

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 October 2008 (23.10.2008)

PCT

(10) International Publication Number
WO 2008/128064 A2

(51) International Patent Classification:
A61K 31/4995 (2006.01)

(21) International Application Number:
PCT/US2008/060064

(22) International Filing Date: 11 April 2008 (11.04.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/911,618 13 April 2007 (13.04.2007) US

(71) Applicant (for all designated States except US):
TRUSTEES OF TUFTS COLLEGE [US/US]; 136
Harrison Avenue, Boston, MA 02111 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BACHOVCHIN, William, W.** [US/US]; 75 Cambridge Parkway, Apt. E609, Cambridge, MA 02142 (US). **SANFORD, David, George** [US/US]; 52 Hopkins Street, Reading, MA 01867 (US). **LAI, Hung-sen** [US/US]; 11 Seten Circle, Andover, MA 01810 (US).

(74) Agents: **GORDON, Dana, M.** et al.; Patent Group Foley Hoag LLP, Seaport World Trade Center West, 155 Seaport Blvd., Boston, MA 02210-2600 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

(54) Title: TRUNCATED ANALOGS OF PEPTIDE AND POLYPEPTIDE THERAPEUTICS

(57) Abstract: One aspect of the present invention relates to biologically active truncated peptides and peptide analogs that are resistant to proteolysis. Another aspect of the invention relates to formulations of and methods for using the compounds of the invention.



WO 2008/128064 A2

Truncated Analogs of Peptide and Polypeptide Therapeutics

Related Applications

5 This application claims the benefit of priority to United States Provisional Patent Application serial number 60/911,618, filed April 13, 2007.

Background of the Invention

 Polypeptide and peptide therapeutics are widely used in medical practice. Their ease of production, either by recombinant DNA technology or peptide synthesizers, ensures
10 their continued use in a variety of circumstances in the years to come. Accordingly, polypeptide therapeutics, such as hormones, cytokines and growth factors, represent an important class of therapeutic agents. Certain native polypeptides, however, can be inactivated rapidly in vivo via proteolysis or isomerization. Such inactivation can be inconvenient in cases where it is desired to maintain a consistent or sustained blood level of
15 the therapeutic over a period of time, as repeated administrations are then necessary. In certain instances, one or more of the proteolytic products of the polypeptide can be antagonistic to the activity of the intact polypeptide. In these cases, administration of additional therapeutic alone may be insufficient to overcome the antagonist effect of the proteolytic products.

20 To further illustrate, one class of peptide hormones whose prolonged presence in the blood may be beneficial include glucagon-like peptides 1 and 2 (GLP-1 and GLP-2, respectively), glucose-dependent insulintropic peptide (GIP), neuropeptide Y (NPY), pancreatic polypeptide (PP), and peptide YY (PYY). GLP-1 is an important polypeptide hormone with regulatory function in glucose metabolism and gastrointestinal secretion and
25 metabolism. Current efforts show that GLP-1 is a growth factor for beta cells in the pancreas and perhaps is involved in cell differentiation in other organs as well. GLP-2 is a 33-amino acid peptide having therapeutic application in the treatment of diseases of the gastrointestinal tract. In particular, it has been determined that GLP-2 acts as a trophic agent to enhance and maintain proper gastrointestinal function, as well as to promote
30 growth of intestinal tissues (See, e.g., U.S. Pat. Nos. 5,834,428; 5,789,379; and 5,990,077; and International Publication No. WO 98/52600). GIP is a 42-amino acid peptide synthesized and secreted from endocrine cells in the small intestine (See Pederson, R. A. et

al. *Endocrinology* **1976**, 99, 780-785; and Usdin, T. B. et al. *Endocrinology* **1993**, 133, 2861-2870). GIP infusions have been shown to inhibit the effects of glucagon on the liver while enhancing those of insulin. Additionally, GIP has dual effects on hepatic blood flow, increasing flow through the portal vein and inhibiting flow through the hepatic artery.

- 5 Neuropeptide Y is a 36-amino acid member of the pancreatic polypeptide family. It is highly concentrated in both the central and peripheral mammalian nervous system, is the most potent substance known to cause an increase in feeding, and may play a role in the genetic basis of Type II Diabetes Mellitus (see U.S. Pat. Nos. 6,410,701, 6,075,009, 5,026,685, 5,328,899, and Tatemoto, K. *Proc. Natl. Acad. Sci. USA* **1982**, 79, 5485-5489).
- 10 Peptide YY (PYY) and pancreatic polypeptide (PP) are structurally related peptide hormones involved in memory loss, depression, anxiety, epilepsy, pain, hypertension, and sleep and eating disorders.

These polypeptide hormones, and other polypeptide factors, are believed to be degraded by members of the post-proline cleaving class of serine proteinase enzymes, such as dipeptidyl peptidase IV (DPP IV). DPP IV is a membrane associated serine peptidase which cleaves N-terminal dipeptides from a peptide chain containing in the penultimate (P1) position, preferably, a proline residue, or an alanine residue if the N-terminal residue (P2) is histidine or a large aromatic, such as tyrosine, tryptophan or phenylalanine. The amino terminus sequences of GLP-1, GIP, and GLP-2 are His-Ala-Glu, Tyr-Ala-Glu, and His-Ala-Asp, respectively. The amino terminal sequences of NPY, PP, and PYY are Tyr-Pro-Ser, Ala-Pro-Leu and Tyr-Pro-Ile, respectively. Hence, DPP IV has been implicated in the regulation of the activity of each of these polypeptide hormones, as well as other polypeptides, in vivo.

DPP IV-mediated removal of Xaa-Ala or Xaa-Pro dipeptides, wherein Xaa is an amino acid residue, from the N-terminus of the bioactive peptide hormones mentioned above renders them inactive, or even antagonistic. Accordingly, cleavage and inactivation of peptide hormones by serine proteinases such as DPP IV is just one example that illustrates the significant limitation imposed by proteolysis for the use of therapeutic polypeptides.

30 Proteins fold to adopt unique three dimensional structures, usually as a result of multiple non-covalent interactions that contribute to their conformational stability. Creighton, T. E. *Proteins: Structures and Molecular Properties*; 2nd ed.; W.H. Freeman: New York, 1993. Removal of hydrophobic surface area from aqueous solvent plays a

dominant role in stabilizing protein structures. Tanford, C. *Science* **1978**, *200*, 1012-1018.

For instance, a buried leucine or phenylalanine residue can contribute ~2–5 kcal/mol in stability when compared to alanine. Although hydrogen bonds and salt bridges, when present in hydrophobic environments, can contribute as much as 3 kcal/mol to protein

5 stability, solvent exposed electrostatic interactions contribute far less, usually ≤ 0.5

kcal/mol. Yu, Y et al. *J. Mol. Biol.* **1996**, *255*, 367-372; Lumb, K. J. et al. *Science* **1995**,

268, 436-439. Hydrogen bonds between small polar side chains and backbone amides can

be worth 1-2 kcal/mol, as seen in the case of N-terminal helical caps. The energetic balance of these intramolecular forces and interactions with the solvent determines the shape and

10 the stability of the fold.

While electrostatic interactions in designed structures can provide conformational specificity at the expense of thermodynamic stability, hydrophobic interactions afford a very powerful driving force for stabilizing structures. Recent studies have focused on the introduction of non-proteinogenic, fluorine containing amino acids as a means for

15 increasing hydrophobicity, without significant concurrent alteration of protein structure.

For example, the low polarizability of fluorine may explain why fluorocarbons have

relatively low propensities for intermolecular interactions. Riess, J. G. *Colloid Surf.- A* **1994**, *84*, 33-48. These unique properties of fluorine simultaneously bestow hydrophobic and lipophobic character to biopolymers with high fluorine content. Marsh, E. N. G. *Chem.*

20 *Biol.* **2000**, *7*, R153-R157.

Introduction of amino acids containing terminal trifluoromethyl groups at appropriate positions on protein folds increases the thermal stability and enhances resistance to chemical denaturants. Furthermore, specific protein-protein interactions can be programmed by the use of fluorocarbon and hydrocarbon side chains. Bilgiçer, B. et al. *J. Am. Chem. Soc.* **2001**, *123*, 11815-11816. Because specificity is determined by the thermodynamic stability of all possible protein-protein interactions, a detailed fundamental understanding of the various combinations is essential.

The so-called “leucine zipper” protein motif, originally discovered in DNA-binding proteins but also found in protein-binding proteins, consists of a set of four or five
30 consecutive leucine residues repeated every seven amino acids in the primary sequence of a protein. In a helical configuration, a protein containing a leucine zipper motif presents a line of leucines on one side of the helix. With two such helixes alongside each other, the arrays of leucines can interdigitate like a zipper and/or form side-to-side contacts, thus

forming a stable link between the two helices. Moreover, an increase in the hydrophobicity of the leucine side chains, e.g., by substitution of hydrogens with fluorines, in a leucine zipper motif should increase the strength of the zipper.

Selective fluorination of biologically active compounds is often accompanied by dramatic changes in physiological activities. See, e.g., Welch, T.; Eswarakrishnan, S. 5 *Fluorine in Bioorganic Chemistry*; Wiley-Interscience: New York, 1991 and references cited therein; (b) *Fluorine-containing Amino Acids*; Kukhar, V. P. et al., Eds.; John Wiley & Sons: Chichester, 1994; (c) Williams, R. M. *Synthesis of Optically Active α -Amino Acids*, Pergamon Press: Oxford, 1989; Tolman, V. *Amino Acids* **1996**, *11*, 15-36. Further, 10 fluorinated amino acids have been synthesized and studied as potential inhibitors of enzymes and as therapeutic agents. Kollonitsch, J. et al. *Nature* **1978**, *274*, 906-908.

The stereochemistry of polypeptides may be described in terms of the topochemical arrangement of the side chains of the amino acid residues about the polypeptide backbone, which is defined by the peptide bonds between the amino acid residues and the α -carbon 15 atoms of the bonded residues. In addition, polypeptide backbones have distinct termini, and thus, direction. Evolution has ensured the almost exclusive occurrence of the more prevalent L-amino acids in naturally occurring proteins. D-Amino acids are the enantiomers of L-amino acids. Virtually all proteases therefore cleave peptide bonds between adjacent L-amino acids; thus, artificial proteins or peptides composed of D-amino acids are largely 20 resistant to proteolytic break-down.

The term "retro-isomer" describes an isomer of a linear peptide in which the direction of the sequence is reversed compared with the parent peptide, *i.e.*, retro peptides are composed of L-amino acids in which the amino acid residues are assembled in opposite direction to the native peptide sequence. An "inverso peptide" is one in which only the 25 chirality of each amino acid is inverted; that is they are peptides corresponding to the native linear peptide sequence and maintain end-group complementarity, but are composed of D-amino acids rather than L-amino acids. It follows, then, that retro-inverso modification of naturally occurring polypeptides involves the synthetic assemblage of amino acids with α -carbon stereochemistry opposite to that of the corresponding L-amino acids, *i.e.* D- or D- 30 *allo*-amino acids, in reverse order with respect to the native peptide sequence. A retro-inverso analog thus has reversed termini and reversed direction of peptide bonds, while approximately maintaining the topology of the side chains as in the native peptide sequence.

The biological activity of a peptide hormone or neurotransmitter depends primarily on its dynamic interaction with a receptor, as well as on transduction process of the peptide-receptor complex. Such interactions are complex processes involving multiple conformational and topological properties. Processes for the synthesis of retro-inverso peptide analogs have been described, prepared, and tested. Importantly, due to the stereospecificity of enzymes with respect to their substrates, replacement of L-amino acid residues with D-amino acid residues in peptide substrates generally abolishes proteolytic enzyme recognition. For example, Jameson has reported an analog of the hairpin loop of the CD4 receptor by combining these two properties: reverse synthesis and a change in chirality. See Jameson et al. *Nature* **1994**, 368, 744; Brady et al. *Nature* **1994**, 368, 692. The net result of combining D-enantiomers and reverse synthesis is that the positions of carbonyl and amino groups in each amide bond are exchanged, while the position of the side-chain groups at each α -carbon is preserved. Jameson reports an increase in biological activity for their reverse D-peptide, which contrasts to the limited activity in vivo of its conventional all-L enantiomer (owing to its susceptibility to proteolysis). A partially modified retro-inverso pseudopeptide has also been reported for use as a non-natural ligand for the human class I histocompatibility molecule, HLA-A2. See Guichard et al. *Med. Chem.* **1996**, 39, 2030. In that case, the authors reported that the non-natural ligands had increased stability and high MHC-binding characteristics.

Accordingly, the discovery of analogs that exhibit stability towards proteolysis, such as DPP IV-mediated inactivation, is therefore of substantial interest. Accordingly, there is a need in the art for proteolysis-resistant peptide hormones.

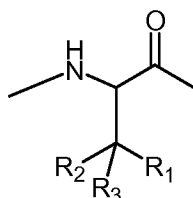
Summary of the Invention

Aspects of the present invention relate to peptide and P'₁ analogs that have increased in vivo half-lives, e.g., resulting from reduced susceptibility to cleavage by proteolytic enzymes, yet retain the desired activity of the original peptide.

In certain embodiments, the invention relates to the discovery that modification of substrates for post-proline cleaving proteinases at the P'₁ position (the residue to the carboxy terminal side of the amide cleavage site) can produce substrate analogs with greatly reduced susceptibility to enzyme-mediated cleavage relative to the native substrate, yet retain the biological activity of the native substrate.

In certain embodiments, the P'₁ residue is replaced with a non-naturally occurring amino acid analog, and even more preferably, with one which is a structural analog, e.g., retaining similar attributes with respect to steric and/or electronic nature.

- In certain embodiments, the present invention provides a modified polypeptide which is rendered less susceptible to proteolysis by a post-proline cleaving proteinases, such as dipeptidylpeptidase IV (DPP-IV), wherein the polypeptide has been modified at the P'₁ position with an amino acid or amino acid analog represented by Formula I:



wherein

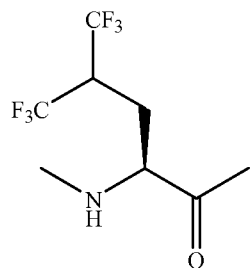
- R₁ and R₂ are independently for each occurrence lower alkyl or halogen; R₃ is selected from the group consisting of lower alkyl, aryl, -OH, -(CH₂)_m-COOH, -(CH₂)_m-NH₂, -(CH₂)_m-N-C(=NH)NH₂, -(CH₂)_m-C(=O)NH₂, -SH, and -(CH₂)_m-S-CH₃; and m is 0, 1, or 2.

- Another aspect of the invention relates to the more general observation that modification of proteinase substrates at the P'₁ residue (of the cleavage site) with an amino acid analog having a tetra-substituted Cβ carbon can markedly increase the in vivo half-life of the resulting analog, e.g., which may have a longer duration of biological action and/or reduced clearance relative to the wild-type polypeptide.

- In certain embodiments, the present invention provides a method for producing the P'₁ analogs of substrates for such proteinases as serine proteinases, metalloproteinases, aspartic proteinases, and cysteine proteinases.

Another aspect of the invention relates to the incorporation of fluorinated side chains on other amino acids that are usually found in hydrophobic cores.

In certain embodiments, the fluorinated species is the trifluoromethyl analog of leucine. In certain embodiments, the analog is 5,5,5,5',5',5'-α-S-hexafluoroleucine:



Another aspect of the present invention relates to retro-inverso analogs of native peptide fragments, such as those selected from the group consisting of secretin, glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), and GLP-1 (7-36) amide.

