

GLUCAGON/GLP-1/GIP TRI-AGONISTS AND THEIR ASSOCIATED
PRODRUGS

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims priority to U.S. Provisional Patent Application No. 63/617,996 filed on January 5, 2024, the disclosure of which is expressly incorporated herein.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED

10 ELECTRONICALLY

Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 104,354 bytes xml file named "418516.xml" created on December 29, 2024.

15 BACKGROUND

- Pre-proglucagon is a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides, including glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin (OXM), that are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. Glucagon is a 29-amino acid peptide that corresponds to amino acids 33 through 61 of pre-proglucagon, while GLP-1 is produced as a 37-amino acid peptide that corresponds to amino acids 72 through 108 of pre-proglucagon.

- 25 When blood glucose begins to fall, glucagon, a hormone produced by the pancreas, signals the liver to break down glycogen and release glucose, causing blood glucose levels to rise toward a normal level. GLP-1 has different biological activities compared to glucagon. Its actions include stimulation of insulin synthesis and secretion, inhibition of glucagon secretion, and inhibition of food intake. GLP-1 has been shown to reduce hyperglycemia (elevated glucose levels) in diabetics. Exendin-4, a peptide from 30 lizard venom that shares about 50% amino acid identity with GLP-1, activates the GLP-1 receptor and likewise has been shown to reduce hyperglycemia in diabetics.

Glucose-dependent insulintropic peptide (GIP) is a 42-amino acid gastrointestinal regulatory peptide that stimulates insulin secretion from pancreatic beta cells in the presence of glucose. It is derived by proteolytic processing from a 133-amino acid precursor, preproGIP.

5 GIP and GLP-1 are known as incretins, and incretin receptor signaling is known to exert physiologically relevant action critical for glucose homeostasis. In normal physiology, GIP and GLP-1 are secreted from the gut following a meal, and these incretins enhance the physiological response to food including sensation of satiety, insulin secretion, and nutrient disposal. T2D patients have impaired incretin responses.

10 Dosing diabetes patients with GLP-1 analogs has been found to be limited by adverse effects, such as nausea and vomiting, and as a consequence dosing most often cannot reach full efficacy for glycemic control and weight loss. GIP alone has very modest glucose-lowering ability in type 2 diabetic humans. Both native GIP and GLP-1 are inactivated rapidly by the ubiquitous protease, DPP IV, and therefore, can only be
15 used for short-term metabolic control.

Peptides that constitute balanced, full potency agonists at the GLP-1, glucagon (Gcg) and GIP receptors have demonstrated transformative pharmacology in the treatment of metabolic diseases, and most notably reduction in body weight. The most advanced drug candidate (retatrutide) is formulated as a subcutaneous injection and
20 administered weekly. This peptide requires a scheduled dose titration to minimize adverse gastrointestinal effects, with monthly increments over the course of a five-month period.

There remains a need for peptides having an increased sustained duration of pharmacological action to minimize frequency in human dosing. Additionally, Gcg/GLP-1/GIP tri-agonists are desired that exhibit reduced peak to trough concentration between
25 injections to support more precise dosing to achieve maximal efficacy (weight lowering) and minimal toxicity (nausea, flatulence and vomiting). The present disclosure is directed to novel Gcg/GLP-1/GIP tri-agonists exhibiting such desired properties relative to existing medicines such as tirzepatide.

30 SUMMARY

As disclosed herein, novel peptides exhibiting agonist activity at each of the glucagon receptor (Gcg), GLP-1 receptor and the GIP receptor are provided. Peptides having Gcg activity, GIP activity and GLP-1 activity are particularly advantageous for inducing weight loss or preventing weight gain, as well as for treating metabolic

syndrome, hyperglycemia, and diabetes. Thus, in one aspect, the invention provides methods for inducing weight loss or preventing weight gain, which involve administering to a patient in need thereof an effective amount of a peptide, that exhibits activity at each of the Gcg, the GIP and the GLP-1 receptors, optionally wherein the peptide has

5 equivalent activity at each of the three receptors.

In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is provided comprising the sequence of

X₁X₂X₃GTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAP
PPS-R₂₀ (SEQ ID NO: 1),

10 X₁X₂X₃GTX₆TSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFX₂₃X₂₄X₂₅LLEGGPSSG
APPPS-R₂₀ (SEQ ID NO: 4),

X₁X₂X₃GTFX₇SDYSIX₁₃LX₁₅X₁₆X₁₇AQX₂₀AFVQWLIAGGPSSGAPPP
S-R₂₀ (SEQ ID NO: 5), or a sequence that differs from SEQ ID NO: 1 or SEQ ID NO: 4
by 1 or 2 amino acid substitutions, optionally wherein the amino acid substitutions are

15 conservative amino acid substitutions,

wherein

X₁ is Tyr or His;

X₂ is an α,α -disubstituted amino acid, optionally Aib;

X₃ is Gln or His;

20 X₆ is an alpha methylated amino acid or Phe, optionally X₆ is alpha
methylated Phe;

X₇ is Thr, (α -Me)T or isoacyl-Thr;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe

or Aib;

25 X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain
that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group,
optionally via a P17 spacer, optionally wherein X₁₇ is an amino acid comprising a
30 (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a
C16-C20 phosphonic fatty acid;

X₁₈ is Ala or Tyr;

X₂₀ is an α,α -disubstituted amino acid, optionally Aib;

X₂₃ is Ile or Val;

X₂₄ is Glu, Asp or Gln;

X₂₅ is Tyr or Trp; and

R₂₀ is COOH or CONH₂, wherein said P17 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment the peptide of SEQ ID NO: 1 is provided wherein X₂ and X₂₀ are both Aib; X₁₃ is (aMe)Leu; X₁₅ is Asp; X₁₆ is Lys and X₁₇ is an acylated Lys, wherein the acyl group of the acylated Lys is a C16-C20 acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally linked via said P17 spacer to the Lys side chain.

In one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is provided comprising the sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3), or

X₁X₂X₃GTX₆TSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFX₂₃X₂₄X₂₅LLEGGPSSGAPPPS-R₂₀ (SEQ ID NO: 4), wherein

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

X₆ is Phe;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe, or Aib;

X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a P17 spacer, optionally wherein X₁₇ is an acylated Lys or dLys;

X₁₈ is Ala or Tyr;

X₂₀ is Aib;

X₂₃ is Ile;

X₂₄ is Glu or Asp;

X₂₅ is Tyr; and

R₂₀ is CONH₂, wherein said P17 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein z is 0, k and q are both 2 and p is 1. In one embodiment the peptide of SEQ ID NO: 4 is provided wherein

X₁₃ is (aMe)Leu; X₁₅ is Asp; X₁₆ is Lys and X₁₇ is an acylated Lys, wherein the acyl group of the acylated Lys is a C16-C20 acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally linked via said P17 spacer to the Lys side chain.

In accordance with one embodiment a prodrug derivative of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1 is provided wherein the peptide is modified by the covalent linkage of one or more dipeptides "A-B" to an amine of said peptide, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated amino acid linked to said peptide through an amide bond between a carboxyl moiety of B and an amine of the peptide (optionally a primary amine), wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid optionally via a spacer.

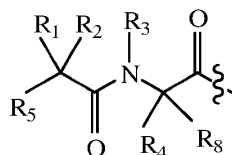
In accordance with one embodiment a prodrug derivative of the GIP/GLP-1 co-agonist peptide of SEQ ID NO: 1 is provided wherein the peptide is modified by the covalent linkage of two or more dipeptides (A-B) via an amide bond to amino acids located at positions selected from positions 1 and 7, relative to SEQ ID NO: 1, wherein X₁₆ is Aib. In one embodiment, the prodrug derivative of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1 is provided wherein the peptide is modified by the covalent linkage of a dipeptides (A-B) via an amide bond N-terminal alpha amine of the amino acid at position 1 and to the primary amine of the isoacyl-Thr at position 7, relative to SEQ ID NO: 1.

In accordance with one embodiment a prodrug derivative of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1 is provided wherein the peptide is modified by the covalent linkage of two or more dipeptides (A-B) via an amide bond to amino acids located at positions selected from positions 1, 7 and 16 relative to SEQ ID NO: 1.

In accordance with one embodiment a prodrug derivative of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 3 is provided wherein the peptide is modified by the

covalent linkage of two or more dipeptides (A-B) via an amide bond to amino acids located at positions selected from positions 1 and 16 relative to SEQ ID NO: 3.

In one embodiment the dipeptides (A-B) present at one or more of positions 1, 7 and 16 relative to SEQ ID NO: 1 or SEQ ID NO: 3 comprise the structure:



5

wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P7 spacer;

10 R₂ is H, or C₁-C₄ alkyl;

R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;;

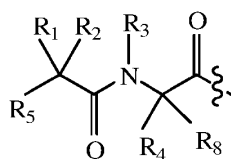
R₈ is H, D, or C₁-C₄ alkyl;

15 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said P7 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, and "D" = deuterium.

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In one embodiment the dipeptide comprises the structure:



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wherein

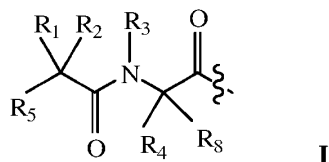
25 R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via a spacer;

R₂, R₄ and R₈ are each H;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂, wherein the spacer is -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In one embodiment the dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P7 spacer;

R₂, is H or C₁-C₄ alkyl;

R₄ and R₈ are each H or D;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein said P7 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein R₄ and R₈ are both D and R₃ is CD₃.

In some embodiments A and/or B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the L stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the L stereoisomer configuration and B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the D stereoisomer configuration. In one embodiment A is dLys and B is an N-alkylated amino acid in the L stereoisomer configuration.

