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(54) **Title**: PEPTIDE-BASED HIV PRE-INTEGRATION COMPLEX INHIBITORS

(57) **Abstract**: The present invention relates to the field of acquired immunodeficiency syndrome (AIDS) therapy, and more particularly to peptide-based Pre- Integration Complex (PIC) inhibitors of the Human Immunodeficiency Virus (HIV).

PEPTIDE-BASED HIV PRE-INTEGRATION COMPLEX INHIBITORS

Field of the Invention

5 The present invention relates to the field of acquired immunodeficiency syndrome (AIDS) therapy, and more particularly to peptide-based inhibitors of the Human Immunodeficiency Virus (HIV).

Background of the Invention

10 The discussion in this section is not limited to subject matter that qualifies as "prior art" against the present invention. Therefore, no admission of such prior art status shall be implied or inferred by reason of inclusion of particular subject matter in this discussion, and no declaration against the present inventors' interests shall be implied by reason of such inclusion.
15 All publications mentioned throughout this application are fully incorporated herein by reference, including all references that would be cited therein.

The HIV is the etiologic agent of AIDS. The commonly used anti-AIDS
20 therapy, referred to as Highly Active Antiretroviral Therapy (HAART), targets mainly three steps of the HIV life cycle (reverse transcription, proteolytic maturation and fusion) which consists of a combination of protease, reverse transcriptase (RT) (non-nucleoside and nucleoside reverse transcriptase inhibitors) and a fusion inhibitor. Although the
25 development of HAART has significantly improved quality of life of patients, it is not curative, and often not well-tolerated by the patients. Moreover they are limited by the rapid emergence of multidrug resistant viruses. Therefore, there is still an urgent need for additional therapeutic approaches that are able to overcome resistant problems and may be
30 useful for multiple drug combination.

Recently a new generation of HIV inhibitors targeting the third viral enzyme HIV-1 integrase (IN) have been introduced into clinics. IN plays an essential role in the HIV life cycle, by catalyzing the insertion of the viral DNA into the host cell chromosome, throughout a multi-steps process taking place during or immediately after reverse transcription of the viral genomic RNA. IN binds the viral DNA, thereby forming a nucleoprotein complex, which constitutes the main component of the pre-integration complex (PIC). Although the composition of the PIC still remains to be clarified, it contains viral components such as RT and Vpr as well as cellular components like BAF, LEDGF-p75, INI1 and HMGA1 (Van Maele *et al.*, 2005; Iordanski *et al.*, 2006; Raghavenda *et al.*, 2010) that stabilize IN/DNA complex, favor its nuclear import and improve IN mediated viral DNA insertion. Within the PIC, IN performs 3'-processing in the cytoplasm, which consists in the cleavage of two terminal nucleotides from both 3'-ends of viral DNA. Then, in the nucleus of infected cell, IN mediates a strand transfer reaction that inserts viral DNA into the cell DNA, resulting in a full-site integration (Fig.1).

The most promising IN inhibitors reported so far inhibit the strand transfer reaction, by interacting directly with the active site of the preassembled IN/viral DNA complex. Two strand transfer inhibitor compounds, Raltegravir (MK-0518, Isentress™) has been approved by the FDA in 2007 and Elvitegravir (GS-9137) is currently in clinical trials. However, as most of the HIV inhibitors targeting catalytic activity of viral enzyme, strand transfer inhibitors have been reported to interact with human proteins that belong to the same polynucleotidyl transferases family, and although they are potent HIV replication inhibitors and highly active on viruses harboring resistance to other antiretroviral classes, these inhibitors rapidly select mutations in IN gene (Malet *et al.*, 2008; Shimura *et al.*, 2008). Therefore the development of inhibitors that do not target directly the IN active site, but its structural organization and functions remain a major challenge.

Therefore, it is a first object of the present invention to provide compounds useful in AIDS therapy which lack the drawbacks of the molecules currently known in the art.

5

It is a further object of the invention to provide compounds having a therapeutically effective HIV antiviral activity without targeting the catalytic activity of viral enzymes.

10 It is another object of the invention to provide compounds capable of disrupting the structural organization of HIV PIC of the HIV and pharmaceutical compositions comprising the same.

It is a further object of the invention to provide uses of the compounds of
15 the invention for the preparation of medicaments as well as methods of treatment of AIDS.

These and other objects of the invention will become apparent as the description proceeds.

20

Summary of the Invention

In a first aspect, the present invention provides an isolated polypeptide comprising or consisting of at least one amino acid sequence denoted by SEQ ID NO: 1:

25

X1-X2-W-L-X5-X6-W-I-P-X10-X11-X12-X13

wherein:

X1 is T, S, or is absent;

30 X2 is K or R;

X5 is T or S;

X6 is E or R;
X10 is W or F;
X11 is T, R or S;
X12 is W or F; and
5 X13 is A, L, or is absent;
said polypeptide being characterized in that it exhibits an antiviral activity against human immunodeficiency virus (HIV).

In some specific embodiments, the isolated polypeptide of the invention 10 comprises or consists of at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 2 – 16.

The isolated polypeptide of the invention is particularly suitable for use as a medicament, especially for the treatment amelioration or prevention of a 15 HIV infection, including HIV-1 and/or HIV-2 infections.

In a second aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of at least one polypeptide according to the above and a pharmaceutically acceptable carrier, diluent, 20 excipient and/or additive.

In a further aspect, the invention provides a method for treating, ameliorating or preventing a HIV infection in a subject, this method comprising administering to said subject a therapeutically effective amount 25 of at least one polypeptide of the invention, or a pharmaceutical composition comprising the same.

In another aspect, the invention provides a monoclonal or polyclonal antibody which specifically recognizes a polypeptide according to the above.

30

In yet another aspect, the invention provides an isolated polynucleotide encoding a polypeptide of the invention, a recombinant expression vector comprising the same, and a host cell comprising this expression vector.

5 In yet another aspect, the invention provides a kit for the treatment, amelioration or prevention of a HIV infection, comprising:

i) a pharmaceutical composition comprising a therapeutically effective amount of at least one polypeptide according to the above or any derivative thereof and a pharmaceutically acceptable carrier, diluent,
10 excipient and/or additive, wherein each of said at least one polypeptide or derivative is optionally comprised within a separate compartment; and

ii) instructions for administrating said pharmaceutical composition.

The invention will be further described on the hand of the following figures,
15 which are illustrative only and are not intended to limit the scope of the invention.

Brief Description of the Figures

Fig. 1 is a scheme presenting the life cycle of the HIV, a particular focus
20 being made on the different steps involved in the integration of the viral cDNA into the genetic material of the host (published in Semenova, 2008, Adv. Pharmacol. 2008;56:199-228);

Fig. 2 is a graph showing the titration binding curve of a GNS-255/IN
25 complex. A fixed concentration of fluorescently labelled peptide (50 nM) was titrated by increasing the concentration of IN up to 2 μM (●). A scrambled-PICI peptide (GNS-264) was used as control for non specific binding (○).

30 **Figs. 3A and 3B** are two graphs showing the effect of PICI peptides on the formation of the complex IN-RT/pt. The formation of the complex IN-RT/pt was monitored by fluorescence spectroscopy

using a FAM-labelled double stranded DNA primer/template (pt). In Fig. 3A, a fixed concentration of RT/pt was titrated by increasing the concentration of IN in the absence (●) or in the presence of 100 (▩), 200 (■) and 1000 nM (□) of GNS-255. A
5 scrambled peptide (GNS-264) was used as a control (○). In Fig. 3B, a fixed concentration of RT/pt was titrated by increasing the concentration IN in the absence (●) or in the presence of 500 nM of either GNS-255 (■) or GNS-261 (□).

10 **Fig. 4** is a graph showing the kinetics of PIC dissociation induced by GNS-265. The disruption of the complex IN-RT/pt was monitored by fluorescence spectroscopy using a FAM-labelled double stranded DNA primer/template (pt). IN-RT/pt complex was preformed using 50 nM of RT and pt, and 200 nM of IN. GNS-265
15 peptide was added at 100 nM (○) and 250 nM (●) to the complex and kinetic of changed in FITC fluorescence was followed. A scrambled-PICI peptide (GNS-Scr2) was used as control for non specific binding (◆).

20 **Detailed Description**

It is to be understood that the invention is not limited in its application to the details set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced out in various ways. Also, it is to be understood that the
25 phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

30 In a first aspect, the invention provides an isolated polypeptide comprising or consisting of at least one amino acid sequence denoted by SEQ ID NO: 1:

X1-X2-W-L-X5-X6-W-I-P-X10-X11-X12-X13

wherein:
X1 is T, S, or is absent;
5 X2 is K or R;
X5 is T or S;
X6 is E or R;
X10 is W or F;
X11 is T, R or S;
10 X12 is W or F; and
X13 is A, L, or is absent;
said polypeptide being characterized in that it exhibits an antiviral activity against human immunodeficiency virus (HIV).

