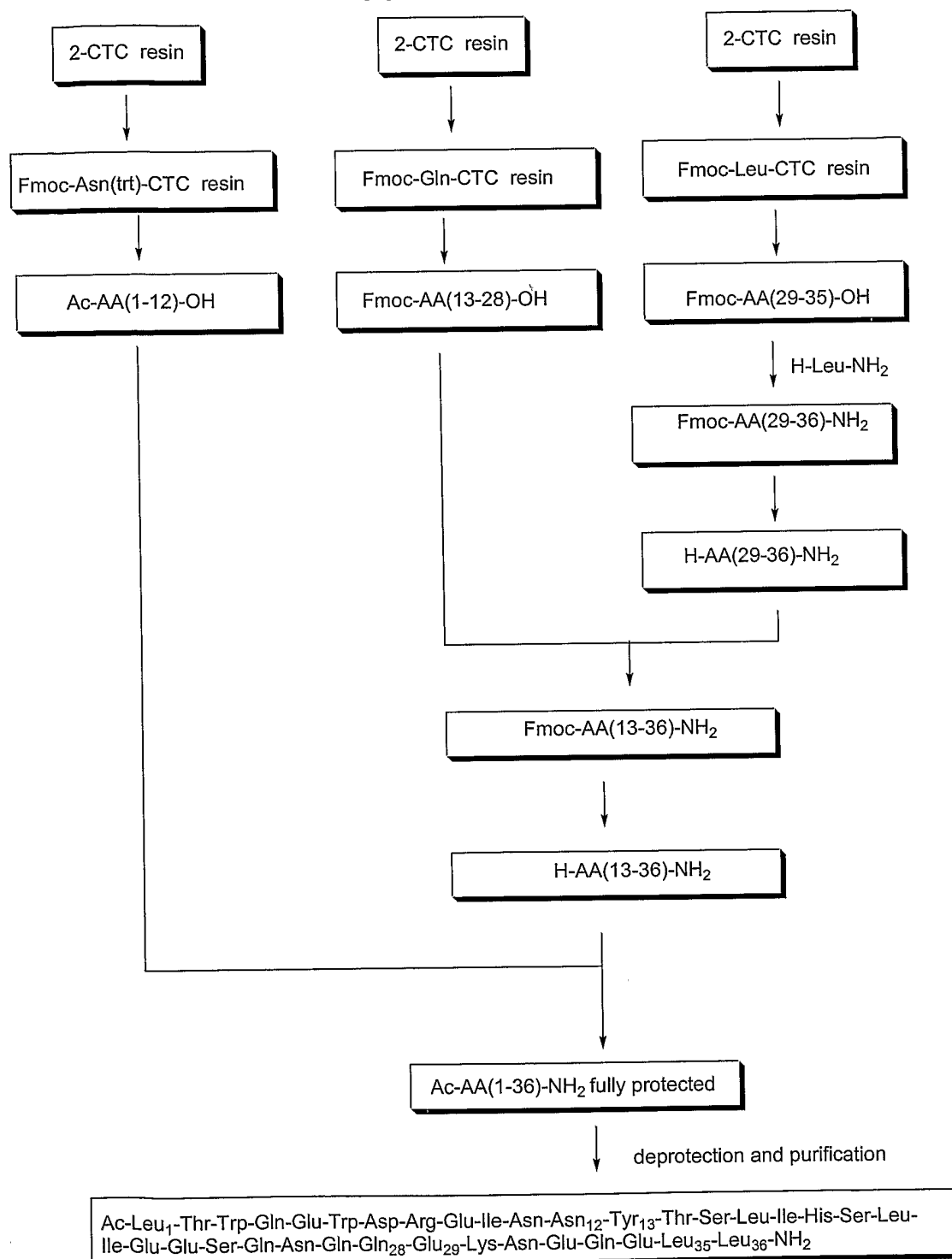


FIG. 3

SEQUENCE LISTING

<110> Trimeris, Inc.
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Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn
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Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
20 25 30

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
35 40 45

Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe
50 55 60

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Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu
20 25 30