In accordance with one embodiment prodrug derivative of a Gcg/GIP/GLP-1 tri-agonist is provided wherein the peptide comprises the sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀

(SEQ ID NO: 3)

wherein

X_1 is Tyr or His;

X_2 and X_{20} are both Aib;

X_3 is Gln or His;

X_{13} is (aMe)Leu,

5 X_{17} is Lys, wherein the lysine side chain is linked via an amide bond to -
[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid, wherein k is 2, and
p and q are independently an integer selected from 1 or 2, and R_{20} is CONH₂, and

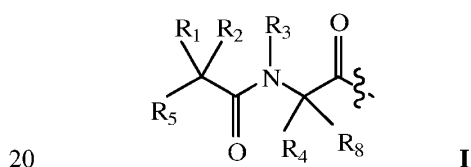
X_{18} is Ala or Tyr, optionally wherein:

10 I) X_1 is Tyr;
 X_3 is Gln; and
 X_{18} is Ala, or

II) X_1 is His;
 X_3 is His; and
 X_{18} is Tyr, or

15 III) X_1 is Tyr;
 X_3 is Gln; and
 X_{18} is Tyr, wherein

a dipeptide is covalently linked via an amide bond to the N-terminal alpha
amine of the peptide, wherein the dipeptide comprises the structure:



wherein

R_1 comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a
C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain,
optionally via a P1 spacer;

25 R_2 is H;

R_4 and R_8 are each H or D;

R_3 is CD₃ or C₁-C₆ alkyl; and

R_5 is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_kNH]_q-
(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0, 1 or 2, optionally
30 wherein R_4 and R_8 are each D and R_3 is CD₃,

optionally wherein

I) R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain via the P1 spacer;

R_2 , R_4 and R_8 are each H;

5 R_3 is C_1 - C_4 alkyl; and

R_5 is NH_2 , wherein said P1 spacers is $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (γ glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1; or

10 II) R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain via the P1 spacer;

R_2 is H;

R_4 and R_8 are each D

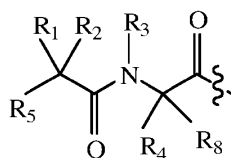
R_3 is CD_3 ; and

15 R_5 is NH_2 , wherein said first and second spacers are independently

$-[COCH_2(OCH_2CH_2)_k-NH]_q-(\gamma \text{ glutamic acid})_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1.

In accordance with one embodiment a prodrug derivative of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or
20 SEQ ID NO: 5 is provided wherein

a dipeptide is covalently linked via an amide bond to the amine of isoacyl-Thr at position 7 of the peptide, and/or at the N-terminal alpha amine, wherein the dipeptide comprises the structure:



I

25 wherein

R_1 comprises a $(C_1$ - C_4 alkyl) NH_2 side chain that has been pegylated, optionally via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R_2 is H, or C_1 - C_4 alkyl;

30 R_4 is H, D, C_1 - C_4 alkyl, C_1 - C_4 alkenyl, C_1 - C_4 alkenyl, $-(CH_2)_nCH_2OH$ or $(C_1$ - C_4 alkyl)phenyl, wherein n is an integer selected from 0-2;

R_8 is H, D, or C₁-C₄ alkyl;

R_3 is CD₃, C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

5 R_5 is NH₂, wherein said first and second spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1,

optionally wherein

10 I) R_1 comprises a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, optionally via a first spacer, with a straight chain or branched polyethylene chain having a combined molecular weight ranging from 20k to 40k;

R_2 , R_4 and R_8 are each H;

R_3 is C₁-C₄ alkyl; and

R_5 is NH₂, wherein said first and second spacers are independently

15 -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1; or

II) R_1 comprises a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, optionally via a second spacer, with a straight chain or branched polyethylene chain having a combined molecular weight ranging from 20k to 40k;

20 R_2 is H;

R_4 and R_8 are each D;

R_3 is CD₃; and

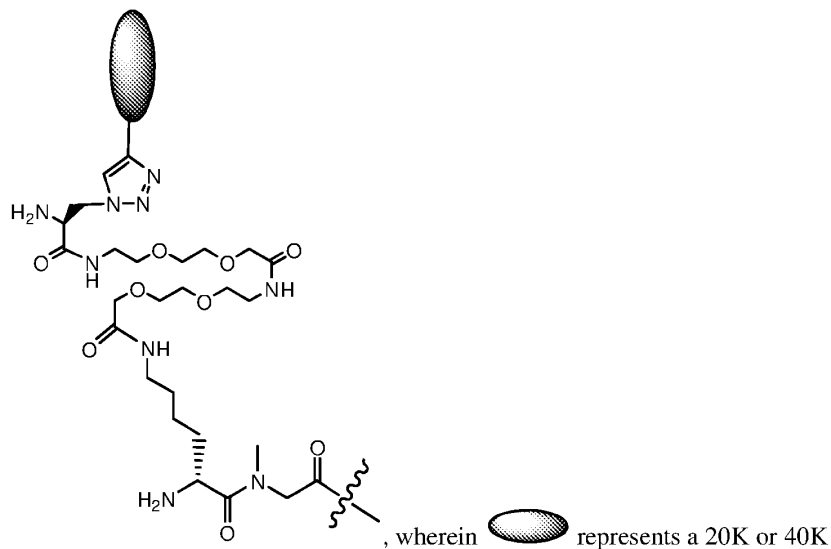
R_5 is NH₂, wherein said first and second spacers are independently

25 -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

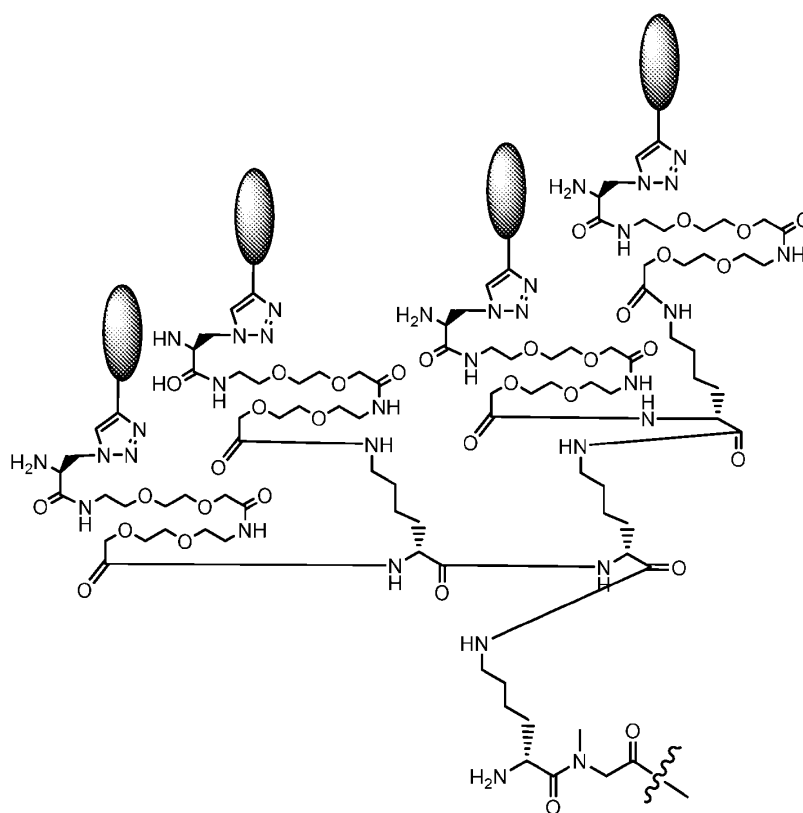
30 In accordance with one embodiment said dipeptide is present only at the N-terminal alpha amine. In one embodiment the dipeptides of the Gcg/GLP-1/GIP tri-agonist peptide, and optionally the amino acid at position 17, comprise a branched PEG chain having a molecular weight of 20K or 40K, wherein the branched PEG chain comprises 2, 3, 4 or five branches. In one embodiment the dipeptide of the prodrug form of the Gcg/GLP-1/GIP tri-agonist peptide comprise a branched PEG chain, and the amino acid at position 17 comprises a straight chain PEG. In one embodiment the branched PEG

chain constructs comprises two branches of 10K each, or two branches of 20K each, optionally linked together via an amino acid extension of lysine residues. In one embodiment the branched polyethylene chain comprises four branches of 5K each, or four branches of 10K each. In one embodiment each of the branches of the branched polyethylene chains are covalently linked to the dipeptide(s) of the Gcg/GLP-1/GIP tri-agonist peptide via a disulfide linkage, wherein the amino acid at position 17 is pegylated via a non-disulfide covalent bond linkage, optionally via a triazole linkage. In one embodiment each of the branches of the branched polyethylene chain are covalently linked to the dipeptide(s), and to the amino acid at position 17, of the Gcg/GLP-1/GIP tri-agonist peptide via a disulfide linkage. The disulfide linkages enhance metabolism and clearance of the polyethylene glycol.

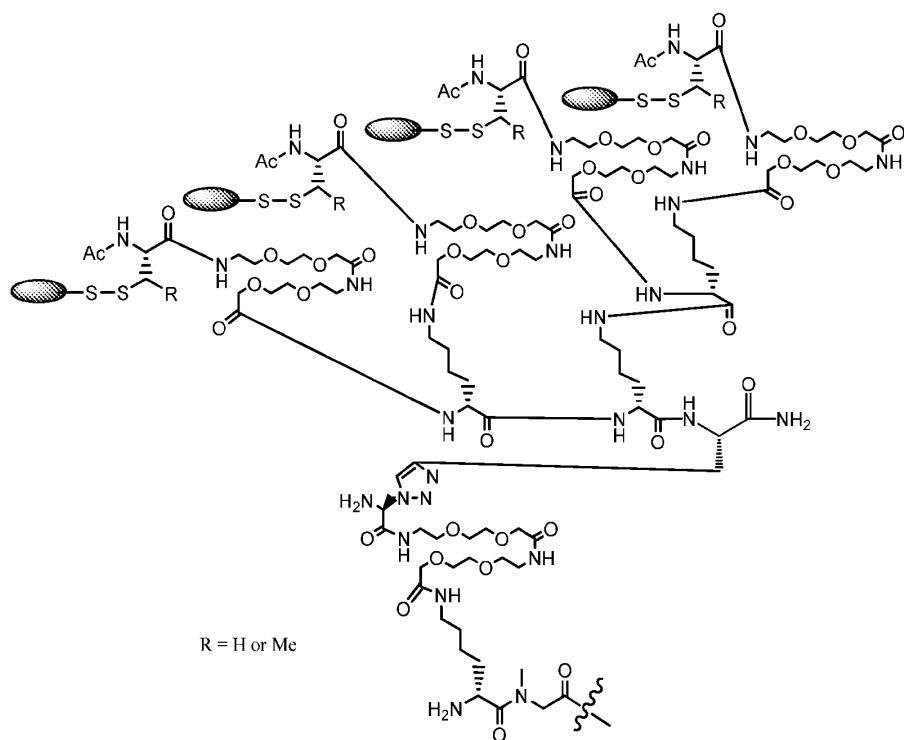
In one embodiment a prodrug derivative of any of the Gcg/GLP-1/GIP tri-agonist peptide disclosed herein is provided, wherein a dipeptide (A-B) is covalently linked to a primary amine of the peptide, optionally at the N-terminal alpha amine, wherein the first
15 amino acid of the dipeptide comprises a structure selected from the group consisting of:



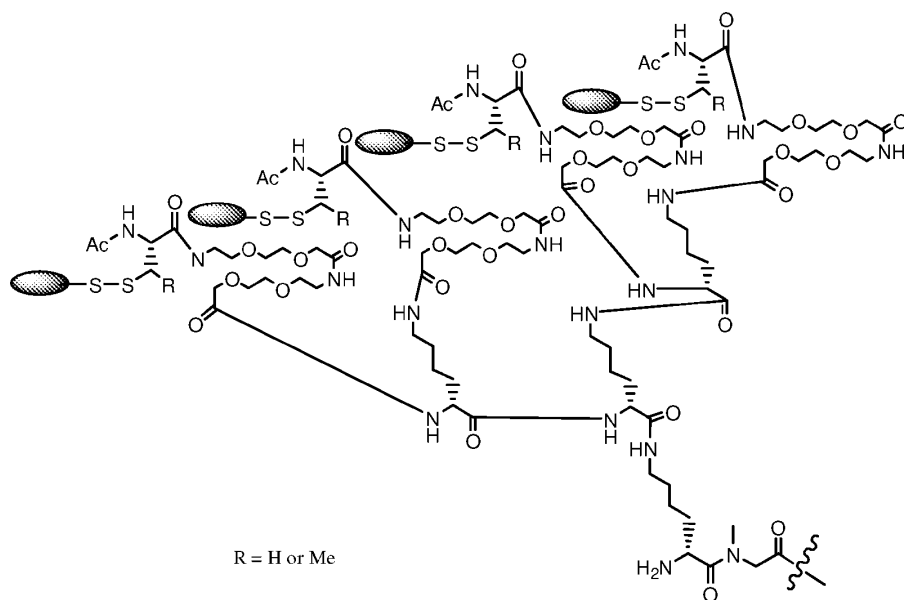
PEG,



wherein  represents a 5K or 10K PEG,



wherein  represents a 5K or 10K PEG and Ac = acyl group, and



wherein  represents a 5K or 10K PEG and Ac = acyl group.

In accordance with one embodiment a GIP/GLP-1 co-agonist peptide of
 $X_1X_2X_3\text{GTFTSDYSIX}_{13}\text{LDKX}_{17}X_{18}\text{QX}_{20}\text{AFVQWLLEGGPSSGAPPPS-R}_{20}$ (SEQ ID
 NO: 3) is provided wherein

X_1 is Tyr or His;

5 X_2 is Aib;

X_3 is Gln;

X_{13} is (α -Me)Leu;

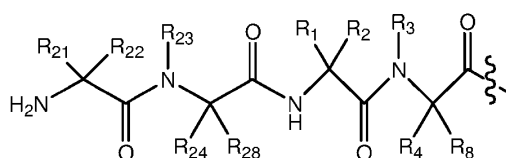
X_{17} is Leu or dLeu acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}(\text{CO}_2\text{H})$ or $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}(\text{PO}_3\text{H}_2)$;

10 X_{18} is Ala;

X_{20} is Aib; and R_{20} is CONH_2 , and a prodrug derivative thereof, wherein a
 dipeptide (A-B) as disclosed herein is covalently linked to the N-terminal alpha amine of
 the Gcg/GLP-1/GIP tri-agonist peptide.

In accordance with one embodiment a prodrug derivative of the Gcg/GLP-1/GIP
 15 tri-agonist peptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or
 SEQ ID NO: 5 is provided wherein the peptide is modified by the covalent linkage of a
 sequential dipeptide "A-B-C-D" to an amine of said peptide, wherein A and C are amino
 acids and B and D are N-alkylated amino acids linked to said peptide through an amide
 bond between a carboxyl moiety of D and an amine of the peptide, wherein the side chain
 20 of at least one of amino acid (A) and (C) is acylated with a C16-C20 fatty acid, a C16-
 C20 phosphonic fatty acid, or a C16-C20 diacid. In one embodiment an amino acid at
 position 1 (via the N-terminal alpha amine), position 7 (via the alpha amine of a isoacyl-
 Thr present at position 7) and/or at position 16 (via the side chain amine of Lys) of SEQ
 ID NO: 1 are covalently linked via an amide bond to the sequential dipeptide "A-B-C-D".
 25 In one embodiment a single sequential dipeptide is linked to a Gcg/GLP-1/GIP tri-agonist
 peptide of the present invention via an amide bond to the N-terminal alpha amine of the
 peptide. In one embodiment a dipeptide "A-B" is covalently linked at one of positions 1,
 7 or 16 and a sequential dipeptide "A-B-C-D" is linked to one of the other the other two
 positions. In one embodiment the sequential dipeptide "A-B-C-D"

30 comprises the structure of



wherein

R_1 is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a second spacer;

5 R_{21} is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a third spacer;

R_2 , and R_{22} are independently H, or C₁-C₄ alkyl;

R_4 , R_8 , R_{24} and R_{28} are independently H, D, or C₁-C₄ alkyl;

10 R_3 is C₁-C₆ alkyl, CD₃ or R_3 and R_4 together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R_{23} is C₁-C₆ alkyl, CD₃ or R_{23} and R_{24} together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or

15 piperidine ring, wherein said second and third spacers are independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

20 In one embodiment the sequential dipeptide "A-B-C-D" is linked to the Gcg/GLP-1/GIP tri-agonist peptide via the N-terminal alpha amine of any one of SEQ ID NO: 1 to SEQ ID NO: 5. In one embodiment a first sequential dipeptide "A-B-C-D" is linked to the Gcg/GLP-1/GIP tri-agonist peptide via the N-terminal alpha amine of the peptide and a second sequential dipeptide "A-B-C-D" is linked to the amino acid at position 7 (via the

25 alpha amine of the isoacyl-Thr at position 7 of SEQ ID NO: 1) via an amide bond. In one embodiment a first sequential dipeptide "A-B-C-D" is linked to the Gcg/GLP-1/GIP tri-agonist peptide via the N-terminal alpha amine of the peptide and a second sequential dipeptide "A-B-C-D" is linked to the amino acid at position 16 (via the side chain amine of Lys). In one embodiment the amino acid at position 1 is linked to the sequential

30 dipeptide "A-B-C-D" via the N-terminal alpha amine of SEQ ID NO: 1, and optionally the amino acids at position 7 (via the alpha amine of the isoacyl-Thr at position 7 of SEQ ID NO: 1) and/or 16 (via the side chain amine of Lys) are covalently linked via an amide bond to the dipeptide "A-B" or the sequential dipeptide "A-B-C-D". In one embodiment the amino acid at position 16 is linked via the side chain amine of Lys to the sequential

dipeptide “A-B-C-D”, and optionally the amino acids at position 7 (via the alpha amine of the isoacyl-Thr of SEQ ID NO: 1) and/or 1 (via the N-terminal alpha amine) are covalently linked via an amide bond to the dipeptide “A-B” or the sequential dipeptide “A-B-C-D”.

- 5 In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide of any one of SEQ ID NOs: 1-5 is provided wherein

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

- 10 X₆ is an alpha methylated amino acid or Phe;

X₇ is Thr or (α-Me)T;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu or Aib;

X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

- 15 X₁₇ is an amino acid comprising a (C4 alkyl)NH₂ side chain that has been pegylated via a first spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

X₁₈ is Ala or Tyr;

X₂₀ is an α,α-disubstituted amino acid, optionally Aib;

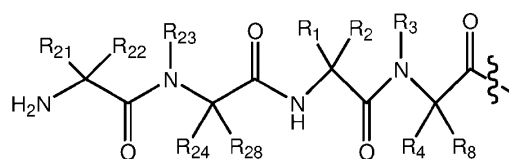
- 20 X₂₃ is Ile or Val;

X₂₄ is Glu, Asp or Gln;

X₂₅ is Tyr or Trp; and

R₃₀ is CONH₂, wherein

a sequential dipeptide “A-B-C-D” comprising the structure of



25

is covalently linked to the N-terminal alpha amine, wherein

R₁ comprises a (C4 alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂₁ comprises (C₄ alkyl)NH₂ or a (C₄ alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

5 R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

10 R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said second and third spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

15 In accordance with one embodiment a pharmaceutical composition is provided comprising any of the novel the Gcg/GIP/GLP-1 tri-agonist, or prodrug derivatives thereof, disclosed herein, preferably at a purity level of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, and a pharmaceutically acceptable diluent, carrier or excipient. Such compositions may contain a Gcg/GIP/GLP-1 tri-agonist, or prodrug
20 derivatives thereof, as disclosed herein at a concentration of about 10mg/ml to 100mg/ml, or about 30mg/ml to about 100mg/ml or about 50 mg/ml to about 100mg/ml, or about 40mg/ml, 50mg/ml, 60 mg/ml, 70 mg/ml, 80mg/ml, 90 mg/ml, or 100 mg/ml, or higher. In one embodiment the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various package containers. In one embodiment
25 aqueous formulations are prepared in dimethyl sulfoxide (DMSO), including about 30% to 100% DMSO, about 50% to 100% DMSO, about 70% to 100% DMSO, or about 90% to 100% DMSO, with the remainder water. In one embodiment formulations are prepared in 100% dimethyl sulfoxide. In other embodiments the pharmaceutical compositions comprise a lyophilized powder. The pharmaceutical compositions can be further
30 packaged as part of a kit that includes a disposable device for administering the composition to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

In accordance with one embodiment an improved method of treating diabetes or metabolic syndrome in patients in need thereof is provided. The method comprises the

steps of administering a Gcg/GIP/GLP-1 tri-agonist, or prodrug derivatives thereof, of the present disclosure in an amount therapeutically effective to lower blood glucose levels.

In one embodiment Gcg/GIP/GLP-1 tri-agonist, or prodrug derivatives thereof, is acylated with one or more a fatty acid or diacid groups having sufficient size to bind
5 serum albumin with high affinity.

In accordance with one embodiment an improved method of reducing weight gain or inducing weight loss in patients in need thereof while reducing adverse side effects is provided. The method comprises the steps of administering a Gcg/GIP/GLP-1 tri-agonist, or prodrug derivative thereof, of the present disclosure in an amount therapeutically
10 effective reducing weight gain or inducing weight loss in a subject. In one embodiment Gcg/GIP/GLP-1 tri-agonist, or prodrug derivatives thereof, is acylated with one or more a fatty acid or diacid groups having sufficient size to bind serum albumin with high affinity.

All therapeutic methods, pharmaceutical compositions, kits and other similar embodiments described herein contemplate that the use of the terms peptides, agonists,
15 tri-agonists, or peptides includes all pharmaceutically acceptable salts or esters thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 provides the structure of native glucagon as well as various analogs of glucagon-related peptides that exhibit co-agonist activity at the GIP/GLP-1 receptors or
20 tri-agonist activity at the Gcg/GIP/GLP-1 receptors. CEX= PSSGAPPPS-NH₂ (SEQ ID NO: 7). Figure discloses SEQ ID NOS 26-31, 8-9, and 32-33, and 14, respectively, in order of appearance.