5 In certain embodiments, the analogs have an enhanced capacity to stimulate insulin production as compared to glucagon or may exhibit enhanced stability in plasma as compared to GLP-1 (7-36) amide or both.

In certain embodiments, the present invention contemplates a retro-inverso modification of the native GLP-1(7-36) amide fragment model, whereby the use of
10 complementary D-amino acid enantiomers constitutes an inversion of the chirality of the amino acid residues in the native sequence (inverso modification), and whereby said D-amino acids are attached in a peptide chain such that the sequence of residues in the resulting analog is exactly opposite of that in the native GLP-1 peptide fragment (retro
modification), furnishing a stable synthetic GLP-1 analog, consistent with greater than
15 about 90% sequence homology with the native GLP-1 (7-36) amide fragment. See Figures 1 and 2.

Another aspect of the present invention relates to methods of treating diabetes, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

20 Another aspect of the present invention relates to methods of reducing blood glucose levels, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

Another aspect of the present invention relates to methods of improving glucose metabolism, comprising administering to a mammal in need thereof a therapeutically
25 effective amount of a compound of the present invention.

Another aspect of the present invention relates to methods of treating hypertension, cardiovascular disease, periodontal disease, retinopathy, glaucoma, renal disease, neuropathy, or ketoacidosis, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

Brief Description of the Figures

Figure 1 shows exemplary embodiments of Formula I, wherein naturally occurring amino acids have been modified at the β -position (3-position) with R_1 and R_2 .

Figure 2 depicts selected modifications that may be made to an amino acid
5 sequence in accordance with the present invention. The variables R^1 , R^2 , R^3 , and R^4 may represent amino acid side chains, and Xaa may represent any amino acid residue.

Figure 3 depicts exemplary truncated analogs of Exendin and GLP-1.

Figure 4 shows the percent change in blood glucose over time for truncated Exendin and truncated stable GLP-1 in 9 week old db/db mice.

10 **Figure 5** depicts generally the design of a retro-inverso GLP-1 analog.

Detailed Description of the Invention

I. P'₁ analogs

Aspects of the present invention relate to peptide and P'₁ analogs that have increased in vivo half-lives, e.g., resulting from reduced susceptibility to cleavage by
15 proteolytic enzymes, yet retain the desired activity of the original substrate peptide. The P'₁ analogs of the present invention include analogs of growth factors, cytokines, encretins, peptide hormones and other polypeptides and peptides whose activity and/or half-life in vivo are ordinarily regulated by proteolytic cleavage.

One aspect of the invention relates to the discovery that modification of substrates
20 for post-proline cleaving proteinases at the P'₁ position (the residue to the carboxy terminal side of the amide cleavage site) can produce substrate analogs with greatly reduced susceptibility to enzyme-mediated cleavage relative to the native substrate, yet retain the biological activity of the native substrate. For example, modification of substrates of the post-proline cleaving serine proteinase DPP IV with an amino acid analog at the P'₁ residue
25 (of the DPP IV cleavage site) results in a substrate analog with reduce susceptibility to cleavage by DPP IV, yet retains the biological activity of the underlying substrate.

In certain embodiments, the present invention provides for the manufacture and use of peptide and P'₁ analogs resistant to proteinase-mediated cleavage. Given a native polypeptide typically cleaved by a particular proteinase (e.g., a metalloproteinase, a
30 cysteine proteinase, an aspartic proteinase, or a serine proteinase), one can readily determine the site within the native polypeptide at which the proteinase cleaves (the cleavage site). Once the cleavage site is identified, P'₁ analogs can be readily made according to the methods of the present invention. Given the depth of understanding in the

art of enzymology, the preferred cleavage sites of a large number of proteinases are known, and the identification of the consensus cleavage site in a given native polypeptide can be rapidly and easily accomplished by simply examining the amino acid sequence.

In the event that the cleavage site within a particular polypeptide is not known or
5 cannot be rapidly determined by simply examining the amino acid sequence, the cleavage site can be determined by simply incubating native polypeptide and proteinase to allow cleavage, separating the cleaved polypeptide species (e.g., by electrophoresis), and sequencing the cleaved peptide fragments. By determining the sequence of the ends of the cleaved peptide fragment, and comparing this sequence to that of the full-length
10 polypeptide sequence, one can rapidly and easily identify or verify the cleavage site within a native polypeptide at which a proteinase acts.

Another exemplary method for rapidly determining the substrate specificity of a proteinase is provided by PCT Publication WO 00/61789.

In certain embodiments, the present invention provides generalizable methods for
15 constructing proteinase resistant P'₁ analogs. In certain embodiments, the present invention contemplates the design and use of P'₁ analogs resistant to metalloproteinases, cysteine proteinases, aspartic proteinases, and serine proteinases. For instance, in certain embodiments, the subject analogs can be rendered resistant to cleavage by proteinases selected from: an aminopeptidase (EC 3.4.11.-), a dipeptidase (EC 3.4.13.-), a dipeptidyl-
20 peptidase or tripeptidyl peptidase (EC 3.4.14.-), a peptidyl-dipeptidase (EC 3.4.15.-), a serine-type carboxypeptidase (EC 3.4.16.-), a metallocarboxypeptidase (EC 3.4.17.-), a cysteine-type carboxypeptidase (EC 3.4.18.-), an omegapeptidase (EC 3.4.19.-), a serine proteinase (EC 3.4.21.-), a cysteine proteinase (EC 3.4.22.-), an aspartic proteinase (EC 3.4.23.-), a metallo proteinase (EC 3.4.24.-), or a proteinase of unknown mechanism (EC
25 3.4.99.-). The EC designation following each class of proteinase is that used in the recommendation of the International Union of Biochemistry and Molecular Biology (1984), and these subclass headings are provided here for reference.

To further illustrate the exemplary proteinases for which proteinase-resistant P'₁ analogs are contemplated, an non-exhaustive list of proteinases include: leucyl
30 aminopeptidase, membrane alanine aminopeptidase, cystinyl aminopeptidase, tripeptide aminopeptidase, prolyl aminopeptidase, aminopeptidase B, glutamyl aminopeptidase, Xaa-Pro aminopeptidase, bacterial leucyl aminopeptidase, clostridial aminopeptidase, cytosol alanyl aminopeptidase, lysyl aminopeptidase, Xaa-Trp aminopeptidase, tryptophanyl

aminopeptidase, methionyl aninopeptidase, D-stereospecific aninopeptidase,
 aminopeptidase Ey, vacuolar aminopeptidase I, Xaa-His dipeptidase, Xaa-Arg dipeptidase,
 Xaa-methyl-His dipeptidase, Cys-Gly dipeptidase, Glu-Glu dipeptidase, Pro-Xaa
 dipeptidase, Xaa-Pro dipeptidase, Met-Xaa dipeptidase, non-stereospecific dipeptidase,
 5 cytosol non-specific dipeptidase, membrane dipeptidase, β -Ala-His dipeptidase, Dipeptidyl-
 peptidase I (DPP I), Dipeptidyl-peptidase II (DPP II), Dipeptidyl-peptidase III (DPP III),
 Dipeptidyl-peptidase IV(DPP IV), Dipeptidyl-dipeptidase, Tripeptidyl-peptidase I,
 Tripeptidyl-peptidase II, Xaa-Pro dipeptidyl-peptidase, peptidyl-dipeptidase A, peptidyl-
 dipeptidase B, peptidyl-dipeptidase Dcp, lysosomal Pro-X carboxypeptidase, Serine-type
 10 D-Ala-D-Ala carboxypeptidase, carboxypeptidase C, carboxypeptidase D,
 carboxypeptidase A, carboxypeptidase B, lysine(arginine) carboxypeptidase, Gly-X
 carboxypeptidase, alanine carboxypeptidase, muramoylpentapeptide carboxypeptidase,
 carboxypeptidase H, glutamate carboxypeptidase, carboxypeptidase M,
 muramoyltetrapeptide carboxypeptidase, zinc D-Ala-D-Ala carboxypeptidase,
 15 carboxypeptidase A2, membrane Pro-X carboxypeptidase, tubuliny-Tyr carboxypeptidase,
 carboxypeptidase T, thermostable carboxypeptidase I, carboxypeptidase U, glutamate
 carboxypeptidase II, metallo-carboxypeptidase D, cysteine-type carboxypeptidase,
 acylaminoacyl-peptidase, peptidyl-glycinamidase, pyroglutamyl-peptidase I, beta-aspartyl-
 peptidase, pyroglutamyl-peptidase II, N-formylmethionyl-peptidase, pteroylpoly-gamma-
 20 glutamate carboxypeptidase, gamma-glutamyl hydrolase, gamma-D-glutamyl-meso-
 diamino- pimelate peptidase I, chymotrypsin, chymotrypsin C, metridin, trypsin, thrombin,
 coagulation factor Xa, plasmin, enteropeptidase, acrosin, alpha-lytic endopeptidase,
 glutamyl endopeptidase, cathepsin G, coagulation factor VIIa, coagulation factor Ixa,
 cucumisin, prolyl oligopeptidase, coagulation factor XIa, bradyurin, plasma kallikrein,
 25 tissue kallikrein, pancreatic elastase, leukocyte elastase, coagulation factor XIIa, chymase,
 complement component C1r, complement component C1s, classical-complement pathway
 C3/C5 convertase, complement factor I, complement factor D, alternative-complement
 pathway C3/C5 convertase, cerevisin, hypodermin C, lysyl endopeptidase, endopeptidase
 La, gamma-renin, venombin AB, leucyl endopeptidase, tryptase, scutellarin, kexin,
 30 subtilisin, oryzin, proteinase K, thermomycolin, thermitase, endopeptidase So, T-
 plasminogen activator, protein C (activated), pancreatic endopeptidase E, pancreatic
 elastase II, IgA-specific serine endopeptidase, U-plasminogen activator, venombin A, furin,
 myeloblastin, semenogelase, granzyme A, granzyme B, streptogrisin A, streptogrisin B,

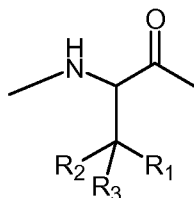
glutamyl endopeptidase II, oligopeptidase B, limulus clotting factor C, limulus clotting factor B, limulus clotting enzyme, omptin, repressor *lexA*, signal peptidase I, togavirin, flavirin, endopeptidase Clp, proprotein convertase 1, proprotein convertase 2, snake venom factor V activator, lactocepin, cathepsin B, papain, ficain, chymopapain, asclepain, clostripain, streptopain, actinidain, cathepsin L, cathepsin H, calpain, cathepsin T, glycyl endopeptidase, cancer procoagulant, cathepsin S, picomain 3C, picornain 2A, caricain, ananain, stem bromelain, fruit bromelain, legumain, histolysin, caspase-1, gingipain R, cathepsin K, pepsin A, pepsin B, gastricsin, chymosin, cathepsin D, neopenthesin, renin, retropepsin, pro-opiomelanocortin converting enzyme, aspergillopepsin I, aspergillopepsin II, penicillopepsin, rhizopuspepsin, endothiapepsin, mucoropepsin, candidapepsin, saccharopepsin, rhodotorulapepsin, physaropepsin, acrocylindropepsin, polyporopepsin, pycnoporopepsin, scytalidopepsin A, scytalidopepsin B, xanthomonapepsin, cathepsin E, barrierpepsin, signal peptidase II, pseudomonapepsin, plasmepsin I, plasmepsin II, phytpepsin, atrolysin A, microbial collagenase, leucolysin, interstitial collagenase, neprilysin, envelysin, IgA-specific metalloendopeptidase, procollagen N-endopeptidase, thimet oligopeptidase, neurolysin, stromelysin 1, meprin A, procollagen C-endopeptidase, peptidyl-Lys metalloendopeptidase, astacin, stromelysin 2, matrilysin, gelatinase A, aeromonolysin, pseudolysin, thermolysin, bacillolysin, aureolysin, coccolysin, mycolysin, beta-lytic metalloendopeptidase, peptidyl-Asp metalloendopeptidase, neutrophil collagenase, gelatinase B, leishmanolysin, saccharolysin, autolysin, deuterolysin, serralysin, atrolysin B, atrolysin C, atroxase, atrolysin E, atrolysin F, adamalysin, horrilysin, ruberlysin, bothropasin, bothrolysin, ophiolysin, trimerelysin I, trimerelysin II, mucrolysin, pitrilysin, insulysin, O-sialoglycoprotein endopeptidase, russellysin, mitochondrial intermediate peptidase, dactylolysin, nardilysin, magnolysin, meprin B, mitochondrial processing peptidase, macrophage elastase, choriolysin L, choriolysin H, tentoxilysin, bontoxilysin, oligopeptidase A, endothelin-converting enzyme 1, fibrolase, jararhagin, fragilysin, and multicatalytic endopeptidase complex.

Another aspect of the present invention relates to a polypeptide sequence encoding for a proteinase-resistant analog of a polypeptide hormone that has an N-terminal sequence selected from $\text{NH}_2\text{-Xaa-Ala-Yaa-}$ and $\text{NH}_2\text{-Xaa-Pro-Yaa-}$, where Xaa and Yaa each independently represent an amino acid residue. In certain embodiments, Xaa is an amino acid with aromatic side chain. In certain embodiments, Xaa is selected from histidine, tyrosine, tryptophan, and phenylalanine. In certain embodiments, Yaa is an amino acid

residue with an acidic side chain. In certain embodiments, Yaa, is selected from aspartic acid and glutamic acid.

In certain embodiments, the proteinase is a serine proteinase. In certain embodiments, the proteinase is a dipeptidyl peptidase. An exemplary dipeptidyl peptidase is dipeptidyl peptidase IV (DPP IV). DPP IV activity alters the biological activity of a large number of bioactive proteins and polypeptides. In addition to the potential DPP IV substrates disclosed in U.S. Patent No. 6,090,786 (incorporated by reference), the present invention is also directed to analogs of GLP-1, GLP-2, and GIP. In certain embodiments, the peptide hormone is a naturally occurring variety found in mammals. In certain embodiments, the peptide hormone is a naturally, or artificially mutated variety of a naturally occurring (wild type) peptide hormone. Thus, natural and synthetic peptide hormones are within the scope of peptide hormones contemplated for the modifications. Thus in certain embodiments, the present invention provides DPP IV proteolysis-resistant analogs of the aforementioned peptide hormones.

While replacing the P'₁ residue with another naturally occurring amino acid is contemplated, in certain embodiments, the P'₁ residue is replaced with a non-naturally occurring amino acid analog, and even more preferably, with one which is a structural analog, e.g., retaining similar attributes with respect to steric and/or electronic nature. To illustrate, in certain embodiments, the present invention provides a modified polypeptide which is rendered less susceptible to proteolysis by a post-proline cleaving proteinases, such as dipeptidylpeptidase IV (DPP-IV), wherein the polypeptide has been modified at the P'₁ position with an amino acid or amino acid analog represented by Formula I:



wherein

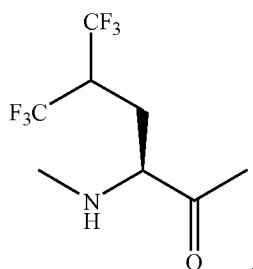
R₁ and R₂ are independently for each occurrence lower alkyl or halogen; R₃ is selected from the group consisting of lower alkyl, aryl, -OH, -(CH₂)_m-COOH, -(CH₂)_m-NH₂, -(CH₂)_m-N-C(=NH)NH₂, -(CH₂)_m-C(=O)NH₂, -SH, and -(CH₂)_m-S-CH₃; and m is 0, 1, or 2.