15 The term “isolated”, “purified” or “substantially purified”, when applied to a peptide, protein or nucleic acid, such as any one of the isolated polypeptides of the invention, denotes that the peptide, protein or nucleic acid is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state, 20 although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as high performance liquid chromatography or polyacrylamide gel electrophoresis. A protein, peptide or nucleic acid which is the predominant species present in a preparation is substantially 25 purified. “Substantially purified” molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are naturally associated. As used herein, the term “purified” or “to purify” also refers to the removal of contaminants from a sample.

30

The term "peptide" or "polypeptide” refers herein interchangeably to a linear or cyclic polymer of amino acid residues without being limited to a

any specific length. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. The terms also include post translation expression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation and the like.

5

"Amino acid sequence" or "peptide sequence" is the order in which amino acid residues, connected by peptide bonds, lie in the chain in peptides and proteins. The sequence is generally reported from the N-terminal end containing free amino group to the C-terminal end containing free
10 carboxyl group. Amino acid sequence is often called peptide, polypeptide, protein sequence if it represents the primary structure of a protein, however one must discern between the terms "Amino acid sequence" or "peptide sequence" and "protein", since a protein is defined as an amino acid sequence folded into a specific three-dimensional configuration and
15 that had typically undergone post-translational modifications, such as phosphorylation, acetylation, glycosylation, sulfhydryl bond formation, cleavage and the likes.

The present invention also concerns derivatives of the isolated polypeptide
20 defined above. Derivatives of the isolated polypeptide of the invention are, for example, where functional groups, such as amino, hydroxyl, mercapto or carboxyl groups, are derivatised, e.g. glycosylated, acylated, amidated or esterified, respectively. In glycosylated derivatives an oligosaccharide is usually linked to asparagine, serine, threonine and/or lysine. Acylated
25 derivatives are especially acylated by a naturally occurring organic or inorganic acid, e.g. acetic acid, phosphoric acid or sulphuric acid, which usually takes place at the N-terminal amino group, or at hydroxy groups, especially of tyrosine or serine, respectively. Esters are those of naturally occurring alcohols, e.g. methanol or ethanol. Further derivatives are salts,
30 especially pharmaceutically acceptable salts, for example metal salts, such as alkali metal and alkaline earth metal salts, e.g. sodium, potassium, magnesium, calcium or zinc salts, or ammonium salts formed with

ammonia or a suitable organic amine, such as a lower alkylamine, e.g. triethylamine, hydroxy-lower alkylamine, e.g. 2-hydroxyethylamine, and the like.

5 The term "amino acids" as used herein, refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., 10 hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Specifically, the term "amino acids" includes L and D isomers. According to some specific embodiments, the isolated polypeptide of the invention comprises at least one D-amino acid residue.

15 “Amino acid analogs” refers to compounds that have the same fundamental chemical structure as a naturally occurring amino acid, i.e., an alpha carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups or 20 modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. “Amino acid mimetics” refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Amino acids may be 25 referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

The polypeptides of the present invention can be synthesized using 30 methods well known in the art. Such methods include recombinant DNA methods and chemical synthesis. Recombinant methods of producing a peptide through expression of a nucleic acid sequence encoding a peptide

in a suitable host cell are well known in the art, such as is described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor, N.Y. (1989), which is incorporated herein by reference.

5

Peptides of the invention can also be produced by chemical synthesis, for example, by the solid phase peptide synthesis of Merrifield (Merrifield et al., J. Am. Chem. Soc., 85:2149 (1964), incorporated herein by reference). Standard solution methods well known in the art also can be used to
10 synthesize a peptide of the present invention (see, for example, Bodanszky, M., Principles of Peptide Synthesis (Springer-Verlag, 1984), which is incorporated herein by reference). Newly synthesized peptides can be purified, for example, by high performance liquid chromatography (HPLC), and can be characterized using, for example, mass spectrometry
15 or amino acid sequence analysis.

In some specific embodiments, the isolated polypeptide of the invention is a cyclic polypeptide, while in some other embodiments, the isolated polypeptide of the invention is linear.

20

As used herein, the term "cyclic peptide" refers to a peptide having an intramolecular bond between two non-adjacent amino acids. The cyclization can be effected through a covalent or non-covalent bond. Intramolecular bonds include, but are not limited to, backbone to
25 backbone, side-chain to backbone and side-chain to side-chain bonds. For instance, a cyclic peptide according to the invention may comprise a disulfide bond or a lactam bridge.

According to some embodiments, the isolated polypeptide of the invention
30 further comprises an amino acid residue at the N-terminus and/or at the C-terminus. Preferably, those supplementary amino-acid residues are selected

from the group consisting of cystein, homocystein and any other alpha or beta thiol residue.

In some specific embodiment of the invention, a cysteine residue (L or D) is added to the N- or C-terminus extremity of the peptide in order to allow cysteine-cysteine cyclisation through disulfide covalent bond formation. In some other embodiments, a cysteine residue (L or D) is added to the N-terminus extremity of the peptide, and a cysteine residue is inserted near the C-terminal extremity or a amino-acid is mutated to a cysteine (L or D). In still other embodiments, a cysteine residue (L or D) is added to C-terminus peptide extremity, and a cysteine residue is inserted near the N-terminal extremity or a amino-acid is mutated to a cysteine (L or D). In still other embodiments of the invention, peptide cyclisation through disulfide covalent bond may be done by modification of C-terminal and N-terminal extremity by addition of amino-ethantiol and thioacetic acid, respectively. According to some other embodiments of the invention, the disulfide bond is not achieved via two cysteine residues but includes either one or two homocysteine and unnatural alpha or beta-thiol amino-acids.

A newly synthesized linear peptide can thus be cyclized by the formation of a bond between reactive amino acid side chains. For example, a peptide containing a cysteine-pair, or any of the cysteine analogs can be synthesized, and a disulfide bridge can be formed by oxidizing the peptide. Alternatively, a lactam, a lysinonorleucine or a dityrosine bond can be formed. Methods for forming these and other bonds are well known in the art and are based on well established principles of chemical reactivity (Morrison and Boyd, Organic Chemistry, 6th Ed. (Prentice Hall, 1992), which is incorporated herein by reference).

As discussed above, a peptide of the present invention also can be cyclized by forming a peptide bond between non-adjacent amino acid residues as described, for example, by Schiller et al., Int. J. Pept. Prot. Res. 25:171

(1985), which is incorporated herein by reference. Peptides can be synthesized on the Merrifield resin by assembling the linear peptide chain using N-Fmoc-amino acids and Boc and tertiary-butyl proteins. Following release of the peptide from the resin, a peptide bond can be formed
5 between the amino and carboxyl termini.

In some specific embodiments, the isolated polypeptide of the invention comprises or consists of at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 16 (GNS-255 to GNS-261,
10 GNS-265, GNS-267 to GNS-272, GNS-299, and GNS-300).

All the peptides of the invention exhibit an antiviral activity against human immunodeficiency virus (HIV). As used herein, the term "HIV" encompasses all HIV types, groups, clades, strains and mutants of HIV,
15 such as HIV-1 and HIV-2 types.

As used herein, the expression "antiviral activity" refers to the activity of an agent that prevents, inhibits, or reduces the viral activity of a virus or the activity of a compound which destroys a virus, preferably HIV. As used
20 herein, “viral activity” refers to the ability of a virus to replicate, multiply, reproduce or infect a cell or a subject.

The term "prevent" means that the progression of a virus infection, preferably a HIV infection, is reduced and/or eliminated, or that the onset
25 of the virus infection, preferably the HIV infection, is delayed or eliminated, in a subject having been exposed to (i.e. in contact with) said virus.

The term "inhibition" as referred to herein, relates to the retardation,
30 attenuation, retraining or reduction of a process, said process being related to a virus infection, preferably a HIV infection. The degree of inhibition of a specific peptide may be assessed for instance by

determining the half maximal inhibitory concentration (IC_{50}) of said peptide towards the target virus, by using techniques well known in the art. The peptides of the invention, either alone or coupled to a cell delivery agent, typically exhibit an IC_{50} towards HIV under a value selected from 5 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, and 0.5 nM.