Figs. 2A-2D compare the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4244, 4293, 4295 and 4296) at the human GLP-1 (Fig. 2A; SEQ ID NOS
25 8, 34, and 10-12, respectively, in order of appearance), GIP (Fig. 2B; SEQ ID NOS 8, 34, and 10-12, respectively, in order of appearance) and Gcg (Fig. 2C; SEQ ID NOS 8, 34, and 10-12, respectively, in order of appearance) receptors. Results are reported relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides relative to MBX 4244 is reported and the analysis as a function of individual test
30 dates 1, 2, 3, and 4 for this study is reported (Fig. 2D). B=(α -Me)T; X= (α -Me)Leu; Z= K((miniPEG)2- γ E-COC18H36CO₂H) and CEX= PSSGAPPPS-NH₂ (SEQ ID NO: 7).

Fig. 3 compares the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (Cmp 1: MBX 4244, Cmp 2: MBX 4262, Cmp 3: MBX 4301, Cmp 4: MBX 4293, Cmp

5: MBX 4298, Cmp 6: MBX 4305, Cmp 7: MBX 4304 and Cmp 8: MBX 4303) at the human Gcg, GIP and GLP-1 receptors. Results are reported relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides relative to MBX 4244 is reported and the analysis as a function of individual dates for study is reported.

Fig. 4 presents the percent body weight lowering results from ten days of observations in DIO-Mice with MBX tri-agonists (MBX 4244, 4262, 4301, 4305, and 4312) administered subcutaneously daily at 2 nmol/kg for three days and subsequently every other day starting on day 5. The (alpha-Me)-Phe6 TZP GIP/GLP-1 co-agonist analog MBX 4062 was administered as a comparative drug agonist. Observations were made through day 14 with dosing beginning on day 0.

Fig. 5 presents the food consumption results from ten days of observations in DIO-Mice with MBX tri-agonists (MBX 4244, 4262, 4301, 4305, and 4312) administered subcutaneously daily at 2 nmol/kg for three days and subsequently every other day starting on day 5. The (alpha-Me)-Phe6 TZP GIP/GLP-1 co-agonist analog MBX 4062 was administered as a comparative drug agonist. Observations were made through day 14 with dosing beginning on day 0.

Fig. 6 compares the average *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4244, 4301, 4318, 4320, 4321, 4322, and 4323) at the human Gcg, GIP and GLP-1 receptors in the period of Dec 8-22, 2023. Results are reported relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides relative to MBX 4244 is reported. Figure discloses SEQ ID NO: 8.

Figs. 7A-7D compare the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4244, 4301, 4318 and 4319) at the human Gcg (Fig. 7A), GLP-1 (Fig. 7B) and GIP (Fig. 7C) receptors. Results report relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides relative to MBX 4244 is reported and the analysis is a function of a single specific date (Fig. 7D (SEQ ID NO: 8)).

Figs. 8A-8D compare the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4244, 4301, 4318, 4319, 4320 and 4321) at the human Gcg, Fig. 8A), GLP-1 (Fig. 8B) and GIP (Fig. 8C) receptors. Results are reported relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides relative to MBX 4244 is reported and the analysis is a function of a single specific date (Fig. 8D (SEQ ID NO: 8)).

Figs. 9A-9D compare the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4244, 4301, 4318, 4320, 4321, 4322 and 4323) at the human Gcg (Fig. 9A), GLP-1 (Fig. 9B) and GIP (Fig. 9C) receptors. Results are reported relative potency to MBX 4244 (LLY3437943). The difference in sequence of the individual peptides
5 relative to MBX 4244 is reported and the analysis is a function of a single specific date (Fig. 9D (SEQ ID NO: 8)).

Figs. 10A-10C compare the *in vitro* bioactivity of various Gcg/GIP/GLP-1 tri-agonists (MBX 4301 and 4359) at the human Gcg (Fig. 10A), GLP-1 (Fig. 10B) and GIP (Fig. 10C) receptors. MBX 4301 and 4359 differ from one another only in the acylation,
10 with MBX 4301 acylated with a fatty diacid and 4359 acylated with a phosphonic fatty acid. Comparison is made relative to the native glucagon, GLP-1 and GIP native peptides at their respective receptors.

Fig. 11 presents the percent body weight lowering results from ten days of observations in DIO-Mice with MBX tri-agonists (MBX 4301 and 4359) administered
15 subcutaneously daily at 2 nmol/kg for three days and subsequently every other day starting on day 5.

Fig. 12 provides a table of Gcg/GIP/GLP-1 tri-agonists, their compound reference number and corresponding sequence identifiers.

20 DETAILED DESCRIPTION

DEFINITIONS

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The term "about" as used herein means greater or lesser than the value or range of
25 values stated by 10 percent but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the
30 standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable. Many of the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or
5 carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary
10 amines.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic
15 acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

As used herein, the term "treating" includes alleviation of the symptoms associated with a specific disorder or condition, or the eliminating said symptoms. For
20 example, as used herein the term "treating diabetes" will refer in general to altering glucose blood levels in the direction of normal levels and may include increasing or decreasing blood glucose levels depending on a given situation.

As used herein an "effective" amount or a "therapeutically effective amount" of a glucagon peptide refers to a nontoxic but sufficient amount of the peptide to provide the
25 desired effect. For example one desired effect would be the prevention or treatment of hypoglycemia, as measured, for example, by an increase in blood glucose level. An alternative desired effect for the glucagon peptides of the present disclosure would include treating hyperglycemia, e.g., as measured by a change in blood glucose level closer to normal, or inducing weight loss/preventing weight gain, e.g., as measured by
30 reduction in body weight, or preventing or reducing an increase in body weight, or normalizing body fat distribution. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, mode of administration, and the like. Thus, it is not always possible to specify an exact "effective

amount." However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous.

5 As used herein, the term "purified" and like terms relate to the isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment.

As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. The term "purified polypeptide" is used herein to
10 describe a polypeptide which has been separated from other compounds including, but not limited to nucleic acid molecules, lipids and carbohydrates.

The term "isolated" requires that the referenced material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide present in a living animal is not isolated,
15 but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated.

As used herein, the term "peptide" encompasses a sequence of 2 or more amino acids and typically less than 50 amino acids, wherein the amino acids are naturally occurring or non-naturally occurring amino acids. Non-naturally occurring amino acids
20 refer to amino acids that do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein.

As used herein, the terms "polypeptide" and "protein" are terms that are used interchangeably to refer to a polymer of amino acids, without regard to the length of the polymer. Typically, polypeptides and proteins have a polymer length that is greater than
25 that of "peptides."

As used herein an amino acid "modification" refers to a substitution, addition or deletion of an amino acid, and includes substitution with or addition of any of the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids. Commercial sources of atypical amino acids include Sigma-
30 Aldrich (Milwaukee, WI), ChemPep Inc. (Miami, FL), and Genzyme Pharmaceuticals (Cambridge, MA). Atypical amino acids may be purchased from commercial suppliers, synthesized de novo, or chemically modified or derivatized from other amino acids.

As used herein an amino acid "substitution" refers to the replacement of one amino acid residue by a different amino acid residue.

As used herein, the term "conservative amino acid substitution" is defined herein as exchanges within one of the following five groups:

- I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly;
- 5 II. Polar, negatively charged residues and their amides and esters:
Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;
- III. Polar, positively charged residues:
His, Arg, Lys; Ornithine (Orn)
- IV. Large, aliphatic, nonpolar residues:
10 Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine
- V. Large, aromatic residues:
Phe, Tyr, Trp, acetyl phenylalanine

As used herein a general reference to a peptide is intended to encompass peptides
15 that have modified amino and carboxy termini. For example, an amino acid chain comprising an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

As used herein a "linker" is a bond, molecule or group of molecules that binds two
20 separate entities to one another. Linkers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups.

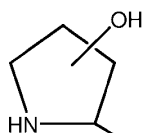
As used herein a "dimer" is a complex comprising two subunits covalently bound
25 to one another via a linker. The term dimer, when used absent any qualifying language, encompasses both homodimers and heterodimers. A homodimer comprises two identical subunits, whereas a heterodimer comprises two subunits that differ, although the two subunits are substantially similar to one another.

As used herein the term "charged amino acid" refers to an amino acid that
30 comprises a side chain that is negatively charged (i.e., de-protonated) or positively charged (i.e., protonated) in aqueous solution at physiological pH. For example, negatively charged amino acids include aspartic acid, glutamic acid, cysteic acid, homocysteic acid, and homoglutamic acid, whereas positively charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino

acids among the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids.

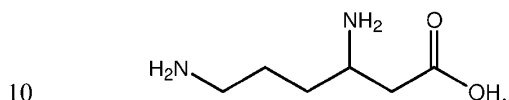
As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety, including for example, a carboxylic acid or sulfonic acid group.

- 5 As used herein the term "hydroxyproline" absent any further elaboration encompasses compounds having the structure:

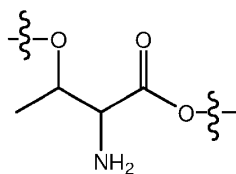


COOH, and includes the specific compounds 3- hydroxyproline, 4- hydroxyproline and 5- hydroxyproline and all the allo-stereoisomers.

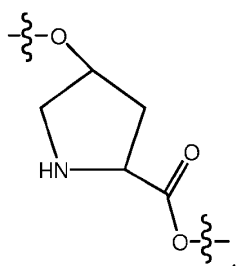
As used herein "beta-Lys" is an amino acid having the structure:



- As used herein the term "isoacyl-peptide" defines a peptide having an ester linked amino acid. A "isoacyl-Thr" is a threonine residue that is linked to another amino acid via an ester bond, rather than a peptide bond, wherein the ester bond is formed using the side chain hydroxyl group of threonine. In a similar manner isoacyl-peptide can be prepared using serine, hydroxyproline or a hydroxylated amino acid. Accordingly,
15 isoacyl-Thr has the structure:



, and a isoacyl-4-hydroxyproline comprises the structure:

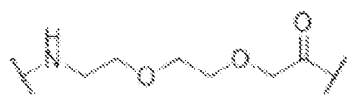


- 20 As used herein the general term "polyethylene glycol chain" or "PEG chain", refers to mixtures of condensation polymers of ethylene oxide and water, in a branched or

straight chain, represented by the general formula $H(OCH_2CH_2)_nOH$, wherein n is at least 9. Absent any further characterization, the term is intended to include polymers of ethylene glycol with an average total molecular weight selected from the range of 500 to 40,000 Daltons. "polyethylene glycol chain" or "PEG chain" is used in combination with a numeric suffix to indicate the approximate average molecular weight thereof. For example, PEG-5,000 (or PEG 5K) refers to polyethylene glycol chain having a total molecular weight average of about 5,000.