Another aspect of the invention relates to the more general observation that modification of proteinase substrates at the P'₁ residue (of the cleavage site) with an amino

acid analog having a tetra-substituted C β carbon can markedly increase the in vivo half-life of the resulting analog, e.g., which may have a longer duration of biological action and/or reduced clearance relative to the wild-type polypeptide. Based on this discovery, and its applicability to substrates cleaved by a diverse range of proteinases, the present invention provides a method for producing the P'₁ analogs of substrates for such proteinases as serine proteinases, metalloproteinases, aspartic proteinases, and cysteine proteinases.

II. Fluorinated Analogs

Another aspect of the invention relates to the incorporation of fluorinated side chains on other amino acids that are usually found in hydrophobic cores. In certain embodiments, the fluorinated species is the trifluoromethyl analog of leucine. In certain embodiments, the analog is 5,5,5,5',5',5'- α -S-hexafluoroleucine:



The synthesis of this analog and methods for incorporating it into peptide analogs are provided in U.S. Patent No. 7,132,559 (incorporated by reference).

III. Retro-Inverso Analogs

Another aspect of the present invention relates to retro-inverso truncated analogs of peptides.

In certain embodiments, the truncated analogs share sequence homology with peptide fragments of secretin, glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), and GLP-1 (7-36) amide.

In certain embodiments, the analogs have an enhanced capacity to stimulate insulin production as compared to glucagon or may exhibit enhanced stability in plasma as compared to GLP-1 (7-36) amide or both. Either of these properties will enhance the potency of an analog as a therapeutic.

Another aspect of the present invention relates to methods of treating diabetes, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

Another aspect of the present invention relates to methods of reducing blood glucose levels, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

Another aspect of the present invention relates to methods of improving glucose metabolism, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

Another aspect of the present invention relates to methods of treating hypertension, cardiovascular disease, periodontal disease, retinopathy, glaucoma, renal disease, neuropathy, or ketoacidosis, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the present invention.

In certain embodiments, the truncated analog is represented by the sequence depicted in Scheme 1:

Scheme 1

GLP-1(7-34) amide truncated analog, all L-amino acids
[N-terminus] H⁷ A E G T¹¹ F T¹³ S D V S S Y L E G Q A A K E F I²⁹ A W L V K [C-terminus]

In certain embodiments, the present invention contemplates a retro-inverso modification of the GLP-1 (7-34) amide truncated analog, whereby the use of complementary D-amino acid enantiomers constitutes an inversion of the chirality of the amino acid residues in the native sequence (inverso modification), and whereby said D-amino acids are attached in a peptide chain such that the sequence of residues in the resulting analog is exactly opposite of that in the native GLP-1 amide truncated analog (retro modification), furnishing a truncated analog with greater than about 90% sequence homology with the native GLP-1 (7-34) amide truncated analog (see Figures 1 and 2).

In certain embodiments, the present invention makes use of complementarily diastereoisomeric D-allo amino acids as a conservative substitution for the two threonine and one isoleucine residues of the model sequence in the preparation of a variant peptide retro-inverso GLP-1 analog,), furnishing a truncated analog with greater than about 90% sequence homology with the native GLP-1 (7-34) amide truncated analog (Figure 3).

As described previously, the biological activity of native GLP-1 is curtailed by rapid removal of the N-terminal dipeptide by DPP-IV. It is envisaged that terminus modifications could increase resistance to DPP-IV degradation, thus leading to prolonged duration of action in vivo, and/or optionally enhance receptor interactions.

The use of “protecting groups” as described herein may also be advantageous in this regard (see definitions). Further examples of N-terminal modifications include glycation (e.g., *N*-glucitol), *N*-pyroglutamyl, *N*-acetyl, *N*-methylation (*N*-Me, α -Me), desamination, and substitution with imidazole-lactic acid.

5 Much less attention has focused on elaboration at the C-terminus. As described above, the GLP-1 derivative CJC-1131 was engineered with a reactive C-terminus chemical linker that allows covalent bonding to endogenous serum albumin (vide supra).

 In certain embodiments, the present invention provides for synthetic peptide analogs that may be optionally derivatized at the terminal residues, independently for each
10 occurrence. In certain embodiments, the present invention considers a retro-inverso modified GLP-1 analog, as described above, which is optionally derivatized, whereby the corresponding C-terminal histidine carboxylate is replaced with a geminal-amino D-histidine residue to afford a stable synthetic GLP-1 analog, consistent with greater than about 90% sequence homology with native GLP-1(7-36) amide (Figure 3).

15 In certain embodiments, the sequence of the retro-inverso modified GLP-1 analog can be extended by a number of amino acid residues, e.g., whereby the corresponding *N*-terminus is extended by up to about nine amino acids to furnish a variant peptide. In certain embodiments, said variant peptide may be a retro-inverso modified GLP-1 analog as described above, wherein the corresponding *N*-terminus has been extended further with
20 nine amino acid residues corresponding to those found in the C-terminal sequence of native extendin-4: P S S G A P P P S.

 According to U.S. Patent No. 6,583,111 (incorporated by reference), as many as about 14 substitutions can be made for amino acid residues along the native GLP-1 sequence to afford variant peptide GLP-1 compounds. In certain embodiments, aspects of
25 the present invention provide for substitution of as many as about 14 residues in the sequence of a retro-inverso modified GLP-1 analog.

 In the formulas representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although often not specifically shown, may be understood to be in the form they would assume at physiological pH values, unless
30 otherwise specified. Thus, the *N*-terminal- H_2^+ and *C*-terminal- O^- at physiological pH may be understood to be present, though not necessarily specified and shown, either in specific examples or in generic formulas.

The foregoing describes the status of the termini at neutral pH; it is understood, of course, that the acid addition salts or the basic salts of the peptides are also included within the scope of the invention. At high pH, basic salts of the C-terminus and carboxyl-containing side chains may be formed from nontoxic pharmaceutically acceptable bases, and suitable counter-ions include, for example, Na⁺, K⁺, Ca²⁺, and the like. Suitable pharmaceutically acceptable nontoxic organic cations can also be used as counter ions. In addition, as set forth herein, the peptides may be prepared as the corresponding amides. Suitable acid addition salts with respect to the N-terminus or amino group-containing side chains include the salts formed from inorganic acids such as hydrochloric, sulfuric, or phosphoric acid and those formed from organic acids such as acetic, citric, or other pharmaceutically acceptable nontoxic acids.

IV. *Methods of Use*

In certain embodiments, the peptide hormone analogs of the invention may be used in radiolabeled or unlabeled form to diagnose or treat a variety of disease states including but not limited to those associated with glucose metabolism, lipid metabolism, food intake, and hypertension.

In certain embodiments, radiolabeled complexes of the compounds of the invention are used for such diagnoses and treatments. Radiolabeled embodiments, of the compounds of the invention may be used in radioisotope guided surgery, as described in WO 93/18797 and in Woltering, et al. Surgery, **1994**, 116, 1139-1147.

In certain embodiments, a complex of a gamma-emitting radionuclide such as ⁹⁹Tc and a compound of the invention is used to diagnose an SSTR-expressing tumor, and subsequently, a complex of beta-emitting radionuclide such as ¹⁸⁸Re or ¹⁸⁶Re with the compound is used to treat the tumor.

In certain embodiments, for diagnostic purposes, an effective diagnostic amount of the diagnostic or radiodiagnostic agent of the invention is administered, preferably intravenously. An effective diagnostic amount is defined as the amount of diagnostic or radiodiagnostic agent necessary to effect localization and detection of the label in vivo using conventional methodologies such as magnetic resonance, computerized tomography, gamma scintigraphy, SPECT, PET, and the like.

In certain embodiments, for diagnosis using scintigraphic imaging, ⁹⁹Tc-labeled compounds of the invention are administered in a single unit injectable dose. The ⁹⁹Tc-labeled compounds provided by the invention may be administered intravenously in any

conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 50 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging in vivo can take place in a matter of a few minutes. However, imaging can take place, if desired, hours or even longer after the radiolabeled compound is injected into a patient. In certain embodiments, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

V. Methods of Treatment

P₁' truncated analogs provide improved methods of treating any disease or condition that can be treated with a given polypeptide therapeutic composition, wherein the polypeptide is normally cleaved in vivo by a proteinase. Given that proteolysis decreases or eliminates the availability of the therapeutic, and in some instances leads to the production of functionally antagonistic products, the safety and efficacy of many polypeptide therapeutics which can be used to treat particular diseases and conditions is greatly compromised. Accordingly, the methods and compositions of proteinase resistant P₁' truncated analogs provides improved methods of treating any of a number of diverse diseases and conditions.

To more explicitly illustrate the applicability of P₁' truncated analogs in improved methods of treating a variety of diseases and conditions, we provide the following non-limiting examples:

In certain embodiments, the P₁' truncated analogs of the present invention are peptide hormone analogs. These peptide hormones possess, in certain embodiments, the ability to lower blood glucose levels, to relieve obesity, to alleviate impaired glucose tolerance, to inhibit hepatic glucose neogenesis, and to lower blood lipid levels and to inhibit aldose reductase. They are thus useful for the prevention and/or therapy of congestive heart failure, hyperglycemia, obesity, hyperlipidemia, diabetic complications (including retinopathy, nephropathy, neuropathy, cataracts, coronary artery disease and arteriosclerosis) and furthermore for obesity-related hypertension and osteoporosis. Thus one aspect of the present invention is a method for treating a disease in a patient or subject

comprising administering a therapeutically effective amount of one or more peptide hormone analogs, such as the peptide hormone analogs disclosed herein.

In certain embodiments, the present invention relates to a method for modifying glucose metabolism. P₁ truncated analogs of GLP-1 peptides may be administered to patient suffering from diabetes mellitus. Diabetes mellitus is a disease characterized by hyperglycemia occurring from a relative or absolute decrease in insulin secretion, decreased insulin sensitivity, or insulin resistance. The morbidity and mortality of this disease result from vascular, renal, and neurological complications. An oral glucose tolerance test is a clinical test used to diagnose diabetes. In an oral glucose tolerance test, a patient's physiological response to a glucose load or challenge is evaluated. After ingesting the glucose, the patient's physiological response to the glucose challenge is evaluated. Generally, this is accomplished by determining the patient's blood glucose levels (the concentration of glucose in the patient's plasma, serum, or whole blood) for several predetermined points in time.

Thus, in certain embodiments, aspects of the present invention relate to therapeutic and related uses of proteolysis-resistant GLP-1 truncated analogs for treating heart-related ailments, hyperglycemia, obesity, hyperlipidemia, diabetic complications (including retinopathy, nephropathy, neuropathy, cataracts, coronary artery disease and arteriosclerosis) and furthermore for obesity-related hypertension and osteoporosis.

In certain embodiments, the subject GLP-1 truncated analogs can be used as part of treatment regimens for various heart-related ailments. Exemplary heart related ailments include myocardial infarction, ischemia-reperfusion injury, congestive heart failure, and cardiac arrest. The subject GLP-1 truncated analogs can also be used in the prevention of heart related ailments.

In certain embodiments, the subject analogs can be used to induce arousal for the treatment or amelioration of depression, schizoaffective disorders, sleep apnea, attention deficit syndromes with poor concentration, memory loss, forgetfulness, and narcolepsy.

In certain embodiments, therapeutically effective amounts of proteolysis-resistant GLP-2 truncated analogs may be administered to patients suffering from gastrointestinal diseases. It has been determined that GLP-2 acts as a trophic agent, to promote growth of gastrointestinal tissue. The effect of GLP-2 is marked particularly by increased growth of the small bowel, and is therefore herein referred to as an "intestinotrophic" effect.

Thus, in certain embodiments, aspects of the present invention relate to therapeutic and related uses of GLP-2 truncated analogs for promoting the growth and proliferation of gastrointestinal tissue, most particularly small bowel tissue. For instance, in certain embodiments, the subject method can be used as part of a regimen for treating injury,
5 inflammation or resection of intestinal tissue, e.g., where enhanced growth and repair of the intestinal mucosal epithelial is desired.

In certain embodiments, with respect to small bowel tissue, such growth is measured conveniently as an increase in small bowel mass and length, relative to an untreated control. The effect of subject GLP-2 truncated analogs on small bowel also manifests as an increase
10 in the height of the crypt plus villus axis. Such activity is referred to herein as an "intestinitrophic" activity. The efficacy of the subject method may also be detectable as an increase in crypt cell proliferation and/or a decrease in small bowel epithelium apoptosis. These cellular effects may be noted most significantly in relation to the jejunum, including the distal jejunum and particularly the proximal jejunum, and also in the distal ileum. A
15 compound is considered to have "intestinitrophic effect" if a test animal exhibits significantly increased small bowel weight, increased height of the crypt plus villus axis, or increased crypt cell proliferation or decreased small bowel epithelium apoptosis when treated with the compound (or genetically engineered to express it themselves). A model suitable for determining such gastrointestinal growth is described by U.S. Patent No.
20 5,834,428 (incorporated by reference).

In certain embodiments, patients who would benefit from either increased small intestinal mass and consequent increased small bowel mucosal function are candidates for treatment by the subject method. Particular conditions that may be treated include the various forms of sprue including celiac sprue which results from a toxic reaction to α -
25 gliadin from wheat, and is marked by a tremendous loss of villi of the bowel; tropical sprue which results from infection and is marked by partial flattening of the villi; hypogammaglobulinemic sprue which is observed commonly in patients with common variable immunodeficiency or hypogammaglobulinemia and is marked by significant decrease in villus height. In certain embodiments, the therapeutic efficacy of the treatment
30 may be monitored by enteric biopsy to examine the villus morphology, by biochemical assessment of nutrient absorption, by patient weight gain, or by amelioration of the symptoms associated with these conditions. Other conditions that may be treated by the subject method, or for which the subject method may be useful prophylactically, include

radiation enteritis, infectious or post-infectious enteritis, regional enteritis (Crohn's disease), small intestinal damage due to toxic or other chemotherapeutic agents, and patients with short bowel syndrome.

In certain embodiments, aspects of the present invention provide a therapeutic method for treating digestive tract diseases. The term "digestive tract" as used herein means a tube through which food passes, including stomach and intestine. The term "digestive tract diseases" as used herein means diseases accompanied by a qualitative or quantitative abnormality in the digestive tract mucosa, which include, e.g., ulceric or inflammatory bowel disease; congenital or acquired digestion and absorption disorder including malabsorption syndrome; disease caused by loss of a mucosal barrier function of the gut; and protein-losing gastroenteropathy. The ulceric disease includes, e.g., gastric ulcer, duodenal ulcer, small intestinal ulcer, colonic ulcer and rectal ulcer. The inflammatory bowel disease includes, e.g., esophagitis, gastritis, duodenitis, enteritis, colitis, Crohn's disease, proctitis, gastrointestinal Behcet, radiation enteritis, radiation colitis, radiation proctitis, enteritis and medicamentosa. The malabsorption syndromes includes the essential malabsorption syndrome such as disaccharide-decomposing enzyme deficiency, glucose-galactose malabsorption, fructose malabsorption; secondary malabsorption syndromes, e.g., the disorder caused by a mucosal atrophy in the digestive tract through the intravenous or parenteral nutrition or elemental diet, the disease caused by the resection and shunt of the small intestine such as short gut syndrome, cul-de-sac syndrome; and indigestible malabsorption syndrome such as the disease caused by resection of the stomach, e.g., dumping syndrome.

The term "therapeutic agent for digestive tract diseases" as used herein means the agents for the prevention and treatment of the digestive tract diseases, which include, e.g., the therapeutic agent for digestive tract ulcer, the therapeutic agent for inflammatory digestive tract disease, the therapeutic agent for mucosal atrophy in the digestive tract and the therapeutic agent for digestive tract wound, the amelioration agent for the function of the digestive tract including the agent for recovery of the mucosal barrier function and the amelioration agent for digestive and absorptive function. The ulcers include digestive ulcers and erosions, acute ulcers, namely, acute mucosal lesions.