The term "decrease" or "reduce", as referred to herein, relates to a decrease in value, amount, or rate. A decrease or a reduction as referred to 10 herein, relate to the a reduction or lessening of a quantity or process by any one of about 1% to 5%, about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, about 25% to 30%, about 30% to 35%, about 35% to 40%, about 40% to 45%, about 45% to 50%, about 50% to 55%, about 55% to 60%, about 60% to 65%, about 65% to 70%, about 75% 15 to 80%, about 80% to 85% about 85% to 90%, about 90% to 95%, about 95% to 99%, or about 99% to 99.9%.

It was surprisingly found by the inventors that the PICI peptides of the invention exhibit very characteristic common features.

20

First, the PICI peptides of the invention tightly bind IN. As shown in Example 4 below, the peptides form stable complex with HIV-1 IN (either recombinant or expressed in cells) in pull-down assays and steady-state fluorescence titrations. Stable interactions correspond to dissociation 25 constant (Kd) in the nanomolar range, typically under a value selected from 300 nM, 250 nM, 200 nM, 150 nM, 100 nM and 50 nM. This characteristic of the peptide of the invention make them suitable for use as capturing agent for recombinant IN or IN contained in cell lysate (H9 expressing GAG-POL gene products of HIV-1, or HA-tagged IN or Hela) 30 when peptides are for instance covalently associated to CNBr sepharose beads.

Second, PICI peptides of the invention poorly interact with RT as monitored by fluorescence spectroscopy, with Kd values typically above a value selected from 2.5 μM, 5 μM, 10 μM, 15 μM and 20 μM (50 to 100-fold higher than for IN). Furthermore, the peptide of the invention poorly
5 inhibits RT polymerase activity, the inhibition constant (Ki) being typically above a value selected from 35 μM, 40 μM, 45 μM, 50 μM, 100 μM and 200 μM. Accordingly, peptides of the invention do not retain RT on resin. Peptides block IN/DNA interaction and promote IN/DNA complex dissociation at sub-micromolar concentrations. In contrast peptides do not
10 block RT/DNA (RT/pt) interaction.

Furthermore, it was surprisingly found that the PICI peptides of the invention block Integrase/Reverse Transcriptase complex (IN-RT/pt) formation and induce IN-RT/pt complex dissociation (see Figs 3A, 3B and
15 4). The different peptides block the formation of the "core" of the pre-integration complex (IN-RT/pt) and induced its dissociation. Formation and dissociation of RT/IN have been evaluated using fluorescence spectroscopy. The binding of IN to RT/pt is associated to a large increase in the primer/template fluorescence leading to a Kd of IN for RT/pt of 54
20 nM. A concentration of peptides below a value selected from 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 50 nM, and 25 nM, is sufficient to completely block the formation of the complex and to induce its dissociation (see Example 5 below).

25 It was also surprisingly found that the PICI peptides of the invention does not induce the apparition of drug resistance for a period selected from at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 1 year, at least 2 years, at least 5 years, and at least 10 years (see Example 8)

30

According to some specific embodiments, the isolated polypeptide of the invention exhibits an antiviral activity against all the HIV types, groups,

clades, strains and mutants. In some other embodiments, the isolated polypeptide of the invention exhibits an antiviral activity against at least one HIV-1 strain selected from the group consisting of LAI, 1650, RF, ADA, Ba/L, SF162, 2914, 4110, NDK, 1647, 2165, 2338, 3191, 3187, HIV-1 215Y, HIV-1 67N 70R 215F 219Q, HIV-1 74V, HIV-1 N119/181C, HIV-1 41L 74V 106A 215Y, HIV-1 T69SSG 108I 210W 215, HIV-1 62V 65R 75L 100I 103N 116Y 118L 151M 184V and HIV-1 N155H/Q148K.

In some embodiments, the peptide of the invention is coupled to a cell delivery agent. In some specific embodiments, this cell delivery agent is a non-covalent peptide carrier vector, such as one comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 21 (see Table 1).

As used herein, the term "coupled" means that the polypeptide of the present invention (named cargo) and the cell delivery agent are non-covalently.

The term "cell delivery agent" refers to a compound capable of delivering or enhancing the delivery of a polypeptide inside the cells *in vitro* and/or *in vivo* (*i.e.*, facilitating the cellular uptake of a polypeptide). Non-limitative examples of cell delivery agents are cell-penetrating peptides (CPP), liposomes, nanoparticles and polycationic molecules (such as cationic lipids). Cell delivery agents are well known to one skilled in the art. The term "cell-penetrating peptide" refers to a peptide of less than 40 amino-acids, preferably less than 30 amino acids, derived from natural or unnatural protein or chimeric sequences, and capable to trigger the movement of the polypeptide of the invention (the cargo) across the cell membrane into the cytoplasm. CPP can be either polycationic, essentially containing clusters of polyarginine in its primary sequence or amphipathic. Advantageaous cell delivery agents are described previously (Divita *et al.*, 2001; Morris *et al.*, 2010), such as the peptides vector Pep-1

(SEQ ID NO: 20), Pep-3 (SEQ ID NO: 21) and Pep-3a (SEQ ID NO: 22). In specific embodiments, the polypeptide of the present invention is non-covalently coupled to a peptide vector, preferably the peptides Pep-1, Pep-3 and Pep-3a.

5

The invention further provides an isolated polypeptide according to the above or any derivative thereof for use as a medicament. In some specific embodiments, the invention provides an isolated polypeptide for use in the treatment, amelioration or prevention of a viral infection, preferably a HIV
10 infection, and more preferably a HIV-1 infection.

As used herein, the term "treatment" refers to an improvement of at least one undesired manifestation of the viral infection. The term "amelioration" as referred to herein, relates to the inhibition or reduction of at least one
15 of clinical symptoms associated with the viral infection. The term "prevention" or "prophylaxis" refer to the reduction of the risk of occurrence of a biological or medical event associated with the viral infection.

20 In a further aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of at least one polypeptide of the invention or derivatives thereof and a pharmaceutically acceptable carrier, diluent, excipient and/or additive.

25 The term “pharmaceutical composition”, as used herein refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

30

As used herein the term “active ingredient” refers to peptides alone or coupled to a cell delivery agents of the present invention accountable for

the intended biological effect. It will be appreciated that a polynucleotide encoding a peptide of the present invention may be administered directly into a subject (as is, or part of a pharmaceutical composition) where it is translated in the target cells i.e. by gene therapy. Accordingly, the phrase
5 "active ingredient" also includes such polynucleotides.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents and the like. The use of such media and agents for pharmaceutical active
10 substances is well known in the art. Except as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic composition is contemplated.

A carrier should be both pharmaceutically and physiologically acceptable
15 in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by
20 any methods well known in the art of pharmacy. The nature, availability and sources, and the administration of all such compounds including the effective amounts necessary to produce desirable effects in a subject are well known in the art and need not be further described herein.

25 Excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gum tragacanth or gelatin, natural and synthetic gums such as
30 acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl

cellulose), polyvinyl pyrrolidinones, methyl cellulose, pro-gelatinized starch, hydroxypropyl methyl cellulose, microcrystalline cellulose, and mixtures thereof.

5 Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof.

10

Disintegrants can be used in the pharmaceutical compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets containing too much disintegrant might disintegrate in storage, while those containing too little might not disintegrate at a
15 desired rate or under desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form the pharmaceutical compositions and solid oral dosage forms described herein. The amount of disintegrant used varies based upon the type of
20 formulation, and is readily discernible to those of ordinary skill in the art. Disintegrants that can be used in pharmaceutical compositions and oral or mucosal dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, Primogel, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium,
25 sodium starch glycolate, corn, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage
30 forms of the invention include, but are not limited to, calcium stearate, magnesium stearate or Sterotes, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium

lauryl sulfate, talc, hydrogenated vegetable oil (e. g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W. R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL03 (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated. A glidant such as colloidal silicon dioxide can also be used.

Pharmaceutical compositions comprising the peptide of the present invention are useful for parenteral administration, i.e., intraperitoneally (i.p.), subcutaneously (s.c.), intramuscularly (i.m.) and intravenously (i.v.), as well as for oral and topical application. The compositions for parenteral administration commonly comprise a solution of the peptide or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate. The concentration of the peptides of the invention in these formulations can vary widely, i.e., from less than about 0.01%, usually at least about 0.1% to as much as 5% by weight and will be selected primarily based on fluid volumes, and viscosities in accordance with the particular mode of administration selected.