As used herein, the term "pegylated" and like terms refers to a compound that has been modified from its native state by linking a polyethylene glycol chain to the compound. A "pegylated glucagon peptide" is a glucagon peptide that has a PEG chain covalently bound to the glucagon peptide.

As used herein the term "miniPEG" or "OEG" defines a functionalized polyethylene compound comprising the structure:



As used herein the term "phosphonic fatty acid" defines a lipid comprising both a carboxy acid and a phosphoric acid group wherein the carboxy acid and phosphoric acid groups are separated by a long carbon chain.

As used herein, the term "prodrug" is defined as any compound that undergoes chemical modification before exhibiting its full pharmacological effects.

As used herein the term "chemical cleavage" absent any further designation encompasses a non-enzymatic reaction that results in the breakage of a covalent chemical bond.

Physiological conditions as disclosed herein are intended to include a temperature of about 35 to 40 °C and a pH of about 7.0 to about 7.4, and more typically include a pH of 7.2 to 7.4 and a temperature of 36 to 38 °C. Since physiological pH and temperature are tightly regulated in humans within a highly defined range, the speed of conversion from dipeptide/drug complex (prodrug) to drug will exhibit high intra and interpatient reproducibility.

As used herein an "acylated" amino acid is an amino acid comprising an acyl group which is non-native to a naturally occurring amino acid, regardless by the means by

which it is produced. Exemplary methods of producing acylated amino acids and acylated peptides are known in the art and include acylating an amino acid before inclusion in the peptide or peptide synthesis followed by chemical acylation of the peptide. In some embodiments, the acyl group causes the peptide to have one or more of
5 (i) a prolonged half-life in circulation, (ii) a delayed onset of action, (iii) an extended duration of action, and (iv) an improved resistance to proteases, such as DPP-IV.

As used herein, an “alkylated” amino acid is an amino acid comprising an alkyl group which is non-native to a naturally occurring amino acid, regardless of the means by which it is produced. Exemplary methods of producing alkylated amino acids and
10 alkylated peptides are known in the art and include alkylating an amino acid before inclusion in the peptide or peptide synthesis followed by chemical alkylation of the peptide. Without being held to any particular theory, it is believed that alkylation of peptides will achieve similar, if not the same, effects as acylation of the peptides, e.g., a prolonged half-life in circulation, a delayed onset of action, an extended duration of
15 action, and an improved resistance to proteases, such as DPP-IV.

The term “identity” as used herein relates to the similarity between two or more sequences. Identity is measured by dividing the number of identical residues by the total number of residues and multiplying the product by 100 to achieve a percentage. Thus, two copies of exactly the same sequence have 100% identity, whereas two sequences that
20 have amino acid deletions, additions, or substitutions relative to one another have a lower degree of identity. Those skilled in the art will recognize that several computer programs, such as those that employ algorithms such as BLAST (Basic Local Alignment Search Tool, Altschul et al. (1993) J. Mol. Biol. 215:403-410) are available for determining sequence identity.

As used herein, the term “selectivity” of a molecule for a first receptor relative to a second receptor refers to the following comparison: Establishing a first ratio representing the EC₅₀ of the test molecule divided by the EC₅₀ of first receptor native hormone at the first receptor, followed by establishing a second ratio representing the EC₅₀ of the test molecule at a second receptor divided by the EC₅₀ of second receptor
30 natural hormone, and comparing the first ratio to the second ratio. For example, a molecule that has a first ratio of two at a first receptor and a second ratio of one at a second receptor has 2-fold selectivity for the first receptor relative to the second receptor. For example, a molecule that has a first ratio of two at a first receptor and second ratio of

one at a second receptor has 2-fold selectivity for the first receptor relative to the second receptor.

As used herein, “glucagon potency” of a molecule refers to the ratio of the EC50 of the molecule at glucagon receptor divided by the EC50 of native glucagon at glucagon
5 receptor.

As used herein, “GIP potency” of a molecule refers to the ratio of the EC50 of the molecule at GIP receptor divided by the EC50 of native GIP at GIP receptor.

As used herein, “GLP-1 potency” of a molecule refers to the ratio of the EC50 of the molecule at GLP-1 receptor divided by the EC50 of native GLP-1 at GLP-1 receptor.

10 As used herein, the term “alkyl” refers to a linear or branched hydrocarbon containing the indicated number of carbon atoms. Exemplary alkyls include methyl, ethyl, and linear propyl groups. The term “C₁-C_n alkyl” wherein n can be from 1 through 6, as used herein, represents a branched or linear alkyl group having from one to the specified number of carbon atoms. Typical C₁-C₆ alkyl groups include, but are not limited
15 to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-Butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

As used herein, the term “heteroalkyl” refers to a linear or branched hydrocarbon containing the indicated number of carbon atoms and at least one heteroatom in the backbone of the structure. Suitable heteroatoms for purposes herein include but are not
20 limited to N, S, and O.

As used herein, the term “cycloalkyl” refers to a cyclic hydrocarbon group containing the indicated number of carbon atoms, e.g., cyclopropyl, cyclobutyl, cyclohexyl, and cyclopentyl.

As used herein, the term “heterocyclic” refers to a cyclic hydrocarbon group
25 containing the indicated number of carbon atoms and one to three heteroatoms independently selected from the group consisting of oxygen, nitrogen, and sulfur. Nonlimiting examples of heterocycloalkyl groups include piperidine, tetrahydrofuran, tetrahydropyran, dihydrofuran, morpholine, thiophene, and the like.

As used herein, the term “aryl” refers to a monocyclic or polycyclic aromatic
30 group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, containing the indicated number of carbon atoms. Unless otherwise indicated, an aryl group can be unsubstituted or substituted.

As used herein, the term “heteroaryl” refers to a monocyclic or polycyclic aromatic group containing the indicated number of carbon atoms and at least one

heteroatom selected from the group consisting of oxygen, nitrogen, and sulfur. Unless otherwise indicated, an aryl group can be unsubstituted or substituted.

As used herein the inclusion of an “R” group at the carboxy terminus of a peptide with the designation that “‘R’ is COOH or CONH₂” designates that the C-terminal amino acid comprises a natural terminal carboxylic acid or an amide group in place of the natural terminal carboxylic acid.

ABBREVIATIONS:

D = deuterium, a stable isotope of hydrogen
 10 CD₃ = methane with deuterium substituted for all three hydrogens
 Lower case k = D-isomer of lysine
 Upper case K = L-isomer of lysine
 γE = L-isomer of gamma, glutamic acid
 (miniPEG)₂ = COCH₂OCH₂CH₂OCH₂CH₂NH
 15 COC₁₈H₃₂CO₂H = (C20 diacid)
 (α-Me)Leu, (a-Me)Leu, (α-Me)L, (a-Me)L = alpha methyl leucine
 (α-Me)Thr, (a-Me)Thr, (α-Me)T, (a-Me)T = alpha methyl threonine
 (N-Me)G = sarcosine

20 EMBODIMENTS

Peptides having tri-agonist activity at the glucagon, GIP and GLP-1 receptors have demonstrated transformative pharmacology in the treatment of metabolic diseases, and most notably reduction in body weight. Eli Lilly currently is advancing a tri-agonist peptide through clinical trials (“Lilly 3437943”) that is formulated as a subcutaneous
 25 injection and administered weekly. This drug requires a scheduled dose titration to minimize adverse gastrointestinal effects, with monthly increments over the course of a five month period. The structure of Lilly 3437943 is provided in Fig. 1.

As disclosed herein, novel peptides exhibiting balanced, full potency agonists at the glucagon, GLP-1 and GIP receptors are provided. Surprisingly, applicant has
 30 discovered that modification of the Lilly 3437943 peptide by substitutions at positions 1, 3, 18, and 23-25 produces a Gcg/GLP-1/GIP tri-agonist peptide having higher potency and improved receptor balance relative to Lilly 3437943 (See Figs. 5 and 6). Thus, the present tri-agonist analogs can be administered at a lower dosage than Lilly 3437943 to

produce a therapeutic effect with a potentially lower risk of adverse gastrointestinal effects.

In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is provided, wherein the peptide comprises the sequence of

5 $X_1X_2X_3GTX_6X_7SDYSIX_{13}LX_{15}X_{16}X_{17}X_{18}QX_{20}AFVQWLLEGGPSSGAPPPS-R_{20}$ (SEQ ID NO: 1), or a peptide comprising 1, 2, 3, 4 or 5 amino acid substitutions relative to SEQ ID NO: 1,

wherein

X_1 is Tyr or His;
 10 X_2 is an α,α -disubstituted amino acid, optionally Aib;
 X_3 is Gln or His;
 X_6 is an alpha methylated amino acid or Phe;
 X_7 is Thr or isoacyl-Thr;
 X_{13} is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe or Aib;
 15 X_{15} is Glu or Asp;
 X_{16} is Aib, Orn or Lys;
 X_{17} is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, C16-C30 diacid or a C16-C30 alkyl group, optionally via a P17 spacer;
 20 X_{18} is Ala or Tyr;
 X_{20} is an α,α -disubstituted amino acid, optionally Aib; and
 R_{20} is COOH or CONH₂, wherein said P17 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are
 25 independently an integer selected from the range of 0-4.

In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is provided, wherein the peptide comprises the sequence of

$X_1X_2X_3GTFTSDYSIX_{13}LX_{15}X_{16}X_{17}X_{18}QX_{20}AFVQWLLEGGPSSGAPPPS-R_{20}$ (SEQ ID
 30 NO: 2), $X_1X_2X_3GTFX_7SDYSIX_{13}LX_{15}X_{16}X_{17}AQX_{20}AFVQWLIAGGPSSGAPPPS-R_{20}$ (SEQ ID NO: 5) or a peptide that differs from SEQ ID NO: 2 or SEQ ID NO: 5 by 1, 2, 3, 4, or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions,

wherein

X₁ is Tyr or His;
 X₂ is an α,α -disubstituted amino acid, optionally Aib;
 X₃ is Gln or His;
 X₇ is Thr or (α -Me)T;
 5 X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu or Aib;
 X₁₅ is Glu or Asp;
 X₁₆ is Aib, Orn or Lys;
 X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has
 been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17
 10 spacer;
 X₁₈ is Ala or Tyr;
 X₂₀ is an α,α -disubstituted amino acid, optionally Aib; and
 R₂₀ is CONH₂, wherein said P17 spacer is selected from the group
 consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid
 15 dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic
 acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are
 independently an integer selected from the range of 0-4.