The subject method, because of promoting proliferation of intestinal mucosa, can be used in the treatment and prevention of pathologic conditions of insufficiency in digestion and absorption, that is, treatment and prevention of mucosal atrophy or treatment of

hypoplasia of the digestive tract tissues and decrease in these tissues by surgical removal as well as improvement of digestion and absorption. Further, the subject method can be used in the treatment of pathologic mucosal conditions due to inflammatory diseases such as enteritis, Crohn's disease and ulceric colitis and also in the treatment of reduction in
5 function of the digestive tract after operation, for example, in dumping syndrome as well as in the treatment of duodenal ulcer in conjunction with the inhibition of peristalsis of the stomach and rapid migration of food from the stomach to the jejunum. Furthermore, glicentin can effectively be used in promoting cure of surgical invasion as well as in improving functions of the digestive tract. Thus, the present invention also provides a
10 therapeutic agent for atrophy of the digestive tract mucosa, a therapeutic agent for wounds in the digestive tract and a drug for improving functions of the digestive tract which comprise glicentin as active ingredients.

In certain embodiments, the subject method can be used to alter the pharmacokinetics of pancreatic peptide, Peptide YY and neuropeptide Y, all of which are
15 members of the pancreatic polypeptide family. Specifically, DPP IV has been implicated in the processing of those peptides in a manner which alters receptor selectivity, and thus DPP IV resistant analogs of each of these peptides can be readily designed.

Neuropeptide Y (NPY) is believed to act in the regulation of vascular smooth muscle tone, as well as regulation of blood pressure. NPY also decreases cardiac
20 contractility. NPY is also the most powerful appetite stimulant known. Wilding et al. *J. Endocrinology* **1992**, 132, 299-302. The centrally evoked food intake (appetite stimulation) effect is predominantly mediated by NPY Y1 receptors and causes increase in body fat stores. Stanley et al. *Physiology and Behavior* **1989**, 46, 173-177. By way of example, one possible use of NPY analogs is in the manufacture of therapeutics that increase
25 appetite. Although much of the world strives to lose weight, in a number of contexts, the goal is to gain weight. The incidence of eating disorders is on the rise around the world. Over time, individuals with eating disorders suffer from a pathological loss of appetite, and this loss of appetite makes re-feeding extremely difficult. Such difficulty often persists even when the individual's weight has reached a life-threateningly low level. Accordingly, the
30 use of agents which stimulate the appetite would greatly enhance the ability of health care providers to encourage and support re-feeding of severely malnourished eating disorder patients.

The difficulty encountered by individuals attempting to re-feed following prolonged periods of malnutrition is not limited to individuals with eating disorders. Malnutrition due to any cause can result in a serious suppression of appetite and this can be a barrier to quickly and easily facilitating proper nutrition in these individuals. Therapeutics that stimulate appetite would have great utility in the treatment of malnourished individuals.

Loss of appetite and wasting syndromes are often associated with other diseases and conditions. For example, patients with various forms of cancer and AIDS often experience wasting. This significant weight loss, as well as loss of muscle mass, can lead to a variety of other complications including loss of energy and further suppression of the immune system. Accordingly, therapeutics which help to counter the loss of appetite and wasting associated with other diseases and treatments would greatly improve the quality of life of patients battling any of a number of diseases.

Another example relates to the administration of therapeutics that stimulate appetite and stimulate weight gain in the agricultural arena. Such agents could be used to help raise animals, such as commercial livestock, with a higher average weight and/or a higher average fat content. By way of example, such therapeutics could be administered, for example in animal feed or water, to cows, pigs, chickens, sheep, turkeys, goat, buffalo, ostrich, and the like to produce larger animals for sale in the food industry.

Peptide YY (PYY) and pancreatic polypeptide (PP) are involved in eating disorders, gastrointestinal disorders, and pancreatic tumors. See U.S. Patent No. 5,574,010 (incorporated by reference).

DPP IV has also been implicated in the metabolism and inactivation of growth hormone-releasing factor (GHRF). GHRF is a member of the family of homologous peptides that includes glucagon, secretin, vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), pituitary adenylate cyclase activating peptide (PACAP), gastric inhibitory peptide (GIP) and helodermin. Kubiak et al. *Peptide Res.*, **1994**, 7, 153. GHRF is secreted by the hypothalamus, and stimulates the release of growth hormone (GH) from the anterior pituitary. Thus, in certain embodiments, the subject method can be used to improve clinical therapy for certain growth hormone deficient children, and in clinical therapy of adults to improve nutrition and to alter body composition (muscle vs. fat). In certain embodiments, the subject method can also be used in veterinary practice, for example, to develop higher yield milk production and higher yield, leaner livestock.

In certain embodiments the invention contemplates the use of P'₁ analogs in methods of treatment wherein the P'₁ analog alone constitutes the therapeutic regimen, as well as methods of treatment that utilize administration of one or more P'₁ analogs as part of a more complex multi-factorial therapeutic regimen. For example, in the case of methods of treating diabetes and/or complications of diabetes, the present invention contemplates methods of treating diabetes by administering a P'₁ analog such as a GLP-1 analog. In certain embodiments, the present invention further contemplates that, in some circumstances, it may be preferably to administer more than one P'₁ analog. For example, the method of treatment may comprise administration of two or more P'₁ analogs. Such P'₁ analogs may be analogs of the same polypeptide (e.g., two different GLP-1 analogs), or may be analogs of distinct polypeptides. Furthermore the invention contemplates that administering of one or more P'₁ analogs may be used as part of a complex therapeutic regimen. In the case of a method of treating diabetes or complications of diabetes, an exemplary therapeutic regimen may include administration of one or more P'₁ analog, administration of insulin, modulation of diet, and modulation of exercise.

In certain embodiments, as another example of a multi-faceted therapeutic regimen, the invention contemplates the administration of one or more P'₁ analogs and one or more agents that inhibit the enzymatic activity of the particular enzyme that endogenously cleaves the native protein. In the case of GLP-1, an exemplary method would comprise administration of one or more peptide analogs with one or more inhibitors of DPP IV. Inhibitors of a particular enzyme may be specific (e.g., an inhibitor that modulates only the activity of DPP IV) or the inhibitor may be more promiscuous (e.g., an inhibitor that modulates the activity of multiple serine proteases). Additionally, in certain embodiments, the invention contemplates the administration of one or more P'₁ analogs and one or more enzymes that degrade the particular enzyme that endogenously cleaves the native protein. In the case of GLP-1, in certain embodiments, an exemplary method would comprise administration of one or more peptide analogs with one or more enzymes that degrade DPP IV. Such enzymes may be specific (e.g., an enzyme that only degrades DPP IV) or the enzyme may degrade multiple other protein (e.g., an enzyme that degrades several serine proteases).

VI. Definitions

The term "high affinity" as used herein means strong binding affinity between molecules with a dissociation constant K_D of no greater than 1 μ M. In a certain case, the

K_D is less than 100 nM, 10 nM, 1 nM, 100 pM, or even 10 pM or less. In another embodiment, the two molecules can be covalently linked (K_D is essentially 0).

A “patient” or “subject” to be treated by the subject method can mean either a human or non-human subject.

5 The term “ED₅₀” means the dose of a drug that, in 50% of patients, will provide a clinically relevant improvement or change in a physiological measurement, such as glucose responsiveness, increase in hematocrit, decrease in tumor volume, etc.

 The term “IC₅₀” means the dose of a drug that inhibits a biological activity by 50%, e.g., the amount of inhibitor required to inhibit at least 50% of DPPIV (or other PPCE)

10 activity in vivo.

 As used herein, the term “inhibitor” is meant to describe a compound that blocks or reduces an activity of an enzyme (for example, inhibition of proteolytic cleavage of standard fluorogenic peptide substrates such as suc-LLVY-AMC, Box-LLR-AMC and Z-LLE-AMC, inhibition of various catalytic activities of the 20S proteasome). An inhibitor
15 can act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor can bind reversibly or irreversibly, and therefore the term includes compounds that are suicide substrates of an enzyme. An inhibitor can modify one or more sites on or near the active site of the enzyme, or it can cause a conformational change elsewhere on the enzyme.

 A compound is said to have an “insulinotropic activity” if it is able to stimulate, or
20 cause the stimulation of, the synthesis or expression of the hormone insulin.

 The term “interact” as used herein is meant to include all interactions (e.g., biochemical, chemical, or biophysical interactions) between molecules, such as protein-protein, protein-nucleic acid, nucleic acid-nucleic acid, protein-small molecule, nucleic acid-small molecule, or small molecule-small molecule interactions.

25 The term “LD₅₀” means the dose of a drug that is lethal in 50% of test subjects.

 The term “prophylactic or therapeutic” treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, (i.e., it protects the host against
30 developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term “preventing” is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

The term “therapeutic index” refers to the therapeutic index of a drug defined as LD_{50}/ED_{50} .

A “therapeutically effective amount” of a compound, e.g., such as a DPIV inhibitor of the present invention, with respect to the subject method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

A “single oral dosage formulation” is a dosage which provides an amount of drug to produce a serum concentration at least as great as the EC_{50} for that drug, but less than the LD_{50} . Another measure for a single oral dosage formulation is that it provides an amount of drug necessary to produce a serum concentration at least as great as the IC_{50} for that drug, but less than the LD_{50} . By either measure, a single oral dosage formulation is preferably an amount of drug which produces a serum concentration at least 10 percent less than the LD_{50} , and even more preferably at least 50 percent, 75 percent, or even 90 percent less than the drug’s the LD_{50} .

As used herein, the term “inhibitor” is meant to describe a compound that blocks or reduces an activity of an enzyme (for example, inhibition of proteolytic cleavage of standard fluorogenic peptide substrates such as suc-LLVY-AMC, Box-LLR-AMC and Z-LLE-AMC, inhibition of various catalytic activities of the 20S proteasome). An inhibitor can act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor can bind reversibly or irreversibly, and therefore the term includes compounds that are suicide substrates of an enzyme. An inhibitor can modify one or more sites on or near the active site of the enzyme, or it can cause a conformational change elsewhere on the enzyme.

The term “electron-withdrawing group” is recognized in the art, and denotes the tendency of a substituent to attract valence electrons from neighboring atoms, *i.e.*, the substituent is electronegative with respect to neighboring atoms. A quantification of the level of electron-withdrawing capability is given by the Hammett sigma (σ) constant. This well known constant is described in many references, for instance, J. March, *Advanced Organic Chemistry*, McGraw Hill Book Company, New York, (1977 edition) pp. 251-259. The Hammett constant values are generally negative for electron donating groups (σ [P] = -0.66 for NH_2) and positive for electron withdrawing groups (σ [P] = 0.78 for a nitro group), σ [P] indicating para substitution. Exemplary electron-withdrawing groups include nitro, acyl, formyl, sulfonyl, trifluoromethyl, cyano, chloride, and the like. Exemplary electron-donating groups include amino, methoxy, and the like.

The terms “Lewis base” and “Lewis basic” are recognized in the art, and refer to a chemical moiety capable of donating a pair of electrons under certain reaction conditions. Examples of Lewis basic moieties include uncharged compounds such as alcohols, thiols, olefins, and amines, and charged moieties such as alkoxides, thiolates, carbanions, and a variety of other organic anions.

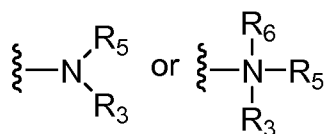
The terms “Lewis acid” and “Lewis acidic” are art-recognized and refer to chemical moieties which can accept a pair of electrons from a Lewis base.

The term “ C_{x-y} alkyl” refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups, such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms “ C_{2-y} alkenyl” and “ C_{2-y} alkynyl” refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, “lower alkyl”, as used herein, means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, and tert-butyl. Likewise, “lower alkenyl” and “lower alkynyl” have similar chain lengths. In certain embodiments, a substituent designated herein as alkyl is a lower alkyl.

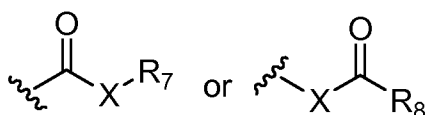
The terms “alkoxyl” or “alkoxy” as used herein refers to an alkyl group, as defined below, having an oxygen moiety attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propoxy, tert-butoxy, and the like. An “ether” is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(CH₂)_m-R₁, where m and R₁ are described below.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formulae:



wherein R₃, R₅, and R₆ each independently represent a hydrogen, an alkyl, an alkenyl, -(CH₂)_m-R₁, or R₃ and R₅ taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R₁ represents an alkenyl, aryl, cycloalkyl, a cycloalkenyl, a heterocyclyl, or a polycyclyl; and m is zero or an integer in the range of 1 to 8. In certain embodiments, only one of R₃ or R₅ can be a carbonyl, e.g., R₃, R₅, and the nitrogen together do not form an imide. In even more certain embodiments, R₃ and R₅ (and optionally R₆) each independently represent a hydrogen, an alkyl, an alkenyl, or -(CH₂)_m-R₁. Thus, the term “alkylamine” as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R₃ and R₅ is an alkyl group. In certain embodiments, an amino group or an alkylamine is basic, meaning it has a conjugate acid with a pK_a ≥ 7.00, i.e., the protonated forms of these functional groups have pK_as relative to water above about 7.00.

The term “carbonyl” is art-recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R₇ represents a hydrogen, an alkyl, an alkenyl, -(CH₂)_m-R₁ or a pharmaceutically acceptable salt, R₈ represents a hydrogen, an alkyl, an alkenyl or -(CH₂)_m-R₁, where m and R₁ are as defined above. Where X is an oxygen and R₇ or R₈ is not hydrogen, the formula represents an “ester.” Where X is an oxygen, and R₇ is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R₇ is a hydrogen, the formula represents a “carboxylic acid.” Where X is an oxygen, and R₈ is a hydrogen, the formula represents a “formate.” In general, where the oxygen atom of the above formula is replaced by a sulfur, the formula represents a “thiocarbonyl” group. Where X is a sulfur and R₇ or R₈ is not hydrogen, the formula represents a “thioester” group. Where X is a sulfur and R₇ is a hydrogen, the formula represents a “thiocarboxylic acid” group. Where X is a sulfur and R₈ is a hydrogen, the formula represents a “thioformate” group. On the other hand, where X is a bond, and R₇ is not hydrogen, the above formula represents a “ketone” group. Where X is a bond, and R₇ is a hydrogen, the above formula represents an “aldehyde” group.

The terms “heterocyclyl” or “heterocyclic group” refer to substituted or unsubstituted non-aromatic 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. The term terms “heterocyclyl” or “heterocyclic group” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term “substituted” refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that “substitution” or “substituted with” includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or

different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

As used herein, the definition of each expression, e.g., alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

The term "amino acid" is intended to embrace all compounds, whether natural or synthetic, which include both an amino functionality and an acid functionality, including amino acid analogs and derivatives. In certain embodiments, the amino acids contemplated in the present invention are those naturally occurring amino acids found in proteins, or the naturally occurring anabolic or catabolic products of such amino acids, which contain amino and carboxyl groups.

Also included are the (D) and (L) stereoisomers of such amino acids when the structure of the amino acid admits of stereoisomeric forms. The configuration of the amino acids and amino acid residues herein are designated by the appropriate symbols (D), (L) or (DL), furthermore when the configuration is not designated, the amino acid or residue can have the configuration (D), (L), or (DL). It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry are included within the scope of this invention. Such isomers can be obtained in substantially pure form by classical separation techniques and by sterically controlled synthesis. For the purposes of this application, unless expressly noted to the contrary, a named amino acid shall be construed to include both the (D) and (L) stereoisomers.