More specifically, injectable compositions that include the peptides of the invention may be prepared in water, saline, isotonic saline, phosphate-buffered saline, citrate-buffered saline, and the like and may optionally be mixed with a nontoxic surfactant. Under ordinary conditions of storage
5 and use, these preparations may contain a preservative to prevent the growth of microorganisms. Pharmaceutical dosage forms suitable for injection or infusion include sterile, aqueous solutions or dispersions or sterile powders comprising an active ingredient which powders are adapted for the extemporaneous preparation of sterile injectable or
10 infusible solutions or dispersions. Preferably, the ultimate dosage form is a sterile fluid and stable under the conditions of manufacture and storage. A liquid carrier or vehicle of the solution, suspension or dispersion may be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol such as glycerol, propylene glycol, or liquid polyethylene
15 glycols and the like, vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. Proper fluidity of solutions, suspensions or dispersions may be maintained, for example, by the formation of liposomes, by the maintenance of the desired particle size, in the case of dispersion, or by the use of nontoxic surfactants. The prevention of the action of
20 microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Isotonic agents such as sugars, buffers, or sodium chloride may be included. Prolonged absorption of the injectable compositions can be brought about by the inclusion in the composition of
25 agents delaying absorption, for example, aluminum monosterate hydrogels and gelatin. Solubility enhancers may be added.

Sterile injectable compositions may be prepared by incorporating the peptides of the invention in the desired amount in the appropriate solvent
30 with various other ingredients, e.g. as enumerated above, and followed by sterilization, as desired, by, for example filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, methods

of preparation include vacuum drying and freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in a previously sterile-filtered solution. Any suitable sterilization process may be employees, such as filter sterilization, e.g. 0.22 micron filter or nanofiltration, gamma or electron beam sterilization.

Still further, the compositions of the invention may be presented in unit dose forms containing a predetermined amount of each active ingredient per dose. Such doses can be provided in a single dose or as a number of discrete doses. The ultimate dose will of course depend on the condition being treated, the route of administration and the age, weight and condition of the patient and will be at the doctor's discretion.

As indicated above, in addition to the parenteral route, the compositions of the invention may be adapted for administration by any other appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal) or vaginal route. Such formulations may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the carrier(s) or excipient(s).

Pharmaceutical formulations adapted for oral administration may be presented as discrete units such as capsules or tablets, powders or granules, solutions or suspensions in aqueous or non-aqueous liquids, edible foams or whips, or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

Pharmaceutical formulations adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time.

Pharmaceutical formulations adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

5 For applications to the eye or other external tissues, for example the mouth and skin, the formulations are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either paraffin or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an 10 oil-in-water cream base or a water-in-oil base.

Pharmaceutical formulations adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

15

Pharmaceutical formulations adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

It should be appreciated that the pharmaceutical composition of the 20 invention may comprise the active compound in free form and be administered directly to the subject to be treated. Alternatively, depending on the size of the active molecule, it may be desirable to conjugate it to a carrier prior to administration. Therapeutic formulations may be administered in any conventional dosage formulation. Formulations 25 typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof.

Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and 30 not injurious to the patient. Formulations include those suitable for topical, oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intraperitoneal (IP), intravenous (IV) and intradermal) administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

In a further aspect, the invention provides a method for the treatment, amelioration or prevention of a viral infection in a subject in need thereof, preferably a HIV infection. The method comprises administering to said subject a therapeutically effective amount of at least one isolated polypeptide of the invention or any derivative thereof, or a pharmaceutical composition comprising the same.

By "patient" or "subject in need" is meant any mammal for which administration of the peptides of the invention, or any pharmaceutical composition of the invention is desired, in order to prevent, overcome or slow down a viral infection. The peptides and compositions of the invention may also be administered to delay the onset of the viral infection, that is, the peptides and compositions of the invention postpone the deterioration of the viral infection or slow down its progress such that clinical signs of said viral infection would appear later than they would without treatment.

As used herein, the term "therapeutically effective amount" means an amount of a compound or composition which is administered to a subject in need thereof, necessary to effect a beneficial change in the severity of a disease or disorder, or prevent such disease, in said subject. This amount should also be within specific pharmacological ranges, to avoid toxic effects by over-dosing. For example, in the present invention, a therapeutically effective amount of at least one of the polypeptides of the invention, for the treatment of a HIV infection would be the amount of these proteins

administered to a subject which would induce a beneficial change in the subject, alleviating, ameliorating, or preventing the recurrence of infection symptoms, without causing detrimental side-effects, or causing only mild side-effects. It is understood that the therapeutically effective amount is not an absolute term and depends on subjective circumstances, such as the subject's age, health, weight, and various other statistics, as described in the and specifically determined by the attendant physician or other person skilled in the art after an evaluation of the subject's conditions and requirements.

It should be further noted that for the method of treatment and prevention provided in the present invention, said therapeutic effective amount, or dosage, is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. In general, dosage is calculated according to body weight or/and body surface area, and may be given once or more daily, weekly, monthly, every 3 months, every 6 months or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the polypeptide used by the invention or any composition of the invention in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the combined composition of the invention is administered in maintenance doses.

The invention still further provides monoclonal or polyclonal antibodies which specifically recognize one or more polypeptides of the invention or any derivative thereof.

5 As employed herein, the term "antibody" generally relates to a naturally derived, or naturally produced antibody, and can be either polyclonal antibodies or monoclonal. Alternatively, the antibodies of the invention may be synthetically produced by e.g. chemical synthesis, or recombinantly produced through the isolation of the specific mRNA from 10 the respective antibody-producing cell or cell line. Said specific mRNA shall then undergo standard molecular biology manipulations (obtaining cDNA, introducing said cDNA into expression vectors, etc.) in order to generate a recombinantly produced antibody. Said techniques are well known to the man skilled in the art.

15

The generation of polyclonal antibodies against proteins is a technique well known to the man skilled in the art, and it is described, *inter alia*, in Chapter 2 of Current Protocols in Immunology, John E. Coligan *et al.* (eds.), Wiley and Sons Inc.

20

Monoclonal antibodies may be prepared from B cells taken from the spleen or lymph nodes of immunized animals with the petpides of the invention, in particular rats or mice, by fusion with immortalized B cells under conditions which favor the growth of hybrid cells. The technique of 25 generating monoclonal antibodies is described in many articles and textbooks, such as the above-noted Chapter 2 of Current Protocols in Immunology. Spleen or lymph node cells of these animals may be used in the same way as spleen or lymph node cells of protein-immunized animals, for the generation of monoclonal antibodies. The techniques used in 30 generating monoclonal antibodies are further described by Kohler and Milstein [Kohler and Milstein (1975) Nature 256; 495-497], and in US 4,376,110.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, scFv, Fv, Fab', Fab, diabody, linear antibody, $F(ab')_2$ antigen binding fragment of an antibody which are
5 capable of binding the peptides of the invention or their derivatives [Wahl *et al.* (1983) *J. Nucl. Med.* 24, 316-325]. Fab and $F(ab')_2$ can be produced for example by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). Additionnally, the Fab and $F(ab')_2$ and other fragments of the antibodies
10 provided by the present invention may be tagged with various tags, according to the intended use. These tags may be detectable tags, to facilitate detection, or toxic tags, which would kill tumor cells, or "inducing" tags, which may induce other cells or substances to kill tumor cells. The antibodies provided by the present invention may be of any
15 isotype, IgG, IgM, IgE, IgA or IgD, particularly the polyclonal antibodies, as well as the monoclonal antibodies.

In a further aspect, the invention provides an isolated polynucleotide encoding at least one polypeptide of the invention.

20

As used herein, the term "polynucleotide" refers to polymer of nucleotides, which may be either single- or double-stranded, based on deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The terms should also be understood to include, as equivalents, analogs of either
25 RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides. The term DNA used herein also encompasses cDNA, i.e. complementary or copy DNA produced from an RNA template by the action of reverse transcriptase (RNA-dependent
30 DNA polymerase).

Due to the degenerative nature of the genetic code it is clear that a plurality of different nucleic acid sequences can be used to code for the polypetides of the invention. It should be appreciated that the codons comprised in the nucleic acid sequence of the invention may be optimized (codon-optimized) for expression in any specific host cell, preferably in host cells capable of expressing large quantities of the desired peptides, and most prferably, commercial quantities of said peptides.

The term "codon-optimized" as it refers to genes or coding regions of nucleic acid molecules for transformation of various hosts, refers to the alteration of codons in the gene or coding regions of the nucleic acid molecules to reflect the typical codon usage of the host organism without altering the polypeptide encoded by the DNA.

It should be appreciated that the invention further ecompasses any derivative, mutant, fragment or homolog of the nucleic acid sequences provided by the invention. Specifically, an analogue or derivative of the nucleic acid sequence that may be used by the methods, compositions and uses of the invention may comprise at least one mutation, point mutation, nonsense mutation, missense mutation, deletion, insertion or rearrangement.