In one embodiment the peptide of SEQ ID NO: 2 is provided wherein X₂ and X₂₀
 are both Aib. In one embodiment the peptide of SEQ ID NO: 2 is provided wherein X₁₃ is
 20 (aMe)Leu. In one embodiment the peptide of SEQ ID NO: 2 is provided wherein X₁₅ is
 Asp and X₁₆ is Lys. In one embodiment the peptide of SEQ ID NO: 2 is provided wherein
 X₂ and X₂₀ are both Aib and X₁₃ is (aMe)Leu. In one embodiment the peptide of SEQ ID
 NO: 2 is provided wherein X₂ and X₂₀ are both Aib and X₁₃ is (aMe)Leu, and X₁₇ is Lys
 having a C16 to C18 fatty acid, a C16-C20 phosphonic fatty acid, or C16 to C20 diacid
 25 linked to the lysine side chain via a spacer comprising the structure: -
 [COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p- wherein k is 2, and p and q are
 independently an integer selected from 1 or 2, optionally wherein X₁₇ is Lys, wherein the
 lysine side chain is linked via an amide bond to (COCH₂(OCH₂CH₂)₂NH)₂- γ E-
 COC₁₈H₃₆CO₂H.

30 In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is
 provided, wherein the peptide comprises the sequence of
 X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
 (SEQ ID NO: 3), or a peptide that differs from SEQ ID NO: 3 by 1, 2, 3, 4, or 5 amino

acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

R_{20} is CONH_2 ;

X_1 is Tyr or His;

5 X_2 and X_{20} are both Aib;

X_3 is Gln or His;

X_{13} is (aMe)Leu;

X_{17} is an amino acid comprising a (C1-C8 alkyl) NH_2 side chain that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a
10 spacer comprising the structure: $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH]}_q\text{-(gamma glutamic acid)}_p\text{-}$;
wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4, optionally wherein
 X_{17} is Lys having a C16 to C18 fatty acid, a C16-C20 phosphonic fatty acid, or a C16 to
C20 diacid linked to the lysine side chain via a spacer comprising the structure: -
 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-}$ wherein k is 2, and p and q are
15 independently an integer selected from 1 or 2, optionally wherein X_{17} is Lys, wherein the
lysine side chain is linked via an amide bond to $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-}$
 $\text{COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$,

X_{18} is Ala or Tyr; and

X_{20} is Aib.

20 In accordance with one embodiment a Gcg/GLP-1/GIP tri-agonist peptide is provided, wherein the peptide comprises the sequence of

$X_1X_2X_3\text{GTFTSDYSIX}_{13}\text{LDKX}_{17}X_{18}\text{QX}_{20}\text{AFVQWLLEGGPSSGAPPPS-R}_{20}$
(SEQ ID NO: 3), or a peptide that differs from SEQ ID NO: 3 by 1, 2, 3, 4, or 5 amino
acid substitutions, optionally wherein the substitutions are conservative amino acid

25 substitutions, wherein R_{20} is CONH_2 , X_1 is Tyr or His, X_2 and X_{20} are each Aib, X_3 is Gln
or His, X_{13} is (aMe)Leu, X_{17} is Lys or dLys, and X_{18} is Ala or Tyr, wherein the lysine side
chain is linked via an amide bond to $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH]}_q\text{-(gamma glutamic acid)}_p\text{-}$
C16-C20 diacid, wherein k is 2, and p and q are independently an integer selected from 1
or 2; further wherein:

30 X_1 is Tyr; X_3 is Gln; and X_{18} is Ala or Tyr; or

X_1 is His; X_3 is Gln; and X_{18} is Tyr; or

X_1 is Tyr; X_3 is His; and X_{18} is Tyr; or

X_1 is His; X_3 is His; and X_{18} is Tyr; or

X_1 is Tyr; X_3 is Gln; and X_{18} is Tyr; or

X₁ is Tyr; X₃ is Gln; and X₁₈ is Ala, optionally wherein X₁₇ is an acylated Lys wherein the lysine side chain is linked via an amide bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H.

The present disclosure also encompasses prodrug derivative of the novel

5 Gcg/GLP-1/GIP tri-agonist peptides disclosed herein. In one embodiment a prodrug derivative of any of the novel Gcg/GLP-1/GIP tri-agonist peptides disclosed herein is provided wherein a dipeptide prodrug element is covalently linked to an active site of the bioactive polypeptide via an amide linkage. In some embodiments the dipeptide is covalently bound to the Gcg/GLP-1/GIP tri-agonist peptides of SEQ ID NO: 1 at a

10 position that interferes with the bioactive polypeptide's ability to interact with its corresponding receptor. The amino acids, A and B of the dipeptide prodrug element are selected to allow the dipeptide prodrug element to chemically cleave under physiological conditions to release the dipeptide (forming a diketopiperazine) and the active Gcg/GLP-1/GIP tri-agonist peptide. In one embodiment a dipeptide prodrug element is

15 covalently linked to one or more amino acids at positions 17 or 16 of the Gcg/GLP-1/GIP tri-agonist peptides of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In some embodiments the dipeptide prodrug element is linked to the N-terminal alpha amine of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1. Subsequent removal of the dipeptide, under physiological conditions and in the absence of enzymatic activity,

20 restores full activity to the Gcg/GLP-1/GIP tri-agonist peptide.

In accordance with one embodiment the peptide of SEQ ID NO: 1 is provided wherein X₇ is isoacyl-Thr, and X₆ is alpha methylated amino acid, further wherein a dipeptide prodrug element is linked via an amide bond to the alpha amine of the isoacyl-Thr of the Gcg/GLP-1/GIP tri-agonist peptide of SEQ ID NO: 1. In accordance with one

25 embodiment the peptide of SEQ ID NO: 1 is provided wherein X₁₆ is Lys, wherein a dipeptide prodrug element is linked via an amide bond to the alpha amine of the Lys side chain.

In some embodiments a dipeptide prodrug element is provided having the general structure of A-B wherein A is an amino acid or a hydroxy acid and B is an N-alkylated

30 amino acid linked to the Gcg/GLP-1/GIP tri-agonist peptide through formation of an amide bond between a carboxyl of B (in A-B) and an amine of the Gcg/GLP-1/GIP tri-agonist peptide. Furthermore, in some embodiments, the chemical cleavage half-life of A-B from the Gcg/GLP-1/GIP tri-agonist peptide is at least about 1 week in PBS under physiological conditions, and in one embodiment is the chemical cleavage half-life of A-

B from the Gcg/GLP-1/GIP tri-agonist peptide is greater than 14 days. In one embodiment the chemical cleavage half-life of A-B from the Gcg/GLP-1/GIP tri-agonist peptide is at least about 8 days up to 21 days or is about 15 days.

In some embodiments A and B are selected to inhibit enzymatic cleavage of the A-B dipeptide from the Gcg/GLP-1/GIP tri-agonist peptide by enzymes found in mammalian serum. In some embodiments A and/or B are selected such that the cleavage of A-B from the Gcg/GLP-1/GIP tri-agonist peptide in PBS under physiological conditions, is not more than two fold the cleavage half-life of A-B from the Gcg/GLP-1/GIP tri-agonist peptide in a solution comprising a DPP-IV protease (i.e., cleavage of A-B from the Gcg/GLP-1/GIP tri-agonist peptide does not occur at a rate more than 2x faster in the presence of DPP-IV protease and physiological conditions relative to identical conditions in the absence of the enzyme). In some embodiments A and/or B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the L stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the L stereoisomer configuration and B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the D stereoisomer configuration.

As disclosed herein prodrugs of the Gcg/GLP-1/GIP tri-agonist peptides disclosed herein can be administered as highly effective therapeutics for lowering body weight in obese rodents. These peptides demonstrate a much sustained duration of pharmacological action that should serve to minimize frequency in human dosing. They also demonstrate a much reduced peak to trough concentration between injections which supports more precise dosing to achieve maximal efficacy (weight lowering) and minimal toxicity (nausea, flatulence and vomiting). The chemical form of the novel peptides is additionally supportive of non-injectable forms of administration, most notably by oral and inhalation routes of administration. However, any of the standard routes of administration, including intramuscular injection, can be used to administer the Gcg/GLP-1/GIP receptor tri-agonist peptides to a patient.

Peptides having glucagon, GIP and GLP-1 receptor agonist activity are particularly advantageous for inducing weight loss or preventing weight gain, as well as for treating metabolic syndrome, hyperglycemia, and diabetes. Thus, in one aspect, the invention provides methods for inducing weight loss or preventing weight gain, as well as methods of treating diabetes and metabolic syndrome, which involve administering to a

patient in need thereof an effective amount of a peptide, that exhibits activity at tri-agonist receptor activity at the glucagon, GIP and the GLP-1 receptors, optionally in the form of a prodrug.

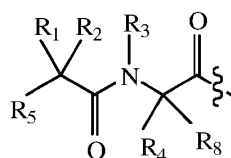
In accordance with one embodiment a isoacyl-peptide derivative of SEQ ID NO: 1 is provided wherein the an ester bond is formed between the amino acids at position 6 and 7, with the proviso that when an ester bond is formed between the amino acids at position 6 and 7, then the amino acid at position 6 is alpha methylated to stabilize against hydrolytic cleavage of the ester. In one embodiment the amino acid at position 7 is serine or threonine. In one embodiment the amino acid at position 7 is a isoacyl-threonine, wherein the ester bond is formed between the side chain hydroxyl group of threonine and the alpha carboxylic acid group of the amino acid at position 6, optionally wherein the amino acid at position 6 is an alpha methyl amino acid.

In accordance with one embodiment a prodrug derivative of any of the Gcg/GLP-1/GIP tri-agonist peptides disclosed herein is provided. In one embodiment a Gcg/GLP-1/GIP tri-agonist peptide selected from SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 is provide, wherein said peptide is modified by the covalent linkage of one or more dipeptides (A-B) to an aliphatic primary amine of the peptide, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated amino acid linked to said peptide through an amide bond between a carboxyl moiety of B and an amine of the peptide, wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid. In accordance with one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1, 7 or 16 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, optional via the N-terminal alpha amine of the peptide. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 16 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, optional via the side chain amine of the amino acid at position 16 of the peptide. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1 and 7 or 1 and 16 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

In one embodiment, the dipeptide linked to the amino acid at position 1 is linked to the N-terminal alpha amine of any of the Gcg/GIP/GLP-1 tri-agonist peptides disclosed herein. In one embodiment, the dipeptide linked to the amino acid at position 7 is linked to the alpha amine of a isoacyl-Thr located at position 7 of any of the Gcg/GIP/GLP-1 tri-

agonist peptides disclosed herein. In one embodiment, the dipeptide linked to the amino acid at position 16 is linked to the side chain amine of the amino acid at position 16 of any of the Gcg/GIP/GLP-1 tri-agonist peptides disclosed herein.