Naturally occurring amino acids are identified throughout by the conventional three-letter and/or one-letter abbreviations, corresponding to the trivial name of the amino acid, in

accordance with the following list. The abbreviations are accepted in the peptide art and are recommended by the IUPAC-IUB commission in biochemical nomenclature.

The term "peptide," as used herein, refers to a sequence of amino acid residues linked together by peptide bonds or by modified peptide bonds. The term "peptide" is intended to encompass peptide analogs, peptide derivatives, peptidomimetics and peptide variants. The term "peptide" is understood to include peptides of any length.

The terms "amino acid residue" and "peptide residue" mean an amino acid or peptide molecule without the -OH of its carboxyl group. In general the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see *Biochemistry* **1972**, *11*, 1726-1732). For instance, Met, Ile, Leu, Ala, and Gly represent "residues" of methionine, isoleucine, leucine, alanine, and glycine, respectively. Residue means a moiety derived from the corresponding α -amino acid by eliminating the OH portion of the carboxyl group and the H portion of the α -amino group. The term "amino acid side chain" is that part of an amino acid exclusive of the $-\text{CH}(\text{NH}_2)\text{COOH}$ portion, as defined by K. D. Kopple, *Peptides and Amino Acids*; Benjamin: New York, 1966; pp. 2 and 33; examples of such side chains of the common amino acids are $-\text{CH}_2\text{CH}_2\text{SCH}_3$ (the side chain of methionine), $-\text{CH}_2(\text{CH}_3)-\text{CH}_2\text{CH}_3$ (the side chain of isoleucine), $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ (the side chain of leucine) or H-(the side chain of glycine).

In certain embodiments, the amino acids used in the present invention are those naturally occurring amino acids found in native proteins, or the naturally occurring anabolic or catabolic products of such amino acids which contain amino and carboxyl groups. Particularly suitable amino acid side chains include side chains selected from those of the following amino acids: glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan, and those amino acids and amino acid analogs which have been identified as constituents of peptidylglycan bacterial cell walls.

The term amino acid residue further includes analogs, derivatives and congeners of any specific amino acid referred to herein, as well as C-terminal or N-terminal protected amino acid derivatives (e.g., modified with an N-terminal or C-terminal protecting group). For example, the present invention contemplates the use of amino acid analogs wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other

reactive precursor functional group for cyclization, as well as amino acid analogs having variant side chains with appropriate functional groups). For instance, the subject compound can include an amino acid analog such as, for example, cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxy-phenylalanine, 5-

5 hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, diaminopimelic acid, ornithine, or diaminobutyric acid. Other naturally occurring amino acid metabolites or precursors having side chains which are suitable herein will be recognized by those skilled in the art and are included in the scope of the present invention.

The term "peptide analog," as used herein, refers to a peptide comprising one or
10 more non-naturally occurring amino acid. Examples of non-naturally occurring amino acids include, but are not limited to, D-amino acids (i.e. an amino acid of an opposite chirality to the naturally occurring form), *N*- α -methyl amino acids, *C*- α -methyl amino acids, β -methyl amino acids, β -alanine (β -Ala), norvaline (Nva), norleucine (Nle), 4-aminobutyric acid (γ -Abu), 2-aminoisobutyric acid (Aib), 6-aminohexanoic acid (ϵ -Ahx), ornithine (orn),
15 hydroxyproline (Hyp), sarcosine, citrulline, cysteic acid, cyclohexylalanine, α -amino isobutyric acid, *t*-butylglycine, *t*-butylalanine, 3-aminopropionic acid, 2,3-diaminopropionic acid (2,3-diaP), D- or L-phenylglycine, D- or L-2-naphthylalanine (2-Nal), 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), D- or L-2-thienylalanine (Thi), D- or L-3-thienylalanine, D- or L-1-, 2-, 3- or 4-pyrenylalanine, D- or L-(2-pyridinyl)-alanine, D- or L-
20 (3-pyridinyl)-alanine, D- or L-(2-pyrazinyl)-alanine, D- or L-(4-isopropyl)-phenylglycine, D-(trifluoromethyl)-phenylglycine, D-(trifluoromethyl)-phenylalanine, D-*p*-fluorophenylalanine, D- or L-*p*-biphenylalanine, D- or L-*p*-methoxybiphenylalanine, methionine sulfoxide (MSO) and homoarginine (Har). Other examples include D- or L-2-indole(alkyl)alanines and D- or L-alkylalanines, wherein alkyl is substituted or unsubstituted
25 methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, or iso-pentyl, and phosphono- or sulfated (e.g., $-\text{SO}_3\text{H}$) non-carboxylate amino acids.

Other examples of non-naturally occurring amino acids include 3-(2-chlorophenyl)-alanine, 3-chloro-phenylalanine, 4-chloro-phenylalanine, 2-fluoro-phenylalanine, 3-fluoro-phenylalanine, 4-fluoro-phenylalanine, 2-bromo-phenylalanine, 3-bromo-phenylalanine, 4-
30 bromo-phenylalanine, homophenylalanine, 2-methyl-phenylalanine, 3-methyl-phenylalanine, 4-methyl-phenylalanine, 2,4-dimethyl-phenylalanine, 2-nitro-phenylalanine, 3-nitro-phenylalanine, 4-nitro-phenylalanine, 2,4-dinitro-phenylalanine, 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid, 1,2,3,4-tetrahydronorharman-3-carboxylic acid,

1-naphthylalanine, 2-naphthylalanine, pentafluorophenylalanine, 2,4-dichloro-phenylalanine, 3,4-dichloro-phenylalanine, 3,4-difluoro-phenylalanine, 3,5-difluoro-phenylalanine, 2,4,5-trifluoro-phenylalanine, 2-trifluoromethyl-phenylalanine, 3-trifluoromethyl-phenylalanine, 4-trifluoromethyl-phenylalanine, 2-cyano-phenylalanine, 3-cyano-phenylalanine, 4-cyano-phenylalanine, 2-iodo-phenylalanine, 3-iodo-phenylalanine, 4-iodo-phenylalanine, 4-methoxyphenylalanine, 2-aminomethyl-phenylalanine, 3-aminomethyl-phenylalanine, 4-aminomethyl-phenylalanine, 2-carbamoyl-phenylalanine, 3-carbamoyl-phenylalanine, 4-carbamoyl-phenylalanine, m-tyrosine, 4-amino-phenylalanine, styrylalanine, 2-amino-5-phenyl-pentanoic acid, 9-anthrylalanine, 4-tert-butyl-phenylalanine, 3,3-diphenylalanine, 4,4'-diphenylalanine, benzoylphenylalanine, α -methyl-phenylalanine, α -methyl-4-fluoro-phenylalanine, 4-thiazolylalanine, 3-benzothienylalanine, 2-thienylalanine, 2-(5-bromothienyl)-alanine, 3-thienylalanine, 2-furylalanine, 2-pyridylalanine, 3-pyridylalanine, 4-pyridylalanine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, allylglycine, 2-amino-4-bromo-4-pentenoic acid, propargylglycine, 4-aminocyclopent-2-enecarboxylic acid, 3-aminocyclopentanecarboxylic acid, 7-amino-heptanoic acid, dipropylglycine, pipercolic acid, azetidine-3-carboxylic acid, cyclopropylglycine, cyclopropylalanine, 2-methoxy-phenylglycine, 2-thienylglycine, 3-thienylglycine, α -benzyl-proline, α -(2-fluoro-benzyl)-proline, α -(3-fluoro-benzyl)-proline, α -(4-fluoro-benzyl)-proline, α -(2-chloro-benzyl)-proline, α -(3-chloro-benzyl)-proline, α -(4-chloro-benzyl)-proline, α -(2-bromo-benzyl)-proline, α -(3-bromo-benzyl)-proline, α -(4-bromo-benzyl)-proline, α -phenethyl-proline, α -(2-methyl-benzyl)-proline, α -(3-methyl-benzyl)-proline, α -(4-methyl-benzyl)-proline, α -(2-nitro-benzyl)-proline, α -(3-nitro-benzyl)-proline, α -(4-nitro-benzyl)-proline, α -(1-naphthalenylmethyl)-proline, α -(2-naphthalenylmethyl)-proline, α -(2,4-dichloro-benzyl)-proline, α -(3,4-dichloro-benzyl)-proline, α -(3,4-difluoro-benzyl)-proline, α -(2-trifluoromethyl-benzyl)-proline, α -(3-trifluoromethyl-benzyl)-proline, α -(4-trifluoromethyl-benzyl)-proline, α -(2-cyano-benzyl)-proline, α -(3-cyano-benzyl)-proline, α -(4-cyano-benzyl)-proline, α -(2-iodo-benzyl)-proline, α -(3-iodo-benzyl)-proline, α -(4-iodo-benzyl)-proline, α -(3-phenyl-allyl)-proline, α -(3-phenyl-propyl)-proline, α -(4-tert-butyl-benzyl)-proline, α -benzhydryl-proline, α -(4-biphenylmethyl)-proline, α -(4-thiazolylmethyl)-proline, α -(3-benzo[b]thiophenylmethyl)-proline, α -(2-thiophenylmethyl)-proline, α -(5-bromo-2-thiophenylmethyl)-proline, α -(3-thiophenylmethyl)-proline, α -(2-furanylmethyl)-proline, α -(2-pyridinylmethyl)-proline, α -(3-pyridinylmethyl)-proline, α -(4-pyridinylmethyl)-proline, α -allyl-proline, α -propynyl-

proline, γ -benzyl-proline, γ -(2-fluoro-benzyl)-proline, γ -(3-fluoro-benzyl)-proline, γ -(4-fluoro-benzyl)-proline, γ -(2-chloro-benzyl)-proline, γ -(3-chloro-benzyl)-proline, γ -(4-chloro-benzyl)-proline, γ -(2-bromo-benzyl)-proline, γ -(3-bromo-benzyl)-proline, γ -(4-bromo-benzyl)-proline, γ -(2-methyl-benzyl)-proline, γ -(3-methyl-benzyl)-proline, γ -(4-methyl-benzyl)-proline, γ -(2-nitro-benzyl)-proline, γ -(3-nitro-benzyl)-proline, γ -(4-nitro-benzyl)-proline, γ -(1-naphthalenylmethyl)-proline, γ -(2-naphthalenylmethyl)-proline, γ -(2,4-dichloro-benzyl)-proline, γ -(3,4-dichloro-benzyl)-proline, γ -(3,4-difluoro-benzyl)-proline, γ -(2-trifluoromethyl-benzyl)-proline, γ -(3-trifluoromethyl-benzyl)-proline, γ -(4-trifluoromethyl-benzyl)-proline, γ -(2-cyano-benzyl)-proline, γ -(3-cyano-benzyl)-proline, γ -(4-cyano-benzyl)-proline, γ -(2-iodo-benzyl)-proline, γ -(3-iodo-benzyl)-proline, γ -(4-iodo-benzyl)-proline, γ -(3-phenyl-allyl-benzyl)-proline, γ -(3-phenyl-propyl-benzyl)-proline, γ -(4-tert-butyl-benzyl)-proline, γ -benzhydryl-proline, γ -(4-biphenylmethyl)-proline, γ -(4-thiazolylmethyl)-proline, γ -(3-benzothioienylmethyl)-proline, γ -(2-thienylmethyl)-proline, γ -(3-thienylmethyl)-proline, γ -(2-furanylmethyl)-proline, γ -(2-pyridinylmethyl)-proline, γ -(3-pyridinylmethyl)-proline, γ -(4-pyridinylmethyl)-proline, γ -allyl-proline, γ -propynyl-proline, trans-4-phenyl-pyrrolidine-3-carboxylic acid, trans-4-(2-fluoro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-fluoro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-fluoro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-chloro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-chloro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-chloro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-bromo-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-bromo-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-bromo-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-methyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-methyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-methyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-nitro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-nitro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-nitro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(1-naphthyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-naphthyl)-pyrrolidine-3-carboxylic acid, trans-4-(2,5-dichloro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2,3-dichloro-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-trifluoromethyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-trifluoromethyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-trifluoromethyl-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-cyano-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-cyano-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-cyano-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-methoxy-phenyl)-pyrrolidine-3-carboxylic acid,

trans-4-(3-methoxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-methoxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-hydroxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-hydroxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-hydroxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2,3-dimethoxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3,4-dimethoxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(3,5-dimethoxy-phenyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-pyridinyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-pyridinyl)-pyrrolidine-3-carboxylic acid, trans-4-(6-methoxy-3-pyridinyl)-pyrrolidine-3-carboxylic acid, trans-4-(4-pyridinyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-thienyl)-pyrrolidine-3-carboxylic acid, trans-4-(3-thienyl)-pyrrolidine-3-carboxylic acid, trans-4-(2-furanyl)-pyrrolidine-3-carboxylic acid, trans-4-isopropyl-pyrrolidine-3-carboxylic acid, 4-phosphonomethyl-phenylalanine, benzyl-phosphothreonine, (1'-amino-2-phenyl-ethyl)oxirane, (1'-amino-2-cyclohexyl-ethyl)oxirane, (1'-amino-2-[3-bromo-phenyl]ethyl)oxirane, (1'-amino-2-[4-(benzyloxy)phenyl]ethyl)oxirane, (1'-amino-2-[3,5-difluoro-phenyl]ethyl)oxirane, (1'-amino-2-[4-carbamoyl-phenyl]ethyl)oxirane, (1'-amino-2-[benzyloxy-ethyl])oxirane, (1'-amino-2-[4-nitro-phenyl]ethyl)oxirane, (1'-amino-3-phenyl-propyl)oxirane, (1'-amino-3-phenyl-propyl)oxirane, and/or salts and/or protecting group variants thereof.

The term "peptide derivative," as used herein, refers to a peptide comprising additional chemical or biochemical moieties not normally a part of a naturally occurring peptide. Peptide derivatives include peptides in which the amino-terminus and/or the carboxy-terminus and/or one or more amino acid side chain has been derivatised with a suitable chemical substituent group, as well as cyclic peptides, dual peptides, multimers of the peptides, peptides fused to other proteins or carriers, glycosylated peptides, phosphorylated peptides, peptides conjugated to lipophilic moieties (for example, caproyl, lauryl, stearoyl moieties) and peptides conjugated to an antibody or other biological ligand. Examples of chemical substituent groups that may be used to derivatise a peptide include, but are not limited to, alkyl, cycloalkyl and aryl groups; acyl groups, including alkanoyl and aroyl groups; esters; amides; halogens; hydroxyls; carbamyls, and the like. The substituent group may also be a blocking group such as Fmoc (fluorenylmethyl-O-CO-), carbobenzoxy (benzyl-O-CO-), monomethoxysuccinyl, naphthyl-NH-CO-, acetylamino-caproyl and adamantyl-NH-CO-. Other derivatives include C-terminal hydroxymethyl derivatives, O-modified derivatives (for example, C-terminal hydroxymethyl benzyl ether) and N-terminally modified derivatives including substituted amides such as alkylamides

and hydrazides. The substituent group may be a “protecting group” as detailed herein.

The term “peptidomimetic,” as used herein, refers to a compound that is structurally similar to a peptide and contains chemical moieties that mimic the function of the peptide.