Trp Asn Trp Phe

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Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu
35

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Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His
1 5 10 15

Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu
20 25 30

Leu Leu Glu Leu
35

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<400> 5

Asn Ala Ser Trp Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met
1 5 10 15

Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile
20 25 30

His Ser Leu Ile
35

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Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp
1 5 10 15

Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu
20 25 30

Glu Ser Gln Asn
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Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile
1 5 10 15

Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn
20 25 30

Gln Gln Glu Lys
35

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Gln	Ile	Trp	Asn	Asn	Met	Thr	Trp	Met	Glu	Trp	Asp	Arg	Glu	Ile	Asn
1				5					10					15	

Asn	Tyr	Thr	Ser	Leu	Ile	His	Ser	Leu	Ile	Glu	Glu	Ser	Gln	Asn	Gln
			20					25					30		

Gln	Glu	Lys	Asn
			35

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Ile	Trp	Asn	Asn	Met	Thr	Trp	Met	Glu	Trp	Asp	Arg	Glu	Ile	Asn	Asn
1				5				10						15	

Tyr	Thr	Ser	Leu	Ile	His	Ser	Leu	Ile	Glu	Glu	Ser	Gln	Asn	Gln	Gln
			20					25					30		

Glu	Lys	Asn	Glu
			35

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Trp	Asn	Asn	Met	Thr	Trp	Met	Glu	Trp	Asp	Arg	Glu	Ile	Asn	Asn	Tyr
1				5				10					15		

Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu
20 25 30

Lys Asn Glu Gln
35

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Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr
1 5 10 15

Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys
20 25 30

Asn Glu Gln Glu
35

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<400> 12

Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser
1 5 10 15

Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn
20 25 30

Glu Gln Glu Leu
35

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<400> 13

Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile
1 5 10 15

His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln
20 25 30

Glu Leu Leu Glu
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Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu Glu Leu
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Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser
1 5 10 15

Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu
20 25 30

Leu Glu Asp
35

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Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu
1 5 10 15

Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu
20 25 30

Glu Leu Asp Lys
35

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Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile
1 5 10 15

Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu
20 25 30

Leu Asp Lys Trp
35

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<400> 18

Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln
1 5 10 15

Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser
20 25 30

Leu Trp Asn Trp
35

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<400> 19

Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu
1 5 10 15

Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp
20 25 30

Asn Trp Phe Asn
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<400> 20

Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys
1 5 10 15

Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn
20 25 30

Trp Phe Asn Ile
35

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<400> 21

Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn
1 5 10 15

Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp
20 25 30

Phe Asn Ile Thr
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<400> 22

Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Glu
1 5 10 15

Arg Glu Ile Asp Asn Tyr Thr Ser Leu Ile Tyr Ser Leu Ile Glu Glu
20 25 30

Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu
35 40

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<400> 23

Asn Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile Asp Asn Tyr Thr
1 5 10 15

Ser Leu Ile Tyr Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys
20 25 30

Asn Glu Gln Glu
35

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<400> 24

Glu Trp Glu Arg Glu Ile Asp Asn Tyr Thr Ser Leu Ile Tyr Ser Leu
1 5 10 15

Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu
20 25 30

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<400> 25

Tyr Thr Asn Thr Ile Tyr Thr Leu Leu Glu Glu Ser Gln Asn Gln Gln
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu
20 25 30

Trp Asn Trp Phe
35

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<400> 26

Tyr Thr Gly Ile Ile Tyr Asn Leu Leu Glu Glu Ser Gln Asn Gln Gln
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Asn Leu
20 25 30

Trp Asn Trp Phe
35

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Tyr Thr Ser Leu Ile Tyr Ser Leu Leu Glu Lys Ser Gln Ile Gln Gln
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu
20 25 30

Trp Asn Trp Phe
35

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Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln
1 5 10 15

Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu
20 25 30

Phe Asn Phe Phe
35

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Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu
1 5 10 15

Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu
20 25 30

Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe
35 40

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Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe
35 40 45

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Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr
1 5 10 15

Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys
20 25 30

Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys
35 40

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Leu Thr Trp Ile Glu Trp Asp Arg Glu Ile Asn Lys Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Lys
35

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<400> 33

Leu Thr Trp Gln Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu
35

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<400> 34

Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Leu Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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Ala Pro Lys Glu Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu
1 5 10 15

Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln
20 25 30

Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu Lys Gln Gly Ile
35 40 45

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Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 38

Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Ala Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 39

Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Ala Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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Met Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Trp Glu Trp Phe
35 40

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
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Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Trp Glu Trp Phe
35 40

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Trp Glu Trp Phe
35 40

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Trp Glu Trp Phe
35

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Trp Glu Trp Phe Gly Gly Ser Gly Gly Ser Thr Thr Trp Glu Ala Trp

1 5 10 15
Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg
20 25 30
Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15
Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Gly Gly Ser Gly Gly Ser Trp Glu Trp Phe
35 40 45

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15
Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Gly Gly Ser Gly Gly Ser Trp
35 40 45

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Trp Gly Gly Ser Gly Gly Ser Thr Thr Trp Glu Ala Trp Asp Arg Ala
1 5 10 15

Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg Ala Ala Gln
20 25 30

Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

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Pro Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala
1 5 10 15

Arg Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn
20 25 30

Glu Ala Ala Leu Arg Glu Leu
35

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Pro Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala
1 5 10 15

Arg Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn

20

25

30

Glu Ala Ala Leu Arg Glu Leu Pro
35 40

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Thr Thr Trp Glu Ala Trp Asp Lys Ala Ile Ala Glu Tyr Ala Ala Lys
1 5 10 15

Ile Glu Ala Leu Ile Lys Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Lys Glu Leu
35

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<400> 51

Thr Thr Trp Glu Ala Trp Asp Arg Ala Trp Gln Glu Trp Glu Gln Lys
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 52

Thr Thr Trp Ala Ala Trp Asp Ala Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 53

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<400> 53

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Ala Tyr Ala Ala Ala
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 54

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Ala Ala Leu Ile Ala Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu

35

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Ala
20 25 30

Ala Ala Leu Ala Glu Leu
35

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Thr Thr Trp Glu Glu Trp Asp Arg Glu Ile Asn Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 57

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Thr Ser Arg
1 5 10 15

Ile Glu Ser Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 58

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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<400> 59

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Leu Glu Leu
35

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<400> 60

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile His Ala Leu Ile Glu Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Asn Tyr Ala Ala Leu
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

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Glu Thr Trp Lys Glu Trp Asp Arg Ala Ile Glu Glu Tyr Lys Lys Arg
1 5 10 15

Ile Glu Glu Leu Ile Lys Ala Ala Glu Asn Gln Gln Glu Lys Asn Lys
20 25 30

Glu Ala Leu Arg Glu Leu
35

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<400> 63

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Ala Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ala Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
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Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Ala
20 25 30

Ala Ala Leu Ala Glu Leu
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Ala Lys Glu Ala Ala Gln Arg Ala Asn Ala Thr Thr Trp Glu Ala Trp
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Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg
20 25 30

Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

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<213> Artificial

<220>

<223> synthesized

<400> 67

Asn Lys Glu Leu Glu Gln Arg Trp Asn Asn Thr Thr Trp Glu Ala Trp
1 5 10 15

Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg
20 25 30

Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

<210> 68
<211> 48
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 68

Glu Lys Ala Ala Arg Gln Ala Glu Asn Ala Ala Arg Trp Glu Ala Trp
1 5 10 15

Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg
20 25 30

Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

<210> 69
<211> 48
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 69

Glu Lys Ser Leu Arg Gln Ile Glu Asn Asn Thr Arg Trp Glu Ala Trp
1 5 10 15

Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile Arg
20 25 30

Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu Leu
35 40 45

<210> 70
<211> 48
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 70

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Ala Ala Arg Glu Ala Ala Trp Arg Trp Phe
35 40 45

<210> 71
<211> 48
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 71

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Asp Lys Arg Glu Ala Leu Trp Arg Trp Phe
35 40 45

<210> 72
<211> 48
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 72

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Asp Lys Arg Glu Ser Leu Trp Arg Trp Phe
35 40 45