In accordance with one embodiment the dipeptide prodrug element comprises the structure:



I

wherein

(I) R_1 and R_2 are independently selected from the group consisting of H, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl)R₇, $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W₁) C_1 - C_{12} alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl;

R_3 is CD₃, or C_1 - C_{18} alkyl;

R_4 and R_8 are independently H or D;

R_5 is NHR₆, or R_5 and R_2 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R_6 is H or C_1 - C_4 alkyl; and,

R_7 is selected from the group consisting of H, OH, halo, $(C_1$ - C_7 alkyl), $(C_2$ - C_7 alkenyl), OCF₃, NO₂, CN, NC, O(C_1 - C_7 alkyl), CO₂H, CO₂(C_1 - C_7 alkyl), NHR₆, aryl, and heteroaryl, with the proviso that when A-B is linked to the alpha amino group on the N-terminus of Q and (i) both R_1 and R_2 are H, and (ii) R_3 is methyl, then Q is not F⁷GLP-1(8-37);

(II) R_1 and R_2 are independently selected from the group consisting of H, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl)R₇, $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W₁) C_1 - C_{12} alkyl, wherein W₁ is

a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

R₄ is selected from the group consisting of D, CH₃, CH₂(C₁-C₁₀ alkyl),

- 5 CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃ alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl), and CH₂(C₀-C₁₂ alkyl)(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected
- 10 from the group consisting of N, S and O, or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H or D;

- 15 R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

- (III) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;
- 20

- 25 R₃ is C₁-C₁₈ alkyl;

- R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂), or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;
- 30

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

- 5 (IV) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is
10 a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

R₄ and R₈ are independently D or H;

- 15 R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

20

- (V) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is
25 a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH (C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(VI) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of D, CH(C₁-C₈ alkyl)₂, CH (C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl.

(VII) R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R_2 and R_8 are both H;

R_4 and R_3 together with the atoms to which they are attached form a
 5 pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a
 hydroxypiperidine ring; and

R_5 is NH_2 ., optionally wherein R_4 and R_3 together with the atoms to which
 they are attached form a hydroxy substituted pyrrolidine ring or a piperidine ring.

(VIII) R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally lLys or dLys, acylated with a C16-C20
 10 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R_2 is H;

R_4 and R_8 are independently H or D;

R_3 is CD_3 or C_1-C_4 alkyl; and

R_5 is NH_2 , optionally wherein R_4 and R_8 are each D, and R_3 is CD_3 .

15

IX) R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally lLys or dLys, acylated with a C16-C20
 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

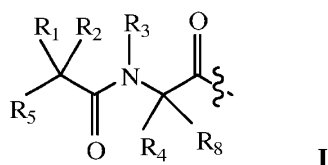
R_2 , R_4 and R_8 are each H;

R_3 is CH_3 ; and

20

R_5 is NH_2 .

In accordance with one embodiment a prodrug derivative of SEQ ID NO: 1 is
 provided wherein the alpha amine group of a isoacyl-Thr present at position 7 is linked to
 a P7 dipeptide via an amide bond, wherein the P7 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of $(C_1-C_4 \text{ alkyl})NH_2$, wherein a C16-C30 fatty
 25 acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said
 side chain, optionally via a P7 spacer;

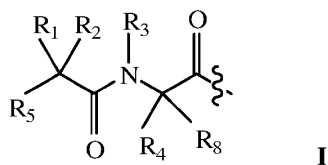
R_2 is H, or C_1-C_4 alkyl;

R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H, D, or C₁-C₄ alkyl; and

R₅ is NH₂, wherein said P7 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In a further embodiment, the prodrug derivative of SEQ ID NO: 1 is provided wherein said P7 dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

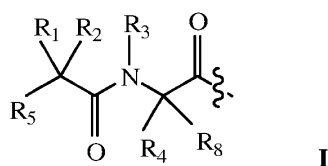
R₂ is H;

R₄ and R₈ are each D or H;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein the P7 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1. In a further embodiment the prodrug derivative of SEQ ID NO: 1 or SEQ ID NO: 3 is provided wherein the P7 spacer is -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid), and the acyl group is a C18-C20 diacid.

In accordance with one embodiment a prodrug derivative of any of the Gcg/GIP/GLP-1 tri-agonist peptide disclosed herein is provided, wherein a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of the peptide, wherein the P1 dipeptide comprises the structure:



wherein

R₁ is selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₂ is H, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl;

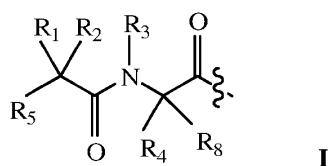
R₄ is selected from the group consisting of D, CH₃, CH₂(C₁-C₁₀ alkyl), CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃ alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl), and CH₂(C₀-C₁₂ alkyl)(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, hydroxypyridine or piperidine ring;

R₈ is H or D;

R₅ is NH₂; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NH₂, aryl, and heteroaryl, optionally wherein R₃ is CD₃ or C₁-C₆ alkyl.

In accordance with one embodiment a prodrug derivative of any of the Gcg/GIP/GLP-1 tri-agonist peptide disclosed herein is provided, wherein a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of the peptide, wherein the P1 dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R₂ is H, or C₁-C₄ alkyl;

5 R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

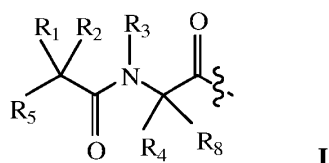
R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

10 R₈ is H, D or C₁-C₄ alkyl; and

R₅ is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z

15 is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In one embodiment the P1 dipeptide comprises the structure:



wherein

20 R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

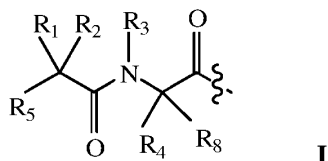
R₂ is H;

R₄ and R₈ are both D or H;

R₃ is CD₃ or C₁-C₆ alkyl; and

- R₅ is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q- (gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally with the proviso that when R₄ and R₈ are both H, R₃ is CD₃, optionally wherein R₄ and R₈ are both D and R₃ is CD₃. In a further embodiment the P1 spacer is -[COCH₂(OCH₂CH₂)₂-NH]- (gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

In one embodiment the P1 dipeptide comprises the structure:



wherein

- R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

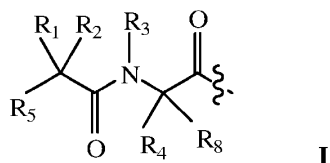
R₂ is H;

R₄ and R₈ are both D;

R₃ is CD₃; and

- R₅ is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q- (gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1. In a further embodiment the P1 spacer is -[COCH₂(OCH₂CH₂)₂-NH]- (gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

- In accordance with one embodiment a prodrug derivative of any of the Gcg/GIP/GLP-1 tri-agonist peptide disclosed herein is provided, wherein a P16 dipeptide is covalently linked via an amide bond to the side chain amine of the Lys at position 16 of the peptide, wherein the P16 dipeptide comprises the structure:



wherein

- R₁ is selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄

alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

5 R₂ is H, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl;

R₄ is selected from the group consisting of D, CH₃, CH₂(C₁-C₁₀ alkyl), CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃

10 alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl), and CH₂(C₀-C₁₂ alkyl)(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperdine or a

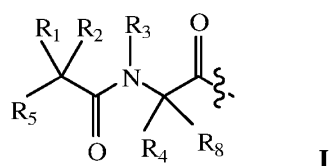
15 hydroxypiperdine ring;

R₈ is H or D;

R₅ is NH₂; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NH₂, aryl, and heteroaryl, optionally wherein R₃ is CD₃ or C₁-C₆ alkyl.

In accordance with one embodiment a prodrug derivative of any of the Gcg/GIP/GLP-1 tri-agonist peptide disclosed herein is provided, wherein a P16 dipeptide is covalently linked via an amide bond to the side chain amine of the amino acid at position 16 of the peptide, wherein the P16 dipeptide comprises the structure:



wherein

25 R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R_2 , is H, or C₁-C₄ alkyl;

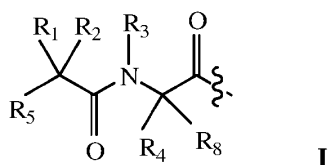
R_4 is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, $-(CH_2)_nCH_2OH$ or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R_3 is CD₃, C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R_8 , is H, D or C₁-C₄ alkyl; and

R_5 is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In one embodiment the P16 dipeptide comprises the structure:



wherein

R_1 comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

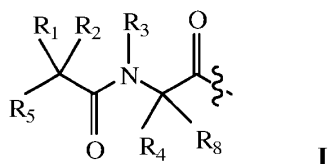
R_2 is H;

R_4 and R_8 are both D or H;

R_3 is CD₃ or C₁-C₆ alkyl; and

R_5 is NH₂, wherein said P16 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, with the proviso that when R_4 and R_8 are both H, R_3 is CD₃, optionally wherein R_4 and R_8 are both D and R_3 is CD₃. In a further embodiment the P1 spacer is -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

In one embodiment the P16 dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

5 R₂ is H;

R₄ and R₈ are both D;

R₃ is CD₃; and

R₅ is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1. In a further
10 embodiment the P1 spacer is -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

In accordance with one embodiment a prodrug derivative of SEQ ID NO: 2 is provided wherein said peptide comprises the sequence of

X₁X₂X₃GTX₆TSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFX₂₃X₂₄X₂₅LLEGGPSSGAPPPS

15 -R₂₀ (SEQ ID NO: 4),

X₁X₂X₃GTFX₇SDYSIX₁₃LX₁₅X₁₆X₁₇AQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀
(SEQ ID NO: 5),

X₁X₂X₃GTFTSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 2), or a peptide that differs from SEQ ID NO: 2 by 1, 2, 3, 4, or 5 amino
20 acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions,

wherein

X₁ is Tyr or His;

X₂ is an α,α-disubstituted amino acid, optionally Aib;

25 X₃ is Gln or His;

X₆ is Phe;

X₇ is Thr or (α-Me)T;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu or Aib;

X₁₅ is Glu or Asp;

30 X₁₆ is Aib, Orn or Lys;

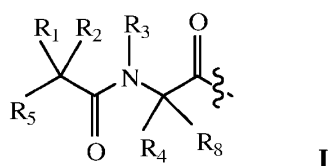
X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17 spacer;

X₁₈ is Ala or Tyr;

X_{20} is an α,α -disubstituted amino acid, optionally Aib; and

R_{20} is CONH_2 , wherein said P17 spacer is selected from the group

consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z\text{-}[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH}]_q\text{-(gamma glutamic acid)}_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, further wherein a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, wherein the P1 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of $(\text{C}_4 \text{ alkyl})\text{NH}_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain via a P1 spacer;

R_2 is H;

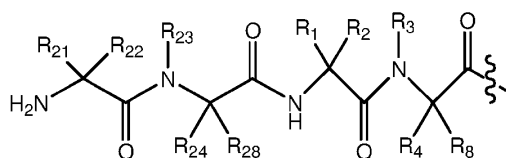
R_4 and R_8 are both D or H;

R_3 is CD_3 or $\text{C}_1\text{-C}_6$ alkyl; and

R_5 is NH_2 , wherein said P1 spacer comprises $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, with the proviso that when R_4 and R_8 are both H, R_3 is CD_3 , optionally wherein R_4 and R_8 are both D and R_3 is CD_3 . In a further embodiment X_{17} is Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$.