A nucleic acid fragment of a sequence according to the invention may comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 101, at least 102, at least 103, at least 104 or at least 121 nucleic acids, or even more.

Still further, the invention provides a recombinant expression vector comprising the polynucleotide described above.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired gene or its fragments, and operably linked genetic control elements that are recognized in a suitable host cell and effect expression of the desired genes. As used herein, the term "construct" encompasses vectors such as plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles, which enable the integration of DNA fragments into the genome of the host. Control elements are capable of effecting expression within a suitable host. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system. This typically includes a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of RNA expression, a sequence that encodes a suitable ribosome binding site, RNA splice junctions, sequences that terminate transcription and translation and so forth. Expression vectors usually contain an origin of replication that allows the vector to replicate independently of the host cell.

A vector may additionally include appropriate restriction sites, antibiotic resistance or other markers for selection of vector-containing cells. Plasmids are the most commonly used form of vector but other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels et al., Cloning Vectors: a Laboratory Manual (1985 and supplements), Elsevier, N.Y.; and Rodriquez, et al. (eds.) Vectors:a Survey of Molecular Cloning Vectors and their Uses, Buttersworth, Boston, Mass (1988), which are incorporated herein by reference.

In a further aspect, the invention is directed to a host cell transformed or transfected with the expression vector according to the invention.

"Cells", "host cells" or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to

the particular subject cells but to the progeny or potential progeny of such a cell. Because certain modification may occur in succeeding generation due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the
5 scope of the term as used herein.

"Host cell" as used herein refers to cells which can be recombinantly transformed with naked DNA or expression vectors constructed using recombinant DNA techniques. A drug resistance or other selectable
10 marker is intended in part to facilitate the selection of the transformants. Additionally, the presence of a selectable marker, such as drug resistance marker may be of use in keeping contaminating microorganisms from multiplying in the culture medium. Such a pure culture of the transformed host cell would be obtained by culturing the cells under conditions which
15 require the induced phenotype for survival.

The host cells of the invention are transformed or transfected with the expression vector described herein to express the peptides of the invention. "Transformation", as used herein, refers to a process in which a cell's
20 genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of at least one of the peptides of the invention. The term "transfection" means the introduction of a nucleic acid, e.g., naked DNA or an expression vector, into a recipient cells by nucleic acid-mediated gene transfer.

25

The invention further provides a kit for the treatment, amelioration or prevention of a HIV infection, comprising:

i) a pharmaceutical composition comprising a therapeutically effective amount of at least one polypeptide of the invention or any derivative
30 thereof and a pharmaceutically acceptable carrier, diluent, excipient and/or additive; wherein each of said at least one polypeptide or derivative is optionally comprised within a separate compartment.

and

ii) instructions for the administration of said pharmaceutical composition.

5 The pharmaceutical composition of the kit may be administered in the form of a single pharmaceutical composition comprising at least one polypeptide of the invention, together with a pharmaceutically acceptable carrier or diluent. Alternatively, the pharmaceutical composition of the kit may have at least one of its components comprised in a pharmaceutical 10 composition stored in a separate compartment. In such a case, the kit includes container means for containing both separate compositions, such as a divided bottle or a divided foil packet. However, the separate compositions may also be contained within a single, undivided container comprising several compartments.

15

It should be appreciated that when the composition of the kit is comprised of at least two dosage forms, for instance one comprising at least one peptide of the invention and another one comprising at least one other peptide of the invention, both dosage forms may be administered 20 simultaneously. Alternatively, said first dosage form and said second dosage form are administered sequentially in either order.

Typically, the kit includes instructions for the administration of the separate components. The kit form is particularly advantageous when 25 separate components are administered in different dosage forms, at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

Disclosed and described, it is to be understood that this invention is not 30 limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose

of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

5 It must be noted that, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise.

Throughout this specification and the claims which follow, unless the 10 context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

15 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related. The following terms are defined for purposes of the invention as described herein.

20 The following Examples are representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications 25 can be made without departing from the spirit and intended scope of the invention.

Example 1

PICI peptides synthesis

30

Short peptide has been selected within the motifs exposed at the surface of the thumb domain of the HIV-1 RT. The peptide obtained was further reduced at both C- and N-terminus to obtain the group of PIC inhibitors

(PICI) sequences as shown in Table 1 below. Two scrambled PICI peptide was used as control in all the reported experiments (GNS-Scr1 and GNS-Scr2). Peptides were synthesized in the laboratory by solid-phase peptide synthesis on a Fmoc-continuous flow apparatus (Pionner, Applied Biosystems, Foster city, CA) starting from Expansin-resin bearing a home-made AEDI-linker and purified by reverse phase HPLC. The PIC inhibitors of the invention inhibit the formation of the PIC or RTC (reverse transcription complex) and exhibit an important antiviral activity on HIV viruses, especially those belonging to the type HIV-1, including resistant strains to non-nucleoside and nucleoside reverse transcriptase inhibitors and to the integrase inhibitor Raltegravir.

Table 1

SEQ. ID N°	Peptide name	Sequences 12345678901234	Comment
1	PICI	XXWLXXWIPXXXX	**Family**
2	GNS-255	TKWLTEWIPWTWAC	Linear peptide
3	GNS-256	TRWLTEWIPWRWAC	Linear peptide
4	GNS-257	SRWLSRWIPWSWAC	Linear peptide
5	GNS-258	TKWLTEWIPWTWLC	Linear peptide
5	GNS-259	TKWLTEWIPFTFAC	Linear peptide
6	GNS-260	CTKWLTEWIPWTWAC	Cysteine cyclic peptide
7	GNS-261	CKWLTEWIPWTWAC	Cysteine cyclic peptide
8	GNS-265	TKWLTEWIPWTWA	Linear peptide
9	GNS-267	TKWLTEWIPWTWA	Lactam cyclic peptide
10	GNS-268	KWLTEWIPWTW	Lactam cyclic peptide
11	GNS-269	TKWLTEWIPWTW	Lactam cyclic peptide
12	GNS-270	T***KW***L ***TE***W ***IP***W ***TW***A	Lactam cyclic peptide

13	GNS-271	T***KW***L***TE***WIP***WT***W	Lactam cyclic peptide
14	GNS-272	TK***W***LT***EW***IPWT***W***	Lactam cyclic peptide
15	GNS-299	KWLTEWIPWTWA	Linear peptide
16	GNS-300	CTRWLTEWIPWRWAC	Cysteine cyclic peptide
18	GNS-Scr1	LTEWTWAWIPKWTC	Scrambled peptide 1
19	GNS-Scr2	TKLATEIWPTWAAC	Scrambled peptide 2
20	Pep-1	Ac-KETWWETWWTEWSQPKKKRKV-Cya	Peptide carrier
21	Pep-3	Ac-KWFETWFTEWPKKRK-Cya	Peptide carrier
22	Pep-3a	Ac-KWFETWFTEWPKRRK-Cya	Peptide carrier

Amino-acids in bold and italic are D-amino acid residues.

Example 2

5

PICI peptides exhibit marked anti-HIV activity in cell lines.

The ability of the PICI peptides family to block viral progression was evaluated on MT4 cells infected with HIV-1 LAI. In order to overcome any
10 cellular uptake limitation, PICI peptides were coupled to the peptide-based cell delivery agent Pep-1. Pep-1 has been used for the delivery of peptides and proteins in numerous cell lines as well as *in vivo* (Divita *et al.*, 2001; Morris *et al.*, 2010). When associated with Pep-1 and similar peptide carrier as Pep-3 and Pep-3a, PICI peptides show an improved
15 inhibition of HIV-1 proliferation. In fact, the cellular uptake of PICI/Pep-1 complexes formed at a molar ratio ranging from 1/10 to 1/40, preferentially 1/20, was analyzed by using fluorescently-labelled PICI peptides. When associated to Pep-1 nanoparticles, peptide rapidly enters the cells and localized in both cytoplasm and nucleus. PICI peptides showed a
20 significant antiviral activity at sub micromolar concentrations on HIV-1 LAI. Specifically, the IN-tight binder peptide GNS-255 and GNS-258 exhibited strong anti-HIV activity with an IC_{50} of 0.6 ± 0.1 nM. Moreover, GNS-255 is not toxic up to 500 μM, which leads to a selectivity index of

about 5000, as determined by MTT/MT4 assay. As a control, empty Pep-1 particles do not show any antiviral activity.