<210> 73
<211> 49
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 73

Gly Ala Lys Glu Ala Ala Gln Arg Ala Asn Ala Thr Thr Trp Glu Ala
1 5 10 15

Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile
20 25 30

Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu
35 40 45

Leu

<210> 74
<211> 49
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 74

Gly Glu Lys Ala Ala Arg Gln Ala Glu Asn Ala Ala Arg Trp Glu Ala
1 5 10 15

Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile
20 25 30

Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu
35 40 45

Leu

<210> 75
<211> 37
<212> PRT
<213> Artificial

<220>

<223> synthesized

<400> 75

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu
35

<210> 76

<211> 36

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 76

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg
35

<210> 77

<211> 35

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 77

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu
35

<210> 78
<211> 33
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 78

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala

<210> 79
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 79

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ile Leu Arg Glu Leu
35

<210> 80
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 80

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Leu Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 81
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 81

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Leu Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ile Leu Arg Glu Leu
35

<210> 82
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 82

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Leu Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 83
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 83

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Leu Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 84
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 84

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ala Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 85
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 85

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Ala Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 86
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 86

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Ala
35

<210> 87
<211> 36
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 87

Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu
1 5 10 15

Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala
20 25 30

Leu Arg Glu Leu
35

<210> 88
<211> 37
<212> PRT
<213> Artificial

<220>

<223> synthesized

<400> 88

Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu
1 5 10 15

Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala
20 25 30

Leu Arg Glu Leu Ala
35

<210> 89

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 89

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Ala Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 90

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 90

Glu Thr Trp Lys Glu Trp Asp Arg Ala Ile Glu Glu Tyr Lys Lys Arg
1 5 10 15

Ile Glu Glu Leu Ile Lys Ala Ala Glu Asn Gln Gln Glu Lys Asn Lys
20 25 30

Glu Ala Leu Arg Glu Leu
35

<210> 91
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 91

Met Ala Trp Met Glu Trp Asp Arg Arg Ile Glu Ala Tyr Ala Arg Leu
1 5 10 15

Ile Ala Glu Leu Ile Ala Arg Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 92
<211> 41
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 92

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Gln Gln Leu Arg Glu Trp Glu Trp Phe
35 40

<210> 93
<211> 41
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 93

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Trp Glu Trp Ile
35 40

<210> 94

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 94

Thr Thr Trp Asp Ala Trp Asp Arg Ala Ile Ala Asp Tyr Ala Ala Arg
1 5 10 15

Ile Asp Ala Leu Ile Arg Ala Ala Gln Asp Gln Gln Glu Lys Asn Asp
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 95

<211> 41

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 95

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Ala Glu
20 25 30

Ala Ala Leu Arg Glu Trp Glu Trp Phe
35 40

<210> 96
<211> 52
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 96

Trp Ala Ser Leu Trp Glu Trp Phe Gly Gly Ser Gly Gly Ser Thr Thr
1 5 10 15

Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu
20 25 30

Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala
35 40 45

Leu Arg Glu Leu
50

<210> 97
<211> 52
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 97

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Gly Gly Ser Gly Gly Ser Trp Ala Ser Leu
35 40 45

Trp Glu Trp Phe
50

<210> 98
<211> 38
<212> PRT
<213> Artificial

<220>

<223> synthesized

<400> 98

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Arg Glu Leu
35

<210> 99

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 99

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Ala Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 100

<211> 41

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 100

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Trp Trp Trp Trp
35 40

<210> 101
<211> 47
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 101

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Asp Lys Trp Ser Leu Trp Arg Trp Phe
35 40 45

<210> 102
<211> 47
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 102

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Ala Leu Asp Lys Trp Glu Ala Leu Trp Arg Phe
35 40 45

<210> 103
<211> 41
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 103

Thr Thr Trp Glu Ala Trp Asp Arg Ala Trp Gln Glu Trp Glu Gln Lys
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Trp Glu Trp Phe
35 40

<210> 104

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 104

Leu Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 105

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 105

Thr Thr Trp Met Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 106
<211> 55
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 106