For example, if a peptide contains two charged chemical moieties having functional

5 activity, a mimetic places two charged chemical moieties in a spatial orientation and constrained structure so that the charged chemical function is maintained in three-

dimensional space. The term peptidomimetic thus is intended to include isosteres. The

term “isostere,” as used herein, refers to a chemical structure that can be substituted for a peptide because the steric conformation of the chemical structure is similar, for example,

10 the structure fits a binding site specific for the peptide. Examples of peptidomimetics include peptides comprising one or more backbone modifications (i.e. amide bond

mimetics), which are well known in the art. Examples of amide bond mimetics include, but are not limited to, $-\text{CH}_2\text{NH}-$, $-\text{CH}_2\text{S}-$, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}=\text{CH}-$ (cis and trans), $-\text{COCH}_2-$,

$-\text{CH}(\text{OH})\text{CH}_2-$, $-\text{CH}_2\text{SO}-$, $-\text{CS}-\text{NH}-$ and $-\text{NH}-\text{CO}-$ (i.e. a reversed peptide bond) (see,

15 for example, Spatola, Vega Data Vol. 1, Issue 3, (1983); Spatola, in *Chemistry and*

Biochemistry of Amino Acids Peptides and Proteins, Weinstein, ed., Marcel Dekker, New York, p. 267 (1983); Morley, J. S., *Trends Pharm. Sci.* pp. 463-468 (1980); Hudson et al.,

Int. J. Pept. Prot. Res. 14:177-185 (1979); Spatola et al., *Life Sci.* 38:1243-1249 (1986);

Hann, J; *Chem. Soc. Perkin Trans. I*, 307-314 (1982); Almquist et al., *J. Med Chem.*

20 23:1392-1398 (1980); Jennings-White et al., *Tetrahedron Lett.* 23:2533 (1982); Szelke et al., EP 45665 (1982); Holladay et al., *Tetrahedron Lett.* 24:4401-4404 (1983); and Hruby,

Life Sci. 31:189-199 (1982)). Other examples of peptidomimetics include peptides

substituted with one or more benzodiazepine molecules (see, for example, James, G. L. et al. (1993) *Science* 260:1937-1942) and peptides comprising backbones cross-linked to form

25 lactams or other cyclic structures.

The term “variant peptide,” as used herein, refers to a peptide in which one or more amino acid residue has been deleted, added or substituted in comparison to the amino acid sequence to which the peptide corresponds. Typically, when a variant contains one or more

amino acid substitutions they are “conservative” substitutions. A conservative substitution

30 involves the replacement of one amino acid residue by another residue having similar side chain properties. As is known in the art, the twenty naturally occurring amino acids can be grouped according to the physicochemical properties of their side chains. Suitable

groupings include: alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine

and tryptophan (hydrophobic side chains); glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine (polar, uncharged side chains); aspartic acid and glutamic acid (acidic side chains) and lysine, arginine and histidine (basic side chains). Another grouping of amino acids is phenylalanine, tryptophan, and tyrosine (aromatic side chains). A conservative substitution involves the substitution of an amino acid with another amino acid from the same group.

The terms “percent (%) amino acid sequence identity” or “percent amino acid sequence homology” as used herein with respect to a reference polypeptide is defined as the percentage of amino acid residues in a candidate peptide sequence that are identical with the amino acid residues in the reference polypeptide sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, without considering any conservative substitutions as part of the sequence identity. Alignment for the purpose of determining percent amino acid sequence identity can be achieved by various techniques known in the art, for instance, using publicly available computer software such as ALIGN or Megalign (DNASTAR). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the peptide sequence being used in the comparison. In the context of the present invention, an analog of GLP-1 is said to share “substantial homology” with GLP-1 if the amino acid sequences of said compound are at least about 80%, at least about 90%, at least about 95%, or substantially the same as that of native GLP-1.

As used herein, the terminology “GLP-1 compound,” be it the native sequence, synthetic versions and variants thereof, truncated analogs and derivatives thereof, including those of the present invention, also includes pharmaceutically acceptable salts of said compounds described, and in accordance with the detailed definitions herein. A GLP-1 compound of this invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such salts include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate,

monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like. Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, and the like.

The term “retro modified,” as used herein, refers to a peptide that is made up of L-amino acids in which the amino acid residues are assembled in the opposite direction to the native peptide with respect to which it is retro modified (see Figure 2).

The term “inverso modified,” as used herein, refers to a peptide that is made up of D-amino acids in which the amino acid residues are assembled in the same direction as the native peptide with respect to which it is inverso modified (see Figure 2).

The term “retro-inverso modified,” as used herein, refers to a peptide that is made up of D-amino acids in which the amino acid residues are assembled in the opposite direction to the native peptide with respect to which it is retro-inverso modified (see Figure 2).

Retro-inverso truncated analogues of peptides may be prepared for peptides according to the following protocol. A peptide sequence (e.g., an incretin hormone) is selected as a model peptide for design and synthesis using D-amino acids by attaching the amino acids in a peptide chain such that the sequence of amino acids in a retro-inverso peptide truncated analog is opposite that in the selected model peptide. To illustrate, if the peptide model is a peptide formed of L-amino acids having the sequence ABC, the retro-inverso peptide truncated analog formed of D-amino acids would have the sequence CBA. The procedures for synthesizing a chain of D-amino acids to form the retro-inverso peptides are known in the art, some of which are illustrated in the references cited herein.

Truncated analogs can differ from the native peptides by amino acid sequence or by modifications that do not affect the sequence or both. Certain analogs include peptides

whose sequences differ from the wild-type sequence (i.e., the sequence of the homologous portion of the naturally occurring peptide) only by conservative amino acid substitutions, preferably by only one, two, or three, substitutions; for example, differing by substitution of one amino acid for another with similar characteristics (e.g., valine for glycine, arginine for lysine) or by one or more non-conservative amino acid substitutions, deletions, or insertions, which do not abolish the peptide's biological activity. Modifications that do not usually alter primary sequence include in vivo or in vitro chemical derivatization of peptides (e.g., acetylation or carboxylation). Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a peptide during its synthesis and processing or in further processing steps, e.g., by exposing the peptide to enzymes (e.g., mammalian glycosylating or deglycosylating enzymes) that affect glycosylation. Also included are sequences that have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine. The invention also includes analogs in which one or more peptide bonds have been replaced with an alternative type of covalent bond (a "peptide mimetic"), which is less susceptible to cleavage by peptidases. Where proteolytic degradation of the peptides following injection into a subject is a problem, replacement of a particularly sensitive peptide bond with a non-cleavable peptide mimetic will make the resulting peptide more stable and thus likely to be more useful as a therapeutic agent. Such amino acid mimetics, and methods of incorporating them into peptides, are well known in the art. Protecting groups are also useful.

Native peptide sequences set out herein are written according to the generally accepted convention whereby the *N*-terminal amino acid is on the left, and the *C*-terminal amino acid is on the right. The sequences of the peptide analogs, however, may run in the same direction as that of the corresponding sequence in the native peptide (i.e., the *N*-terminus of the peptide analog corresponds to the *N*-terminal end of the corresponding amino acid sequence in the native peptide), or the sequence of the peptide may be inverted (i.e., the *N*-terminus of the peptide analog corresponds to the *C*-terminal end of the corresponding amino acid sequence in the native peptide). For example, for a peptide region having a sequence from *N*- to *C*-terminus: 123456, the sequence of a retro-modified peptide corresponding to this region would be from *N*- to *C*-terminus: 654321, or could be optionally represented from *C*-terminus to *N*-terminus as 123456, so long as the termini are clearly identified in the depiction (see, e.g., Figures 1 and 2).

The term "regioisomers" refers to compounds which have the same molecular formula but differ in the connectivity of the atoms. Accordingly, a "regioselective process" is one which favors the production of a particular regioisomer over others, e.g., the reaction produces a statistically significant preponderance of a certain regioisomer.

5 The phrase "protecting group" as used herein means substituents which protect the reactive functional group from undesirable chemical reactions. Examples of such protecting groups include esters of carboxylic acids and boronic acids, ethers of alcohols, and acetals and ketals of aldehydes and ketones. For instance, the phrase "N-terminal protecting group" or "amino-protecting group" as used herein refers to various amino-
10 protecting groups which can be employed to protect the N-terminus of an amino acid or peptide against undesirable reactions during synthetic procedures. Examples of suitable groups include acyl protecting groups such as, to illustrate, formyl, dansyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; aromatic urethane protecting groups as, for example, benzyloxycarbonyl (Cbz); and aliphatic urethane protecting groups such as t-
15 butoxycarbonyl (Boc) or 9-Fluorenylmethoxycarbonyl (Fmoc).

 The term "amino-protecting group" or "N-terminal protecting group" refers to those groups intended to protect the α -N-terminal of an amino acid or peptide or to otherwise protect the amino group of an amino acid or peptide against undesirable reactions during synthetic procedures. Commonly used N-protecting groups are disclosed in Greene,
20 *Protective Groups In Organic Synthesis*, (John Wiley & Sons, New York (1981)), which is hereby incorporated by reference. Additionally, protecting groups may be used as pro-drugs which are readily cleaved in vivo, for example, by enzymatic hydrolysis, to release the biologically active parent. α -N-Protecting groups comprise lower alkanoyl groups such as formyl, acetyl ("Ac"), propionyl, pivaloyl, t-butylacetyl and the like; other acyl groups
25 include 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, -chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl and the like; carbamate forming groups such as benzyloxycarbonyl, p-
chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-
30 nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-ethoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenyl)-1-methylethoxycarbonyl, α,α -dimethyl-

3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropylloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentylloxycarbonyl, 5 adamantylloxycarbonyl, cyclohexylloxycarbonyl, phenylthiocarbonyl and the like; arylalkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl, 9-fluorenylmethyloxycarbonyl (Fmoc) and the like and silyl groups such as trimethylsilyl and the like. Still other examples include theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelalyl, dansyl, benzyloxycarbonyl, methoxyazelalyl, methoxyadipyl, methoxysuberyl, and 2,4- 10 dinitrophenyl.

The term "carboxy protecting group" or "C-terminal protecting group" refers to a carboxylic acid protecting ester or amide group employed to block or protect the carboxylic acid functionality while the reactions involving other functional sites of the compound are performed. Carboxy protecting groups are disclosed in Greene, *Protective Groups in*

15 *Organic Synthesis* pp. 152-186 (1981), which is hereby incorporated by reference.

Additionally, a carboxy protecting group may be used as a pro-drug whereby the carboxy protecting group can be readily cleaved in vivo, for example by enzymatic hydrolysis, to release the biologically active parent. Such carboxy protecting groups are well known to those skilled in the art, having been extensively used in the protection of carboxyl groups in

20 the penicillin and cephalosporin fields as described in U.S. Patent Nos. 3,840,556 (incorporated by reference) and 3,719,667 (incorporated by reference). Representative

carboxy protecting groups are C₁-C₈ loweralkyl (e.g., methyl, ethyl or t-butyl and the like); arylalkyl such as phenethyl or benzyl and substituted derivatives thereof such as

25 alkoxybenzyl or nitrobenzyl groups and the like; arylalkenyl such as phenylethenyl and the like; aryl and substituted derivatives thereof such as 5-indanyl and the like;

dialkylaminoalkyl such as dimethylaminoethyl and the like); alkanoyloxyalkyl groups such as acetoxymethyl, butyryloxymethyl, valeryloxymethyl, isobutyryloxymethyl, isovaleryloxymethyl, 1-(propionyloxy)-1-ethyl, 1-(pivaloyloxy)-1-ethyl, 1-methyl-1-(propionyloxy)-1-ethyl, pivaloyloxymethyl, propionyloxymethyl and the like;

30 cycloalkanoyloxyalkyl groups such as cyclopropylcarbonyloxymethyl,

cyclobutylcarbonyloxymethyl, cyclopentylcarbonyloxymethyl,

cyclohexylcarbonyloxymethyl and the like; aroyloxyalkyl such as benzoyloxymethyl,

benzoyloxyethyl and the like; arylalkylcarbonyloxyalkyl such as benzylcarbonyloxymethyl,

2-benzylcarbonyloxyethyl and the like; alkoxy carbonylalkyl or cycloalkyloxy carbonylalkyl such as methoxycarbonylmethyl, cyclohexyloxy carbonylmethyl, 1-methoxycarbonyl-1-ethyl and the like; alkoxy carbonyloxyalkyl or cycloalkyloxy carbonyloxyalkyl such as methoxycarbonyloxymethyl, t-butyloxy carbonyloxymethyl, 1-ethoxycarbonyloxy-1-ethyl, 5 1-cyclohexyloxy carbonyloxy-1-ethyl and the like; aryloxy carbonyloxyalkyl such as 2-(phenoxycarbonyloxy)ethyl, 2-(5-indanyloxy carbonyloxy)ethyl and the like; alkoxyalkyl carbonyloxyalkyl such as 2-(1-methoxy-2-methylpropan-2-oyloxy)ethyl and like; arylalkyloxy carbonyloxyalkyl such as 2-(benzyloxy carbonyloxy)ethyl and the like; arylalkenyloxy carbonyloxyalkyl such as 2-(3-phenylpropen-2-yloxy carbonyloxy)ethyl and 10 the like; alkoxy carbonylaminoalkyl such as t-butyloxy carbonylaminomethyl and the like; alkylaminocarbonylaminoalkyl such as methylaminocarbonylaminomethyl and the like; alkanoylaminoalkyl such as acetylaminomethyl and the like; heterocyclic carbonyloxyalkyl such as 4-methylpiperazinyl carbonyloxymethyl and the like; dialkylaminocarbonylalkyl such as dimethylaminocarbonylmethyl, diethylaminocarbonylmethyl and the like; (5- 15 (loweralkyl)-2-oxo-1,3-dioxolen-4-yl)alkyl such as (5-t-butyl-2-oxo-1,3-dioxolen-4-yl)methyl and the like; and (5-phenyl-2-oxo-1,3-dioxolen-4-yl)alkyl such as (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl and the like. Representative amide carboxy protecting groups are aminocarbonyl and loweralkylaminocarbonyl groups. For example, aspartic acid may be protected at the α -C-terminal by an acid labile group (e.g., t-butyl) and 20 protected at the β -C-terminal by a hydrogenation labile group (e.g., benzyl) then deprotected selectively during synthesis. As mentioned above, the protected carboxy group may also be a loweralkyl, cycloalkyl or arylalkyl ester, for example, methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, sec-butyl ester, isobutyl ester, amyl ester, isoamyl ester, octyl ester, cyclohexyl ester, phenylethyl ester and the like or an 25 alkanoyloxyalkyl, cycloalkanoyloxyalkyl, aroyloxyalkyl or an arylalkyl carbonyloxyalkyl ester.

As noted above, certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, *R*- and *S*-enantiomers, diastereomers, (D)- 30 isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomer. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomer.

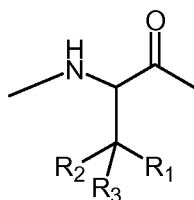
For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 67th ed., 1986-87, inside cover.

A compound is said to have an "insulinotropic activity" if it is able to stimulate, or cause the stimulation of, the synthesis or expression of the hormone insulin.

Another aspect of the present invention relates to pharmaceutical compositions of dipeptidylpeptidase inhibitors, particularly inhibitor(s) and their uses in treating and/or preventing disorders which can be improved by altering the homeostasis of peptide hormones. In an embodiment, the inhibitors have hypoglycemic and antidiabetic activities, and may be used in the treatment of disorders marked by aberrant glucose metabolism (including storage). In particular embodiments, the compositions of the subject methods are useful as insulinotropic agents, or to potentiate the insulinotropic effects of such molecules as GLP-1. In this regard, the present method can be useful for the treatment and/or prophylaxis of a variety of disorders, including one or more of: hyperlipemia, hyperglycemia, obesity, glucose tolerance insufficiency, insulin resistance, and diabetic complications.