5

Example 3

PICI peptides exhibit marked anti-HIV activity in primary cells

A) The ability of PICI peptides, without cell delivery agent, to block viral progression was evaluated on Phytohemaglutinin-P (PHA-P)-activated
10 peripheral blood mononuclear cells (PBMC). PHA-P-activated PBMC were 30 minutes pre-treated by increasing concentrations of GNS 271 and GNS 272 compounds and then infected with the reference HIV-1-LAI strain (Barré-Sinoussi et al., 1983; One hundred and twenty five 50% tissue culture infectious doses (TCID50) per 125,000 cells). This virus was
15 amplified in vitro on PHA-P-activated PBMC and the viral stock was previously titrated using PHA-P-activated PBMC and the Kärber's formula (Kärber, 1931). Seven days post-infection, cell culture supernatants were collected and stored at -20°C. Viral replication was then measured in these cell culture supernatants by quantifying the
20 retrotranscriptase activity using Lenti RT activity kit ELISA (Cavidi). GNS 271 and GNS 272 show a significant antiviral activity with an EC_{50} of 25 nM and 37 nM respectively. At day 7 of culture, cytotoxicity of PICI peptides tested was evaluated in uninfected PHA-P-activated PBMC using the methyltetrazolium salt (MTS/PMS) assay (Promega). GNS-271 and
25 GNS-272 are not toxic up to 50 μM, which leads to a selectivity index of about 2000 for GNS-271 and 1351 for GNS-272.

B) Anti-HIV activity of the four peptides, GNS-255, GNS-256, GNS-260 and GNS-261, was evaluated on Cord Blood Mononuclear Cells (CBMC).
30 PICI peptides were coupled to the cell based delivery agent Pep-1 as above at a molar ratio of 1/20. Complexes were prepared at a concentration of 0.4 mM in a solution of 5% DMSO in water. Five-fold dilutions were made in

media, beginning at 5000 nM which was filter-sterilized. Raltegravir was used as a control compound. CBMC were subjected to an acute infection with HIV-IIIb (Wild-type) lab strain at an MOI: 0.1 (2 h incubation). The compounds were added post-infection, for a duration of 7 days, with a
5 media & drug change at day 3. On day 7, aliquots of the supernatant from each well were assayed for RT activity. Mock-infected CBMC, treated with the same drug dilutions and conditions, were counted on day 7 to determine the cytotoxicity ($CCID_{50}$) of the compounds. The IC_{50} (for anti-HIV activity) and $CCID_{50}$ (for cytotoxicity) were calculated using the
10 Prism program dose-response curve analysis (one-site competition). Results are displayed in Table 2 below.

Table 2

Results	GNS-261/Pep-1	GNS-260/Pep-1	GNS-255/Pep-1	GNS-256/Pep-1	Raltegravir
IC_{50} (nM)	0.706	2.218	0.380	4.841	18.210
$CCID_{50}$ (nM)	200	200	40 - 200	200	>200,000
S.I.	283	90	105 - 526	41	>11,000

15

In conclusion, it can be seen from the above experiments that the four compounds tested displayed a potent activity as anti-HIV agents when peptides alone displayed an IC_{50} in the 20 – 40 nM range and when
20 associated to the Pep-1 cell delivery agent, an IC_{50} ranging from 0.3 to 5 nM.

Example 4

25 ***PICI peptides tightly bind IN***

The different peptides of the PICI family were screened on their ability to form stable complex with HIV-1 IN either recombinant or expressed in cells, by pull-down and steady-state fluorescence titration. Peptides

covalently associated to CNBr sepharose beads, were incubated with either recombinant IN or cell lysate of H9 and Hela cells, expressing *gag-pol* gene products of HIV-1, or HA-tagged IN, respectively. PICI peptides were able to interact with cellular HA-tagged IN and to retained cellular IN when expressed at low level in H9 cells. The direct interactions between the PICI peptides and either IN or RT were further assessed by steady-state fluorescence spectroscopy using fluorescein-labeled peptides. Upon addition of IN or RT, fluorescence of PICI peptides increased significantly. The titration binding curve of GNS-255 with IN leads to an apparent dissociation constant of Kdapp ≈ 150 nM (Fig. 2). A strong interaction with IN was also reported for GNS-255 to GNS-261. Moreover, this PICI peptides/IN tight interaction has been also demonstrated via pull down experiments.

Example 5

PICI peptides block IN/RT complex formation and are able to dissociate pre-formed IN/RT complex

The impact of the different peptides on the formation of the “core” of the PIC (RT/IN) has been evaluated using fluorescence spectroscopy. A fluorescently labelled double strand 19/36-mer DNA/DNA primer/template (pt), with 5’-TCCCTGTTCGGGCGCCACT-3’ (SEQ ID NO: 23) for the primer strand and 5’-TGTGGAAAATCTCATGCAGTGGCGCCCGAACA GGGA-3’ (SEQ ID NO: 24) for the template-strand was used (obtained for Eurogentec SA, Belgium). The sequence of the template strand corresponds to the sequence of the natural primer binding site (PBS) of HIV-1. The primer was labelled at the 3’-end with 6-carboxyfluorescein (6-FAM) on thymine base. Primer and template oligodeoxynucleotides were separately resuspended in water and diluted to 100 μM in annealing buffer (25 mM Tris pH 7.5 and 50 mM NaCl). Oligonucleotides were mixed together and heated at 95°C for 3 min, then cooled to room temperature

for 1h. As shown in Fig. 3A, the binding of IN to the complex RT/pt is associated to a large increase in the primer/template fluorescence leading to a Kd of IN for RT of 54 nM, and the presence of GNS-255 inhibit the binding of IN onto RT. It was also demonstrated that a concentration of 500 nM of either GNS-255 or GNS-261 peptide is able to dissociate the IN-RT/pt complex (Fig 3B).

Example 6

PICI peptides do not significantly inhibit RT polymerase activity

The ability of the PICI peptide family to block polymerase activity of HIV RT was evaluated using DNA template. RNA-dependent-DNA RT-polymerase activity was measured in a standard reaction assay using poly(rA)-(dT)15 as template/primer as previously described (Restle *et al.*, 1990 , Agopian *et al.*, 2009). Briefly, 10 μL of RT at 20 nM was incubated at 37 °C for 30 min with 20 μL of reaction buffer (50 mM Tris, pH 8.0, 80 mM KCl, 6 mM MgCl2, 5 mM DTT, 0.15 μM poly(rA-dT), 15 μM dTTP, 0.3 μCi 3H-dTTP). For peptide evaluation, HIV-1 RT was incubated with increasing concentrations of peptide inhibitors for 3 hr, and polymerase reaction was initiated by adding reaction buffer. Reactions were stopped by precipitation of nucleic acids with 5 ml of 20% trichloroacetic acid (TCA) solution for 2 h on ice, then filtered using a multiwell-sample collector (Millipore), and washed with 5% TCA solution. Filters were dried at 55°C for 30 min and radionucleotide incorporation was determined by liquid scintillation spectrometry. The inhibition constants Ki_{DNA} were calculated from Dixon plots and reported data correspond to the mean of three separate experiments. As shown in Table 3, PICI peptides (GNS-255, GNS-256, GNS-260 and GNS-261) did not inhibit significantly polymerase activity of HIV RT compared to NRTI (for example Ki_{DNA} = 1.6 μM for Tenofovir diphosphate).

Table 3

PICI peptides	Ki_{DNA} (µM)
GNS-255	45 ± 5
GNS-256	91 ± 10
GNS-260	123 ± 5
GNS-261	98 ± 10

5

Example 7

PICI peptides blocks replication of various HIV-1 subtypes, even those reported as resistant to NRTI, NNRTI, and to Raltegravir

10 GNS-255 coupled to the peptide-based delivery system Pep-1 (or Pep-3) (with a molar ratio of 1/20) was evaluated on different HIV-1 clades (from A to G) as well as on HIV-1 resistant strains to Nucleoside analog Reverse-Transcriptase Inhibitors (NRTI), to Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTI), and to Raltegravir. A comparison of efficacy was also 15 made with azidothymidine (AZT). The results are disclosed in Table 4 below:

Table 4

Strain	Clade	GNS-255 ED_{50} (nM)	AZT ED_{50} (nM)
LAI		0.3	2.3
1650	A	0.53	34
RF	B	2.1	2.1
ADA	B	0.3	3
Ba/L	B	0.5	14
SF162	B	5.4	2.4
2914	C	0.6	>500
4110	C	17	>500
NDK	D	4	NA*
1647	D	0.8	450
2165	E	0.1	42
2338	F	18	>1000
3191	G	4.7	190
3187	G	9.7	70
HIV-1 215Y		0.16	NA*
HIV-1 67N, 70R, 215F, 219Q		0.2	NA*
HIV-1 74V		0.3	NA*
HIV-1 N119/181C		0.4	NA*

HIV-1 41L, 74V, 106A, 215Y		1.4	NA*
HIV-1 T69SSG, 108I, 210W, 215		0.12	NA*
HIV-1 62V, 65R, 75L, 100I, 103N, 116Y, 118L, 151M, 184V		4.3	NA*
HIV-1 N155H/Q148K		0.7	NA*

NA* = Non-Available

The above results show that GNS-255 may be considered as a broad
5 antiviral compound against all HIV-1 subtypes, in particular those which
are resistant to NRTI, NNRTI and/or Raltegravir.