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Gly Gly Ser Gly Gly Ser Gly Gly Ser Trp
35 40 45

Ala Ser Leu Trp Glu Trp Phe
50 55

<210> 107
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 107

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Ala Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 108
<211> 58
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 108

Gly Ala Lys Glu Ala Ala Gln Arg Ala Asn Ala Thr Thr Trp Glu Ala
1 5 10 15

Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg Ile Glu Ala Leu Ile
20 25 30

Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu Ala Ala Leu Arg Glu
35 40 45

Leu Asp Lys Trp Ala Ser Leu Trp Trp Phe
50 55

<210> 109
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 109

Pro Ala Asn Trp Lys Ala Trp Glu Ala Gln Ile Gln Lys Tyr Gln Arg
1 5 10 15

Gln Ile Ala Glu Leu Ile Ala Asn Ala Lys Lys Gln Gln Glu Gln Asn
20 25 30

Glu Lys Ala Leu Arg Glu Leu
35

<210> 110
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 110

Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ile Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu Glu Leu
35

<210> 111
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 111

Thr Thr Trp Glu Glu Trp Asp Arg Glu Ile Asn Glu Tyr Thr Ser Arg
1 5 10 15

Ile Glu Ser Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Ala
20 25 30

Ala Ala Leu Ala Glu Leu
35

<210> 112
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 112

Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ile Gln Asn Ile Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu Glu Leu
35

<210> 113
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 113

Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ile Gln Asn Ile Gln Glu Lys Ile Glu
20 25 30

Gln Glu Leu Leu Glu Leu
35

<210> 114

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 114

Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ile Gln Asn Ile Gln Glu Lys Asn Glu
20 25 30

Gln Ile Leu Leu Glu Leu
35

<210> 115

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 115

Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ala Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Ala Leu Leu Glu Leu
35

<210> 116
<211> 42
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 116

Pro Ala Asn Trp Lys Ala Trp Glu Ala Gln Ile Gln Lys Tyr Gln Arg
1 5 10 15

Gln Ile Ala Glu Leu Ile Ala Asn Ala Lys Lys Gln Gln Glu Gln Asn
20 25 30

Glu Lys Ala Leu Arg Glu Trp Glu Trp Phe
35 40

<210> 117
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 117

Ala Asn Trp Lys Ala Trp Glu Ala Gln Ile Gln Lys Tyr Gln Arg Gln
1 5 10 15

Ile Ala Glu Leu Ile Ala Asn Ala Lys Lys Gln Gln Glu Gln Asn Glu
20 25 30

Lys Ala Leu Arg Glu Leu
35

<210> 118
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 118

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Val Leu Arg Glu Leu
35

<210> 119
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 119

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Val Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 120
<211> 38
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 120

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Ile Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 121
<211> 38
<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 121

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 . 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Leu Gln Glu Lys Asn Glu
20 25 30

Ala Ile Leu Arg Glu Leu
35

<210> 122

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 122

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Leu Gln Glu Leu Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu
35