VII. Exemplary Embodiments

In certain embodiments, the invention relates to a proteinase-resistant truncated analog of a biologically active peptide or polypeptide selected from the group consisting of HAXGTFTSDVSSYLEGQAAKEFIAWLK-NH₂ and HAXGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂; wherein X is an amino acid analog represented by Formula I:



wherein

R_1 and R_2 are independently selected from a lower alkyl or a halogen;

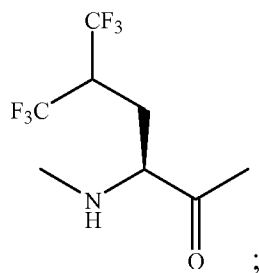
- R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$,
 5 $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$; and
 m is 0, 1, or 2.

In certain embodiments, the invention relates to the aforementioned truncated analog, wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$; and m is 0, 1, or 2.

- In certain embodiments, the invention relates to the aforementioned truncated
 10 analog, wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

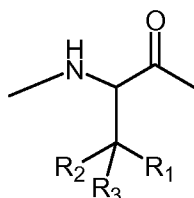
In certain embodiments, the invention relates to a proteinase-resistant truncated analog of a biologically active peptide or polypeptide which has the amino acid sequence: HEGTFTSDLSKQMEEEAVRLFIEWLKN-NH₂.

- In certain embodiments, the invention relates to a proteinase-resistant truncated
 15 analog of a biologically active peptide or polypeptide selected from the group consisting of
 HAXGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,
 HAXGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and
 HEGTFTSDL*SKQMEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for
 each occurrence leucine or an amino acid analog represented by:



20

and X is an amino acid analog represented by Formula I:



wherein

R_1 and R_2 are independently selected from a lower alkyl or a halogen;

R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$,

5 $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$; and

m is 0, 1, or 2.

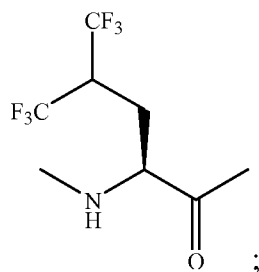
In certain embodiments, the invention relates to the aforementioned truncated analog, wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$; and m is 0, 1, or 2.

10 In certain embodiments, the invention relates to the aforementioned truncated analog, wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

In certain embodiments, the invention relates to a retro-inverso truncated analog of a biologically active peptide or polypeptide selected from the group consisting of HAEGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,

HAEGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and

15 HEGTFTSDL*SKQMEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



said analog has at least about 90% sequence homology to the peptide fragment or polypeptide fragment; and said analog consists essentially of D-amino acids assembled in
20 reversed order along the peptide chain.

In certain embodiments, the invention relates to the aforementioned truncated analog, wherein said analog has at least about 95% sequence homology to the peptide fragment or polypeptide fragment.

In certain embodiments, the invention relates to the aforementioned truncated analog, wherein said analog has at least about 99% sequence homology to the peptide fragment or polypeptide fragment.

In certain embodiments, the invention relates to a retro-inverso truncated analog of a
5 biologically active peptide or polypeptide selected from the group consisting of
HAEGTFTSDVSSYLEGQAAKEFIAWLVK-NH₂,
HAEGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂, and
HGEFTFTSDLSKQMEEEAVRLFIEWLKN-NH₂; wherein said analog has at least about
90% sequence homology to the peptide fragment or polypeptide fragment; and said analog
10 consists essentially of D-amino acids assembled in reversed order along the peptide chain;
and wherein said analog is independently derivatized at one or both of the terminal
residues.

In certain embodiments, the invention relates to the aforementioned truncated
analog, wherein said analog has at least about 95% sequence homology to the peptide or
15 polypeptide.

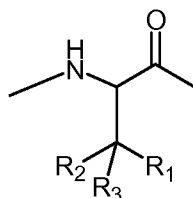
In certain embodiments, the invention relates to the aforementioned truncated
analog, wherein said analog has at least about 99% sequence homology to the peptide or
polypeptide.

In certain embodiments, the invention relates to any one of the aforementioned
20 truncated analogs, wherein the peptide sequence is extended by 1 to about 10 additional
amino acid residues at one or both of the terminal residues.

In certain embodiments, the invention relates to a formulation, comprising a
compound according to any one of the aforementioned truncated analogs; and a
pharmaceutically acceptable excipient.

25 In certain embodiments, the invention relates to a formulation, comprising a
compound according to any one of the aforementioned truncated analogs; and an acceptable
excipient for administration to an animal.

In certain embodiments, the invention relates to a method for treating or preventing
a disease or condition in a mammal, comprising the step of administering to a mammal in
30 need thereof an effective amount of a truncated analog selected from the group consisting
of HAXGTFTSDVSSYLEGQAAKEFIAWLVK-NH₂ and
HAXGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂; wherein X is an amino acid analog
represented by Formula I:



;

wherein R_1 and R_2 are independently selected from a lower alkyl or a halogen;

R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$, $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$;

5 m is 0, 1, or 2; and

said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, 10 gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

In certain embodiments, the invention relates to the aforementioned method,

15 wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$; and m is 0, 1, or 2.

In certain embodiments, the invention relates to the aforementioned method,

wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

In certain embodiments, the invention relates to the aforementioned method,

20 wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

In certain embodiments, the invention relates to the aforementioned method,

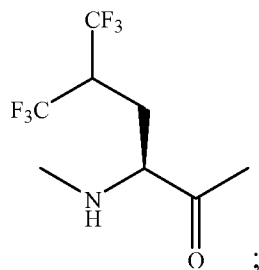
wherein said method modifies glucose metabolism.

In certain embodiments, the invention relates to a method for treating or preventing

25 a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a truncated analog with the amino acid sequence:

HGEGTFTSDL*SKQMEEEAVRL*FIEWL*KN-NH₂;

wherein L* is independently for each occurrence leucine or an amino acid analog represented by:

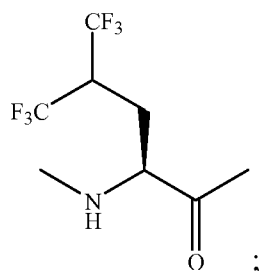


and said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

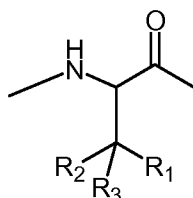
10 In certain embodiments, the invention relates to the aforementioned method, wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

15 In certain embodiments, the invention relates to the aforementioned method, wherein said method modifies glucose metabolism.

In certain embodiments, the invention relates to a method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a truncated analog selected from the group consisting of HAXGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,
 20 HAXGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and HEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



X is an amino acid analog represented by Formula I:



wherein

R_1 and R_2 are independently selected from a lower alkyl or a halogen;

R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$,

5 $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$;

m is 0, 1, or 2; and

said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, 10 gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

15 In certain embodiments, the invention relates to the aforementioned method, wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$; and m is 0, 1, or 2.

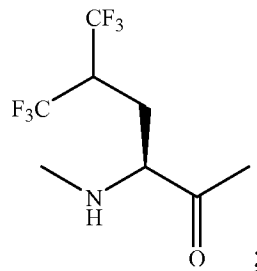
In certain embodiments, the invention relates to the aforementioned method, wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

In certain embodiments, the invention relates to the aforementioned method, 20 wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

In certain embodiments, the invention relates to the aforementioned method, wherein said method modifies glucose metabolism.

25 In certain embodiments, the invention relates to a method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a retro-inverso truncated analog selected from the group consisting of HAEGTFTSDVSSYL*EGQAAKEFIWL*VK-NH₂, HAEGTFTSDVSSYL*EGQAAKEFIWL*KN-NH₂, and

HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



said analog has at least about 90% sequence homology to the peptide fragment or

5 polypeptide fragment; said analog consists essentially of D-amino acids assembled in reversed order along the peptide chain; and said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia,

hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related

complications, obesity-related hypertension, abnormalities of digestive track mucosa, food

10 intake disorder, gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

15 In certain embodiments, the invention relates to the aforementioned method, wherein said analog has at least about 95% sequence homology to the peptide or polypeptide.

In certain embodiments, the invention relates to the aforementioned method, wherein said analog has at least about 99% sequence homology to the peptide or

20 polypeptide.

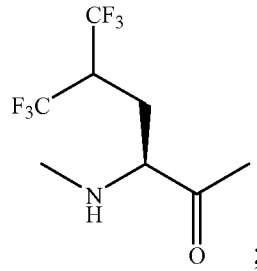
In certain embodiments, the invention relates to any one of the aforementioned methods, wherein the peptide sequence is extended by 1 to about 10 additional amino acid residues at one or both of the terminal residues.

In certain embodiments, the invention relates to any one of the aforementioned

25 methods, wherein said method modifies glucose metabolism.

In certain embodiments, the invention relates to a method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a retro-inverso truncated analog selected from the group consisting of HAEGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,

HAEGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and
 HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for
 each occurrence leucine or an amino acid analog represented by:



- 5 said analog has at least about 90% sequence homology to the peptide fragment or
 polypeptide fragment; said analog consists essentially of D-amino acids assembled in
 reversed order along the peptide chain; said analog is independently derivatized at one or
 both of the terminal residues; and said disease or condition is selected from the group
 consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia,
 10 obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-
 related hypertension, abnormalities of digestive track mucosa, food intake disorder,
 gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease),
 inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-
 reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression,
 15 schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss,
 forgetfulness, and narcolepsy.

In certain embodiments, the invention relates to the aforementioned method,
 wherein said analog has at least about 95% sequence homology to the peptide or
 polypeptide.

- 20 In certain embodiments, the invention relates to the aforementioned method,
 wherein said analog has at least about 99% sequence homology to the peptide or
 polypeptide.

- In certain embodiments, the invention relates to any one of the aforementioned
 methods, wherein the peptide sequence is extended by 1 to about 10 additional amino acid
 25 residues at one or both of the terminal residues.

In certain embodiments, the invention relates to any one of the aforementioned
 methods, wherein said method modifies glucose metabolism.

Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1

Truncated exendin and truncated stable GLP-1 test compounds were tested in 9 week old db/db mice for their effect on blood glucose levels. BKS.Cg-m^{+/+} Lepr(db)/J mice for the study were purchased from Jackson Labs at 7-8 weeks of age. The mice were given minimum of 2 days following delivery to acclimate before beginning experiments.

The compounds were prepared as solutions in sterile buffered saline and administered at 8 µg per animal by IP injection. A pre-dose blood glucose measurement was made with a hand-held glucometer while the animals were anesthetized with isoflurane. The compounds were then injected while the animals were still under anesthesia. The animal's blood glucose was then measured in duplicate or triplicate with a handheld glucometer from a drop of blood from the lateral tail vein at either 60 minutes or 240 minutes post injection, without anesthesia. As shown in Figure 4, truncated stable GLP-1 showed a greater stabilizing effect on blood glucose levels after administration than truncated exendin. The test procedure for this study is listed below.

Detailed Procedure

Test Agent

- Dissolved or diluted from concentrated stock into sterile PBS at 0.02 mg/mL. Made 5 mL of each test agent solution for 10 animals.
- Filled syringes with 0.4 mL (8 ug) drug. Used 1 mL disposable syringes with 25 gauge needles.

Animals:

- BKS.Cg-m^{+/+} Lepr(db)/J mice were purchased from Jackson Labs at 7-8 weeks of age. Allowed a minimum of 2 days following delivery to acclimate before beginning experiments. The mice were exposed to normal lighting with 12 hours light and 12 hours dark.
- Animals were fed standard chow (Teklad 2018) and water ad libitum.
- Diabetic animals were housed no more than 3 per cage. Additional cleaning required as the disease progresses.

Experiment:

- Anesthetized animals with isoflurane. When asleep, moved animal to nose cone and kept anesthetized for initial blood glucose (BG) measurement and injection.
- Measured Blood Glucose using Freestyle BG meter
 - 5 ○ wiped the tail of animal with an alcohol swab.
 - a sterile needle was used to make a nick in the tail vein.
 - wiped away the first drop of blood
 - measured BG using the meter as described in the manual.
 - measurements made in duplicate. If blood glucose differed by more than 30
 - 10 mg/dl, measured a 3rd time and use the two nearest values.
 - Applied pressure with gauze to stop bleeding, if necessary.
- Administered test compound
- Injected 0.4 mL of 0.02 mg/mL drug (dose = 8 ug) by IP injection. This was done quickly so that the animal could be returned to the cage before regaining
- 15 consciousness.
 - recorded the time of injection
 - returned the animal to the cage and observed until awake.
- Measure Blood Glucose at designated time.(60 or 240 min post dose)
 - 20 ○ Anesthetized animals with isoflurane. When asleep, moved animal to nose cone.
 - wiped the tail with an alcohol swab.
 - a sterile needle was used to make a nick in the tail vein.
 - wiped away the first drop of blood
 - measured BG using the meter as described in the manual.
 - 25 ○ measurements were made in duplicate. If blood glucose differed by more than 30 mg/dl, measured a 3rd time and use the two nearest values. apply pressure with gauze to stop bleeding if necessary.
 - recorded time of measurement.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

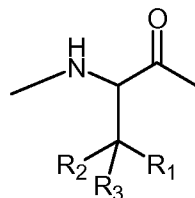
5 claims.

Incorporation by Reference

All of the U.S. patents and U.S. patent application publications cited herein are hereby incorporated by reference.

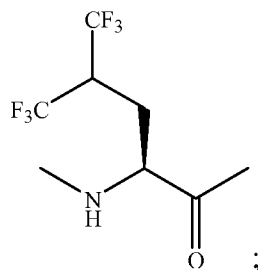
We claim:

1. A proteinase-resistant truncated analog of a biologically active peptide or polypeptide selected from the group consisting of HAXGTFTSDVSSYLEGQAAKEFIAWLK-NH₂ and
- 5 HAXGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂; wherein X is an amino acid analog represented by Formula I:

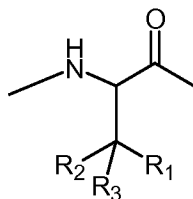


wherein

- R₁ and R₂ are independently selected from a lower alkyl or a halogen;
- 10 R₃ is selected from lower alkyl, aryl, hydroxyl group, -(CH₂)_m-COOH, -(CH₂)_m-NH₂, -(CH₂)_m-N-C(=NH)NH₂, -(CH₂)_m-C(=O)NH₂, -SH, and -(CH₂)_m-S-CH₃; and m is 0, 1, or 2.
 2. The truncated analog of claim 1, wherein R₁ and R₂ are lower alkyl; R₃ is -(CH₂)_m-COOH; and m is 0, 1, or 2.
 - 15 3. The truncated analog of claim 1, wherein R₁ and R₂ are methyl; R₃ is -(CH₂)_m-COOH; and m is 0.
 4. A proteinase-resistant truncated analog of a biologically active peptide or polypeptide which has the amino acid sequence:
HGEFTFTSDLSKQMEEEEAVRLFIEWLKN-NH₂.
 - 20 5. A proteinase-resistant truncated analog of a biologically active peptide or polypeptide selected from the group consisting of HAXGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂, HAXGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and HGEFTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for
 - 25 each occurrence leucine or an amino acid analog represented by:

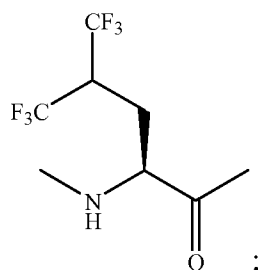


and X is an amino acid analog represented by Formula I:



wherein

- 5 R_1 and R_2 are independently selected from a lower alkyl or a halogen;
 R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$,
 $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$; and
 m is 0, 1, or 2.
6. The truncated analog of claim 5, wherein R_1 and R_2 are lower alkyl; R_3 is
10 $-(CH_2)_m-COOH$; and m is 0, 1, or 2.
7. The truncated analog of claim 5, wherein R_1 and R_2 are methyl; R_3 is
 $-(CH_2)_m-COOH$; and m is 0.
8. A retro-inverso truncated analog of a biologically active peptide or polypeptide
selected from the group consisting of
15 HAEGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,
HAEGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and
HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for
each occurrence leucine or an amino acid analog represented by:



said analog has at least about 90% sequence homology to the peptide fragment or polypeptide fragment; and said analog consists essentially of D-amino acids assembled in reversed order along the peptide chain.