Example 8

10 ***PICI peptides do not induce drug resistance in CBMC***

The selection of HIV resistance to PICI peptides (GNS-299, GNS-265,
GNS-256) was attempted using CBMC and the HIV-IIIb lab strain. In
order to overcome any limitation of cellular uptake, PICI peptides were
15 coupled to the peptide-based cell delivery agent Pep-1 at a molar ratio of
1:20. Phytohemagglutinin-stimulated CBMC were infected with HIV-IIIb
lab strain (MOI of 0.1 to 1.0) for 2 h, incubated at 37°C, and subsequently
resuspended in RPMI 1640 medium (Invitrogen) plus human IL-2 (20
U/ml; ABI Inc.) and 10% fetal bovine serum (FBS) and seeded into a 24-
20 well plate at a density of 2×10^6 to 4×10^6 cells per well. Selections for
resistance were initiated with sub-optimal drug concentrations (below the
EC50 of the drugs) of 0.05 nM for PICI peptides. Drug concentrations
were increased as described by Oliveira and coll. (Prasad VRK et al., 2009
HIV protocols, 2nd ed., vol 485 Humana Press, Totowa, NJ). As a control,
25 HIV-IIIb lab strain was simultaneously passaged without drugs. RT
assays were performed weekly to monitor viral replication as described
previously (Prasad VRK et al., 2009. HIV protocols, 2nd ed, vol 485.
Humana Press, Totowa, NJ). Based on the ratio of the RT values in cell
culture supernatants from control wells to those from test wells at the
30 previous round of replication, drug concentrations were increased at

subsequent passages. The increases in drug concentrations, above those listed above, did not permit residual viral replication.

No drug resistance was obtained over 3 months in tissue culture for GNS-
5 299, GNS-265 and GNS-256. Sequencing of the Integrase gene of viruses recovered from tissue culture after growth in the presence of these compounds did not show evidence of mutagenesis.

Although embodiments of the invention have been described by way of
10 illustration, it will be understood that the invention may be carried out with many variations, modifications, and adaptations, without exceeding the scope of the claims.

15

CLAIMS

5 **1.** An isolated polypeptide comprising or consisting of at least one amino acid sequence denoted by SEQ ID NO: 1:

X1-X2-W-L-X5-X6-W-I-P-X10-X11-X12-X13

10 wherein:

X1 is T, S, or is absent;

X2 is K or R;

X5 is T or S;

X6 is E or R;

15 X10 is W or F;

X11 is T, R or S;

X12 is W or F; and

X13 is A, L, or is absent;

said polypeptide being characterized in that it exhibits an antiviral activity
20 against human immunodeficiency virus (HIV); or any derivative thereof.

2. The isolated polypeptide of claim 1 or a derivative thereof, further comprising an amino acid residue at the N-terminus and/or at the C-terminus, said amino-acid residue being selected from the group consisting
25 of cystein, homocystein and any other alpha or beta thiol residue.

3. The isolated polypeptide of claim 1 or a derivative thereof, wherein at least one of the amino acid residues is a D-amino acid.

30 **4.** The isolated polypeptide of claim 1 or a derivative thereof, wherein said polypeptide is a linear polypeptide.

5. The isolated polypeptide of claim 1 or a derivative thereof, wherein said polypeptide is a cyclic polypeptide.

6. The isolated polypeptide of claim 1 or a derivative thereof, comprising or consisting of at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 16.

5

7. The isolated polypeptide of claim 1 or a derivative thereof, wherein said polypeptide exhibits an antiviral activity against HIV type-1 (HIV-1).

8. The isolated polypeptide of claim 7 or a derivative thereof, wherein
10 said polypeptide exhibits an antiviral activity against at least one HIV-1 strain selected from the group consisting of LAI, 1650, RF, ADA, Ba/L, SF162, 2914, 4110, NDK, 1647, 2165, 2338, 3191, 3187, HIV-1 215Y, HIV-1 67N 70R 215F 219Q, HIV-1 74V, HIV-1 N119/181C, HIV-1 41L 74V 106A 215Y, HIV-1 T69SSG 108I 210W 215, HIV-1 62V 65R 75L 100I 103N 116Y
15 118L 151M 184V and HIV-1 N155H/Q148K.

9. The isolated polypeptide of claim 1 or a derivative thereof, wherein said polypeptide exhibits an antiviral activity against HIV type-2 (HIV-2).

20 **10.** The isolated polypeptide of claim 1 or a derivative thereof, wherein said polypeptide does not induce drug resistance for a period of at least 3 months.

11. The isolated polypeptide of claim 1 or a derivative thereof,
25 characterized in that it is coupled to a cell delivery agent.

12. The isolated polypeptide of claim 11 or a derivative thereof, characterized in that the cell delivery agent is a non-covalent peptide carrier vector.

30

13. The isolated polypeptide of claim 12 or a derivative thereof, characterized in that the peptide vector has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

14. An isolated polypeptide according to any one of claims 1 to 13 or any derivative thereof, for use as a medicament.

5 **15.** An isolated polypeptide according to claim 14 or any derivative thereof for use in the treatment, amelioration or prevention of a HIV infection.

16. An isolated polypeptide according to claim 15 or any derivative
10 thereof for use according to claim 14, wherein the HIV infection is a HIV-1 infection.

17. An isolated polypeptide according to claim 15 or any derivative thereof for use according to claim 14, wherein the HIV infection is a HIV-2
15 infection.

18. A pharmaceutical composition comprising a therapeutically effective amount of at least one isolated polypeptide according to any one of claims 1 to 13 or any derivative thereof and a pharmaceutically acceptable carrier,
20 diluent, excipient and/or additive.

19. A method for the treatment, amelioration or prevention of a HIV infection in a subject, said method comprising administering to said subject a therapeutically effective amount of at least one isolated polypeptide
25 according to any one of claims 1 to 13 or any derivative thereof, or a pharmaceutical composition according to claim 18.

20. A method according to claim 19, wherein the HIV infection is a HIV-1 infection.

30

21. A method according to claim 19, wherein the HIV infection is a HIV-2 infection.

22. A monoclonal or polyclonal antibody which specifically recognizes a polypeptide according to any one of claims 1 to 13 or a derivative thereof.

23. An isolated polynucleotide encoding a polypeptide according to any 5 one of claims 1 to 13.

24. A recombinant expression vector comprising the polynucleotide of claim 23.

10 **25.** A host cell comprising the expression vector of claim 24.

26. A kit for the treatment, amelioration or prevention of a HIV infection, comprising:

i) a pharmaceutical composition comprising a therapeutically effective 15 amount of at least one polypeptide according to any one of claims 1 to 13 or any derivative thereof and a pharmaceutically acceptable carrier, diluent, excipient and/or additive, wherein each of said at least one polypeptide or derivative is optionally comprised within a separate compartment; and

20 ii) instructions for administrating said pharmaceutical composition.

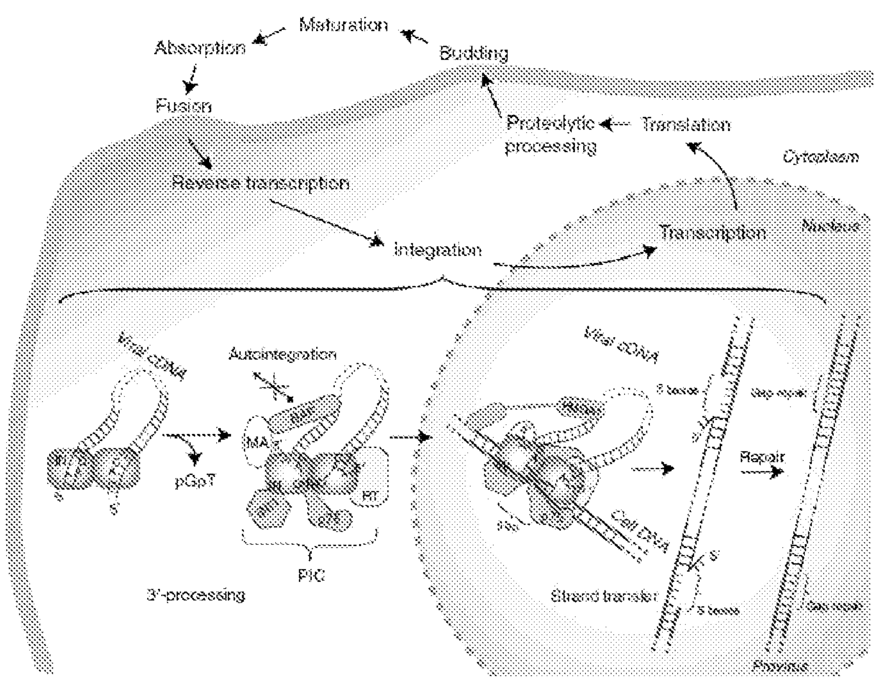

Fig. 1 (PA)