<210> 123

<211> 38

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 123

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Leu Leu Arg Glu Leu
35

<210> 124
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 124

Thr Thr Trp Glu Ala Trp Asp Arg Ala Ile Ala Glu Tyr Ala Ala Arg
1 5 10 15

Ile Glu Ala Leu Ile Arg Ala Ala Gln Glu Gln Gln Glu Lys Asn Glu
20 25 30

Ala Ala Leu Arg Glu Leu Lys
35

<210> 125
<211> 34
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 125

Trp Met Glu Trp Asp Arg Lys Ile Glu Glu Tyr Thr Lys Lys Ile Lys
1 5 10 15

Lys Leu Ile Glu Glu Ser Gln Glu Gln Gln Glu Lys Asn Glu Lys Glu
20 25 30

Leu Lys

<210> 126
<211> 34
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 126

Trp Met Glu Trp Asp Arg Lys Ile Glu Glu Tyr Thr Lys Lys Ile Glu
1 5 10 15

Glu Leu Ile Lys Lys Ser Gln Glu Gln Glu Lys Asn Glu Lys Glu
20 25 30

Leu Lys

<210> 127

<211> 35

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 127

Trp Glu Glu Trp Asp Lys Lys Ile Glu Glu Tyr Thr Lys Lys Ile Glu
1 5 10 15

Glu Leu Ile Lys Lys Ser Glu Glu Gln Gln Lys Lys Asn Glu Glu Glu
20 25 30

Leu Lys Lys
35

<210> 128

<211> 40

<212> PRT

<213> Artificial

<220>

<223> synthesized

<400> 128

Ser Trp Glu Thr Trp Glu Arg Glu Ile Glu Asn Tyr Thr Lys Gln Ile
1 5 10 15

Tyr Lys Ile Leu Ile Glu Glu Ser Gln Gln Asp Arg Asn Glu Lys Asp
20 25 30

Arg Asn Glu Lys Asp Leu Leu Glu
35 40

<210> 129
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 129

Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln
1 5 10 15

Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp
20 25 30

Ala Ser Leu Trp Glu Trp Phe
35

<210> 130
<211> 36
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 130

Trp Gln Glu Trp Glu Gln Lys Val Arg Tyr Leu Glu Ala Asn Ile Thr
1 5 10 15

Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Glu Tyr Glu
20 25 30

Leu Gln Lys Leu
35

<210> 131
<211> 46
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 131

Trp Gln Glu Trp Glu Gln Lys Val Arg Tyr Leu Glu Ala Asn Ile Thr

Leu Gln Lys Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe
35 40 45

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<210> 132
<211> 50
<212> PRT
<213> Artificial
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<220>
<223> synthesized
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<400> 132

Asn Asn Met Thr Trp Gln Glu Trp Glu Gln Lys Val Arg Tyr Leu Glu
1 5 10 15

Ala Asn Ile Thr Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys
20 25 30

Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp Ala Ser Leu Trp Asn
35 40 45

Trp Phe
50

<210>	133
<211>	36
<212>	PRT
<213>	Artificial

<220>
<223> synthesized

<400> 133

Trp Asn Trp Phe Ile Thr Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln
1 5 10 15

Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp Ala Ser Leu
20 25 30

Trp Asn Trp Phe
35

<210> 134
<211> 46
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 134

Trp Gln Glu Trp Asp Arg Glu Ile Ser Asn Tyr Thr Ser Leu Ile Thr
1 5 10 15

Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Glu Tyr Glu
20 25 30

Leu Gln Lys Leu Asp Glu Trp Ala Ser Leu Trp Glu Trp Phe
35 40 45

<210> 135
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthesized

<400> 135

Trp Gln Glu Trp Glu Arg Glu Ile Ser Ala Tyr Thr Ser Leu Ile Thr
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Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Ile Glu Tyr Glu
20 25 30

Leu Gln Lys Leu Glu Trp Glu Trp
35 40

<210> 136
<211> 39
<212> PRT
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Trp Gln Glu Trp Asp Arg Glu Ile Thr Ala Leu Leu Glu Gln Ala Gln

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Trp Gln Glu Trp Asp Arg Glu Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp Glu Trp
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Ala Ser Leu Trp Glu Trp Phe
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<210>	138
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Glu Trp Phe
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Trp Gln Glu Trp Glu Arg Glu Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Glu Trp Phe
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Trp Gln Glu Trp Glu Arg Glu Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Glu Trp Phe
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Trp Gln Glu Trp Glu Arg Glu Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Ile Glu Tyr Glu Leu Gln Lys Leu Asp Glu Trp

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Glu Trp Phe
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Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp
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Ala Ser Leu Trp Asn Trp Phe
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Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp
20 25 30

Ala Gly Leu Trp Glu Trp Phe
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Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Ala Glu Trp
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Ala Gly Leu Trp Ala Trp Phe
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Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln
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Ile Gln Gln Glu Lys Ile Glu Tyr Glu Leu Gln Lys Leu Ile Glu Trp
20 25 30

Glu Trp Phe
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Leu Thr Trp Ile Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu
1 5 10 15

Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu
20 25 30

Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe

35

40

45