9. The truncated analog of claim 8, wherein said analog has at least about 95% sequence homology to the peptide fragment or polypeptide fragment.

10. The truncated analog of claim 8, wherein said analog has at least about 99% sequence homology to the peptide fragment or polypeptide fragment.

11. A retro-inverso truncated analog of a biologically active peptide or polypeptide selected from the group consisting of HAEGTFTSDVSSYLEGQAAKEFIAWLVK-NH₂, HAEGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂, and HEGTFTSDLSKQMEEEEAVRLFIEWLKN-NH₂; wherein said analog has at least about 90% sequence homology to the peptide fragment or polypeptide fragment; and said analog consists essentially of D-amino acids assembled in reversed order along the peptide chain; and wherein said analog is independently derivatized at one or both of the terminal residues.

12. The truncated analog of claim 11, wherein said analog has at least about 95% sequence homology to the peptide or polypeptide.

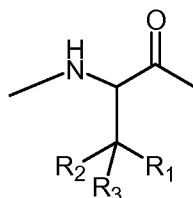
13. The truncated analog of claim 11, wherein said analog has at least about 99% sequence homology to the peptide or polypeptide.

14. The truncated analog of any one of claims 8-13, wherein the peptide sequence is extended by 1 to about 10 additional amino acid residues at one or both of the terminal residues.

15. A formulation, comprising a compound according to any of claims 1-14; and a pharmaceutically acceptable excipient.

16. A formulation, comprising a compound according to any of claims 1-14; and an acceptable excipient for administration to an animal.

17. A method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a truncated analog selected from the group consisting of HAXGTFTSDVSSYLEGQAAKEFIAWLVK-NH₂ and HAXGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂; wherein X is an amino acid analog represented by Formula I:



;

wherein R_1 and R_2 are independently selected from a lower alkyl or a halogen;

R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$, $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$;

5 m is 0, 1, or 2; and

said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, 10 gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

18. The method of claim 17, wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$;

15 and m is 0, 1, or 2.

19. The method of claim 17, wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

20. The method of claim 17, wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia,

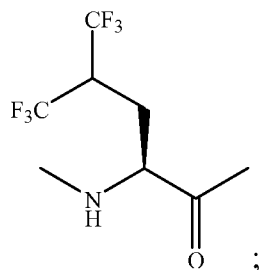
20 obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

21. The method of claim 17, wherein said method modifies glucose metabolism.

22. A method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a truncated analog with the amino acid sequence: HEGTFTSDL*SKQMEEEAVRL*FIEWL*KN-

25 NH_2 ;

wherein L^* is independently for each occurrence leucine or an amino acid analog represented by:

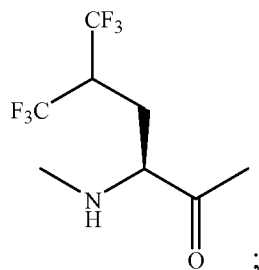


and said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

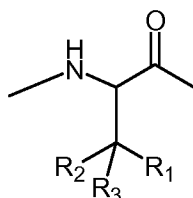
23. The method of claim 22, wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

24. The method of claim 22, wherein said method modifies glucose metabolism.

25. A method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a truncated analog selected from the group consisting of
HAXGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,
HAXGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and
HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



X is an amino acid analog represented by Formula I:



wherein

R_1 and R_2 are independently selected from a lower alkyl or a halogen;

R_3 is selected from lower alkyl, aryl, hydroxyl group, $-(CH_2)_m-COOH$, $-(CH_2)_m-NH_2$,

5 $-(CH_2)_m-N-C(=NH)NH_2$, $-(CH_2)_m-C(=O)NH_2$, $-SH$, and $-(CH_2)_m-S-CH_3$;

m is 0, 1, or 2; and

said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia,

hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension,

10 abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder,

gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease,

heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea,

attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

15 26. The method of claim 25, wherein R_1 and R_2 are lower alkyl; R_3 is $-(CH_2)_m-COOH$; and m is 0, 1, or 2.

27. The method of claim 25, wherein R_1 and R_2 are methyl; R_3 is $-(CH_2)_m-COOH$; and m is 0.

28. The method of claim 25, wherein the disease or condition is selected from the group
20 consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

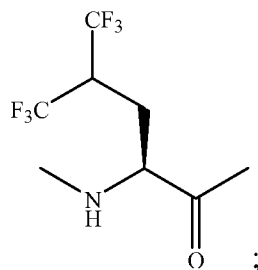
29. The method of claim 25, wherein said method modifies glucose metabolism.

30. A method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a retro-
25 inverso truncated analog selected from the group consisting of

HAEGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,

HAEGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and

HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



said analog has at least about 90% sequence homology to the peptide fragment or polypeptide fragment; said analog consists essentially of D-amino acids assembled in reversed order along the peptide chain; and said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, forgetfulness, and narcolepsy.

31. The method of claim 30, wherein said analog has at least about 95% sequence homology to the peptide or polypeptide.

32. The method of claim 30, wherein said analog has at least about 99% sequence homology to the peptide or polypeptide.

33. The method of any one of claims 30-32, wherein the peptide sequence is extended by 1 to about 10 additional amino acid residues at one or both of the terminal residues.

34. The method of any one of claims 30-33, wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related complications.

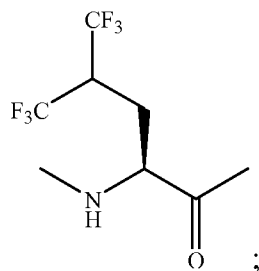
35. The method of any one of claims 30-33, wherein said method modifies glucose metabolism.

36. A method for treating or preventing a disease or condition in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a retro-inverso truncated analog selected from the group consisting of

HAEGTFTSDVSSYL*EGQAAKEFIAWL*VK-NH₂,

HAEGTFTSDVSSYL*EGQAAKEFIAWL*KN-NH₂, and

HGEGTFTSDL*SKQMEEEEAVRL*FIEWL*KN-NH₂; wherein L* is independently for each occurrence leucine or an amino acid analog represented by:



said analog has at least about 90% sequence homology to the peptide fragment or

- 5 polypeptide fragment; said analog consists essentially of D-amino acids assembled in reversed order along the peptide chain; said analog is independently derivatized at one or both of the terminal residues; and said disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, diabetes-related complications, obesity-
- 10 related hypertension, abnormalities of digestive track mucosa, food intake disorder, gastrointestinal disorder, gastrointestinal disease, regional enteritis (Crohn's disease), inflammatory bowel disease, heart-related ailments, myocardial infarction, ischemia-reperfusion injury, congestive heart failure, cardiac arrest, osteoporosis, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss,
- 15 forgetfulness, and narcolepsy.

37. The method of claim 36, wherein said analog has at least about 95% sequence homology to the peptide or polypeptide.

38. The method of claim 36, wherein said analog has at least about 99% sequence homology to the peptide or polypeptide.

- 20 39. The method of any one of claims 36-38, wherein the peptide sequence is extended by 1 to about 10 additional amino acid residues at one or both of the terminal residues.

40. The method of any one of claims 36-39, wherein the disease or condition is selected from the group consisting of insulin resistance, glucose intolerance, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, hyperlipoproteinemia, and diabetes-related

25 complications.

41. The method of any one of claims 36-39, wherein said method modifies glucose metabolism.

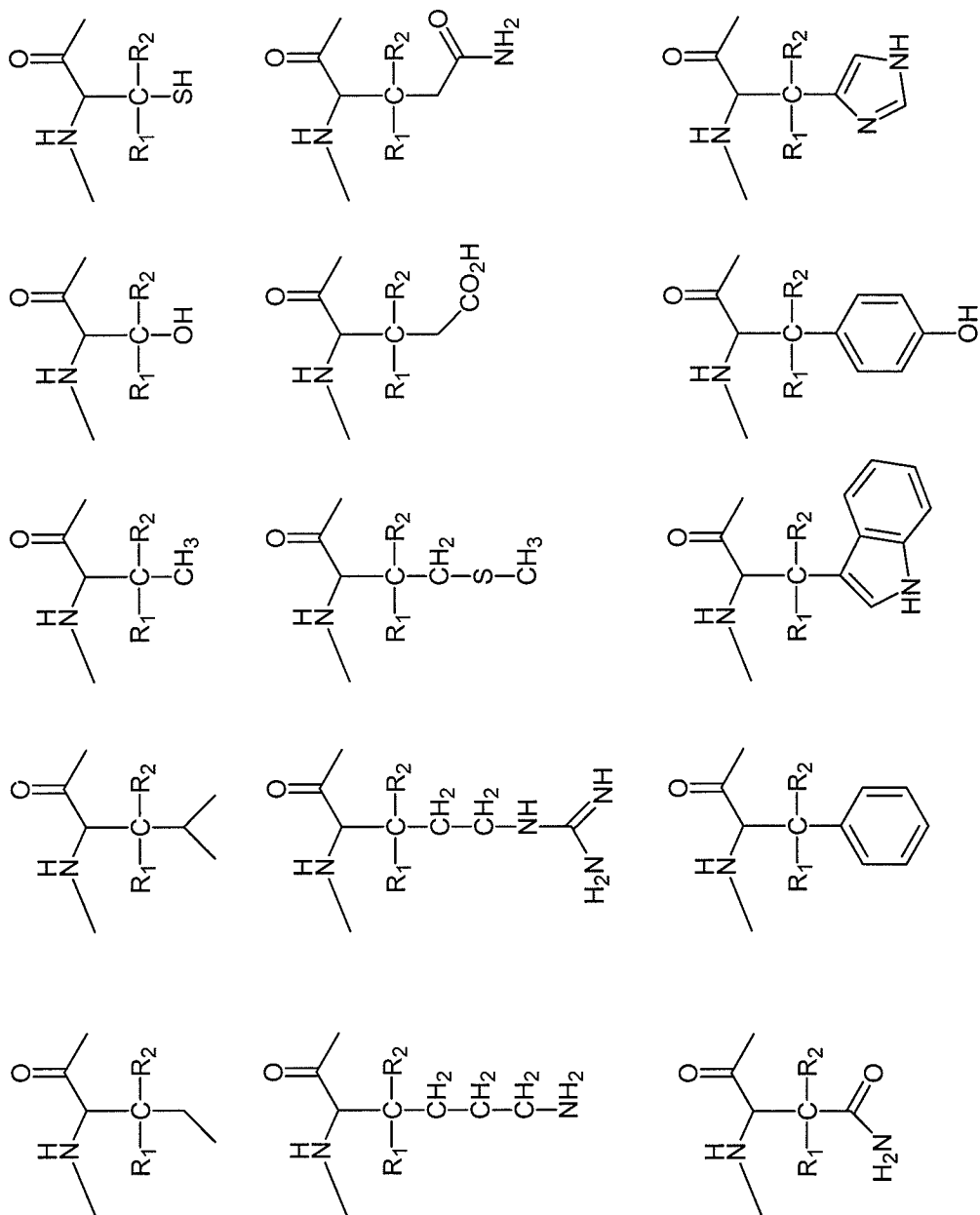


Figure 1

Figure 2

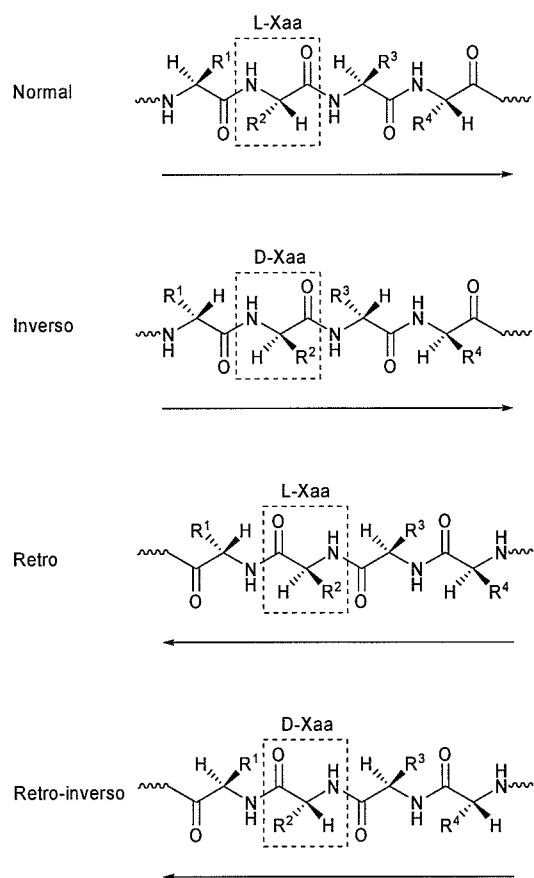


Figure 3*Exemplary Truncated Analogues of Exendin and GLP-1*

- Truncated Exendin
- HEGTFTSDLSKQMEEEAVRLFIEWLKN-NH₂
- GLP-1(1-28)amide ("Truncated Stable GLP-1")
- HAXGTFTSDVSSYLEGQAAKEFIAWLVK-NH₂
- GLP-1(1-28)amide ("version b")
- HAXGTFTSDVSSYLEGQAAKEFIAWLKN-NH₂

X = β -dimethyl Asp

Figure 4

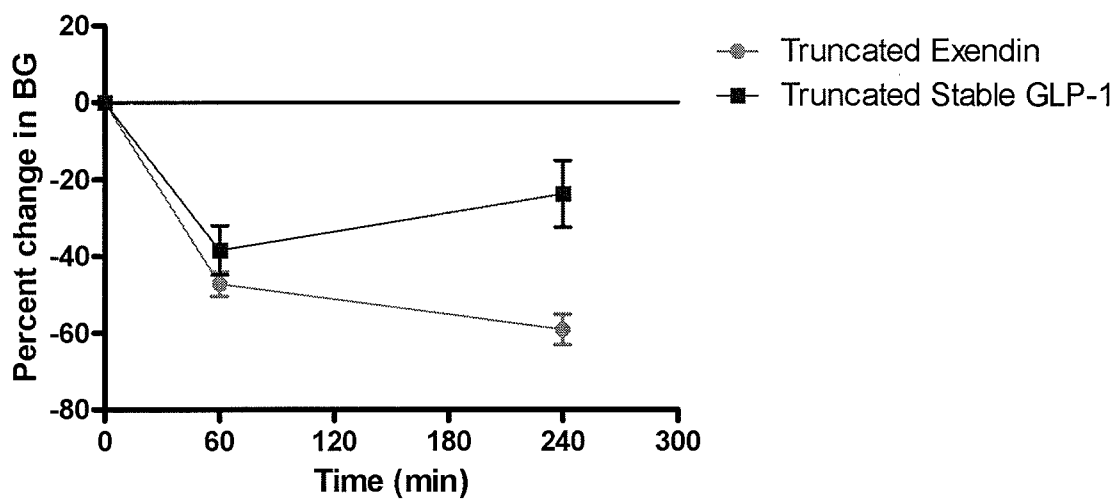


Figure 5