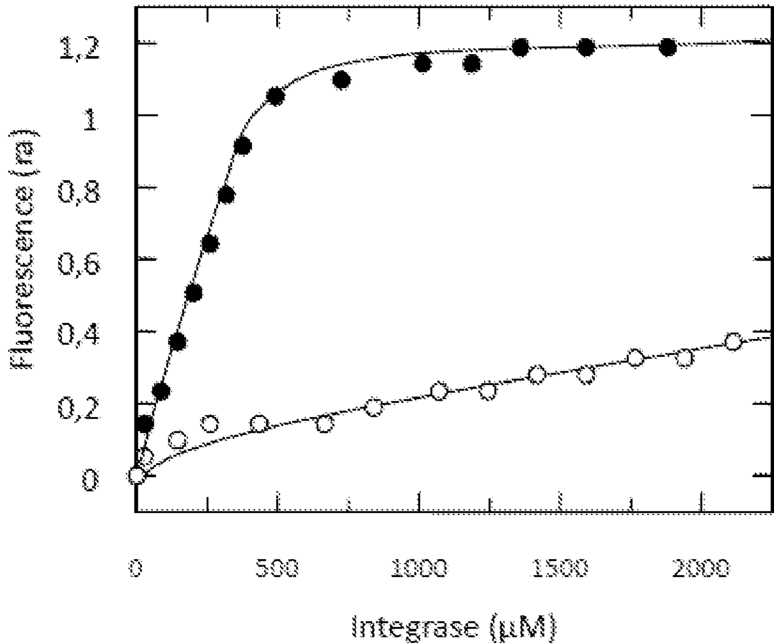

Fig. 2

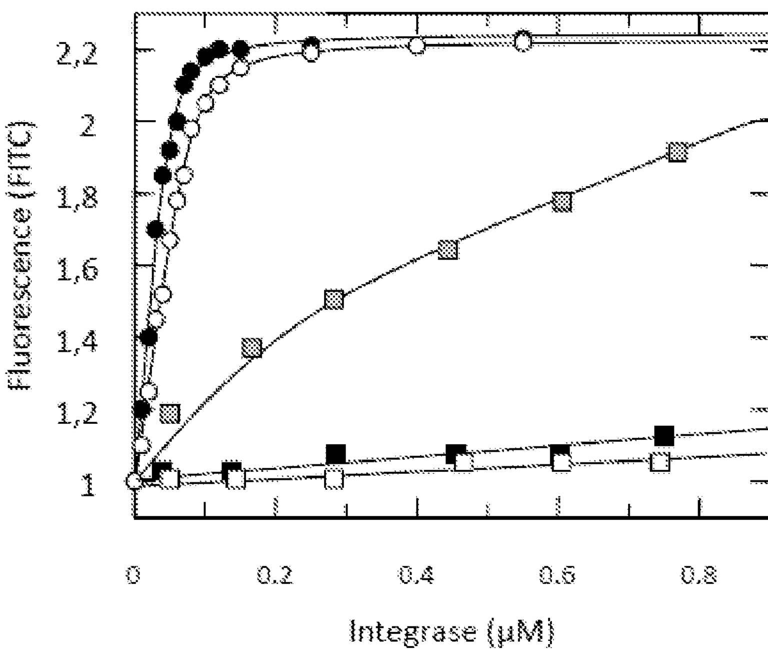

Fig. 3A

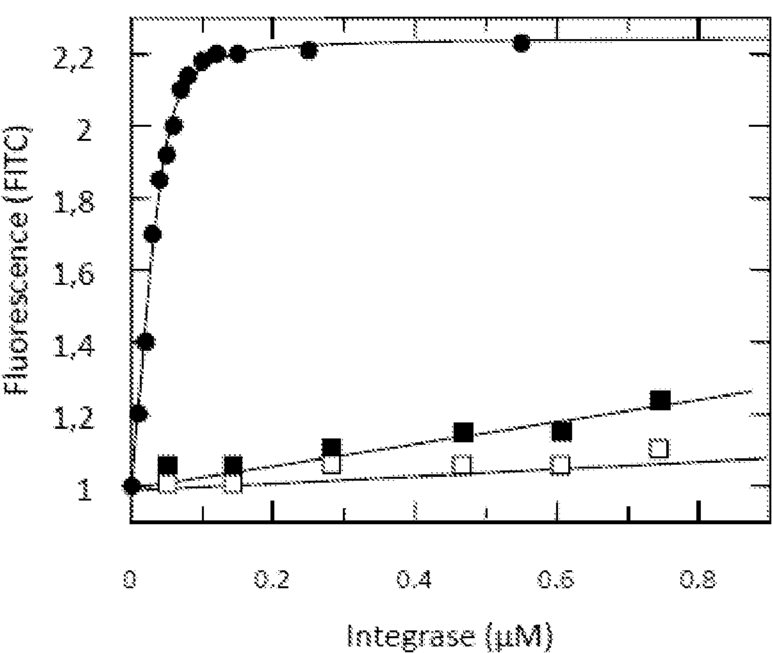

Fig. 3B

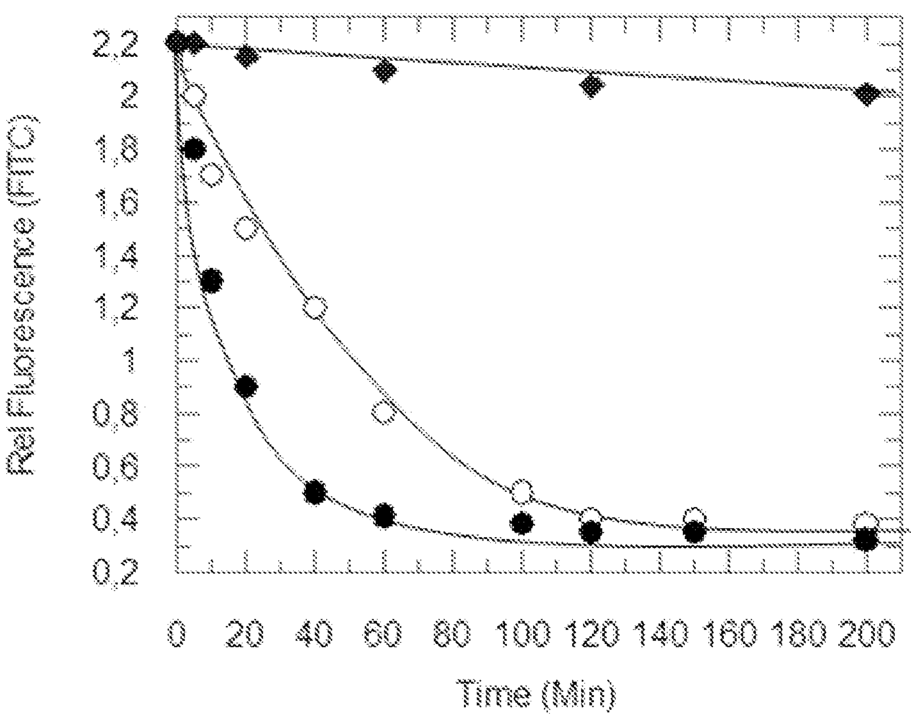

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/001194

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/03 A61P31/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AGOPIAN AUDREY ET AL: "A New Generation of Peptide-based Inhibitors Targeting HIV-1 Reverse Transcriptase Conformational Flexibility", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 284, no. 1, 2 January 2009 (2009-01-02), pages 254-264, XP002511116, ISSN: 0021-9258, DOI: 10.1074/JBC.M802199200 the whole document ----- -/--	1-26

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
14 September 2012	02/10/2012

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	E. Gros et al: "A Potent Cyclic Peptide Inhibitor of HIV-1 Pre-Integration Complex Formation: A New HIV Drug Candidate", , March 2012 (2012-03), XP002683449, Retrieved from the Internet: URL:http://www.retroconference.org/2012b/Abstracts/44327.htm [retrieved on 2012-09-14] the whole document -----	1-26