In accordance with one embodiment, in any of the prodrug derivative disclosed herein, the first amino acid of the dipeptide prodrug element is an amino acid in the D-configuration, optionally wherein the amino acid is dLys.

In accordance with one embodiment a prodrug derivative of any of the Gcg/GIP/GLP-1 tri-agonist peptides is provided wherein a sequential dipeptide structure of



is covalently linked via an amide bond to said peptide, optionally at one or more of amino acid positions 1, 7 and 16, wherein

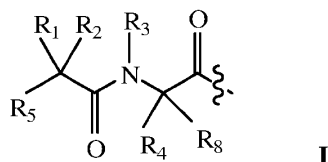
R_1 is selected from an acylated amino acid having a side chain comprising
5 (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

10 R_2 , and R_{22} are independently H, or C₁-C₄ alkyl;

R_4 , R_8 , R_{24} and R_{28} are independently H, D or C₁-C₄ alkyl;

R_3 and R_{23} are independently CD₃, C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said DD1 and DD2 spacers are independently
15 selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment the sequential dipeptide is linked at the N-terminal alpha amine of the
20 peptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, optionally with a dipeptide having the structure of



covalently linked to the amino acid at position 7 or 16 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 wherein

25 R_1 comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R_2 , is H, or C₁-C₄ alkyl;

R_4 is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄
30 alkyl)phenyl, wherein n is an integer selected from 0-2;

R_3 is CD_3 , C_1 - C_6 alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R_8 , is H, D or C_1 - C_4 alkyl; and

5 R_5 is NH_2 , wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z\text{-(COCH}_2\text{(OCH}_2\text{CH}_2)_k\text{-NH)}_q\text{-(gamma glutamic acid)}_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

10

Acylation and alkylation

The Gcg/GIP/GLP-1 tri-agonists of the present invention are acylated or alkylated at position 17 and at the first amino acid of the dipeptide prodrug element of the prodrug derivative of the Gcg/GIP/GLP-1 tri-agonists. Acylation or alkylation can increase the half-life of the Gcg/GIP/GLP-1 tri-agonists peptides in circulation. Acylation or alkylation can advantageously delay the onset of action and/or extend the duration of action at the glucagon, GIP and/or GLP-1 receptors and/or improve resistance to proteases such as DPP-IV and/or improve solubility. Activity at the GLP-1 and/or GIP receptors of the Gcg/GIP/GLP-1 tri-agonists peptide may be maintained after acylation.

15 In some embodiments, the potency of the acylated glucagon peptides is comparable to the unacylated versions of the glucagon peptides. In alternative embodiments, the potency of the acylated glucagon peptides is increased as compared to that of the unacylated version of the glucagon peptides.

In some embodiments, the Gcg/GIP/GLP-1 tri-agonists peptide may further comprise a spacer between the amino acid at position 20 (or at amino acid A of the dipeptide prodrug element) of the Gcg/GIP/GLP-1 tri-agonists peptide and the acyl group or alkyl group. In some embodiments, the acyl group is a fatty acid or diacid, or salt thereof, e.g. a C_4 to C_{30} fatty acid/diacid, a C_4 - C_{30} phosphonic fatty acid, a C_8 to C_{24} fatty acid/diacid, a C_8 - C_{24} phosphonic fatty acid, cholic acid, a C_4 to C_{30} alkyl, a C_8 to C_{24} alkyl, or an alkyl comprising a steroid moiety of a bile acid. The spacer is any moiety with suitable reactive groups for attaching acyl or alkyl groups. In exemplary embodiments, the spacer comprises an amino acid, a dipeptide, a tripeptide, a hydrophilic bifunctional, or a hydrophobic bifunctional spacer. In some embodiments, the spacer is selected from the group consisting of: Trp, Glu, Asp, Cys and a spacer comprising

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$\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_m\text{COOH}$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12. Such acylated or alkylated glucagon peptides may also further comprise a hydrophilic moiety, optionally a polyethylene glycol. Any of the foregoing Gcg/GIP/GLP-1 tri-agonists peptides may comprise two acyl groups or two alkyl groups, or a combination thereof.

The acyl group of the acylated Gcg/GIP/GLP-1 tri-agonist peptide can be of any size, e.g., any length carbon chain, and can be linear or branched. In some specific embodiments of the invention, the acyl group is a C8 to C30 fatty acid, a C8-C30 phosphonic fatty acid, or C8 to C30 diacid. For example, the acyl group can be any of a C8 fatty acid, C10 fatty acid/diacid, C12 fatty acid/diacid, C14 fatty acid/diacid, C16 fatty acid/diacid, C18 fatty acid/diacid, C20 fatty acid/diacid, C22 fatty acid/diacid, C24 fatty acid/diacid, C26 fatty acid/diacid, C28 fatty acid/diacid, or a C30 fatty acid/diacid. In some embodiments, the acyl group is a C8 to C20 fatty acid/diacid, e.g., a C18 fatty acid/diacid or a C20 fatty acid/diacid.

Addition modifications can be made to the tri-agonist peptides of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, including but are not limited to:

(A) Increasing solubility and/or duration of action or half-life in circulation and/or delaying the onset of action by acylation or alkylation of the glucagon peptide, as described herein; (B) Increasing duration of action or half-life in circulation through introducing resistance to dipeptidyl peptidase IV (DPP IV) cleavage by modification of the amino acid at position 1 or 2 including the substitution with D-amino acids.

(C) Non-conservative or conservative substitutions that do not substantially affect activity, for example, conservative substitutions at one or more of positions 5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29; substitution of one or more of these positions with Ala.

Pharmaceutical compositions comprising the Gcg/GIP/GLP-1 tri-agonist peptides, or prodrug derivatives thereof, as disclosed herein can be formulated and administered to patients to using standard pharmaceutically acceptable carriers and routes of administration known to those skilled in the art. Accordingly, the present disclosure also encompasses pharmaceutical compositions comprising one or more of the Gcg/GIP/GLP-1 tri-agonist peptides, or prodrug derivatives thereof, as disclosed herein, in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions may comprise the Gcg/GIP/GLP-1 tri-agonist peptides, or prodrug derivatives thereof, as the sole pharmaceutically active component, or the Gcg/GIP/GLP-1 tri-agonist peptides, or

prodrug derivatives thereof, can be combined with one or more additional active agents, including for example an additional anti-obesity peptide. Suitable anti-obesity peptides include those disclosed in US patents 5,691,309, 6,436,435 or US Patent application 20050176643, and including, but not limited to GLP-1, GIP (Gastric Inhibitory

5 Polypeptide), MP1, PYY, MC-4, amylin, calcitonin, NK2, leptin.

In accordance with some embodiments a pharmaceutical composition is provided comprising any of the novel Gcg/GIP/GLP-1 tri-agonist peptides, or prodrug derivatives thereof, as disclosed herein, preferably sterile and preferably at a purity level of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, and a pharmaceutically
10 acceptable diluent, carrier or excipient. Such compositions may contain a bioactive peptide prodrug derivative as disclosed herein, wherein the resulting active peptide is present at a concentration of at least 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 11 mg/ml, 12 mg/ml, 13 mg/ml, 14 mg/ml, 15 mg/ml, 16 mg/ml, 17 mg/ml, 18 mg/ml, 19 mg/ml, 20 mg/ml, 21 mg/ml, 22
15 mg/ml, 23 mg/ml, 24 mg/ml, 25 mg/ml or higher.

The pharmaceutical composition can comprise any pharmaceutically acceptable ingredient, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents,
20 chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives,
25 sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or
30 wetting agents.

In some embodiments, the pharmaceutical composition comprises any one or a combination of the following components: acacia, acesulfame potassium, acetyltributyl citrate, acetyltriethyl citrate, agar, albumin, alcohol, dehydrated alcohol, denatured alcohol, dilute alcohol, aleuritic acid, alginic acid, aliphatic polyesters, alumina,

aluminum hydroxide, aluminum stearate, amylopectin, α -amylose, ascorbic acid, ascorbyl palmitate, aspartame, bacteriostatic water for injection, bentonite, bentonite magma, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, benzyl benzoate, bronopol, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, 5 butylparaben sodium, calcium alginate, calcium ascorbate, calcium carbonate, calcium cyclamate, dibasic anhydrous calcium phosphate, dibasic dehydrate calcium phosphate, tribasic calcium phosphate, calcium propionate, calcium silicate, calcium sorbate, calcium stearate, calcium sulfate, calcium sulfate hemihydrate, canola oil, carbomer, carbon dioxide, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium, β -carotene, 10 carrageenan, castor oil, hydrogenated castor oil, cationic emulsifying wax, cellulose acetate, cellulose acetate phthalate, ethyl cellulose, microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, sodium carboxymethyl cellulose, cetostearyl alcohol, cetrimide, cetyl alcohol, chlorhexidine, chlorobutanol, chlorocresol, cholesterol, chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, 15 chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC)chlorophenoxyethanol, chloroxylonol, corn syrup solids, anhydrous citric acid, citric acid monohydrate, cocoa butter, coloring agents, corn oil, cottonseed oil, cresol, m-cresol, o-cresol, p-cresol, croscarmellose sodium, crospovidone, cyclamic acid, cyclodextrins, dextrans, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl 20 phthalate, dibutyl sebacate, diethanolamine, diethyl phthalate, difluoroethane (HFC), dimethyl- β -cyclodextrin, cyclodextrin-type compounds such as Captisol®, dimethyl ether, dimethyl phthalate, dipotassium edentate, disodium edentate, disodium hydrogen phosphate, docusate calcium, docusate potassium, docusate sodium, dodecyl gallate, dodecyltrimethylammonium bromide, edentate calcium disodium, edtic acid, eglumine, 25 ethyl alcohol, ethylcellulose, ethyl gallate, ethyl laurate, ethyl maltol, ethyl oleate, ethylparaben, ethylparaben potassium, ethylparaben sodium, ethyl vanillin, fructose, fructose liquid, fructose milled, fructose pyrogen-free, powdered fructose, fumaric acid, gelatin, glucose, liquid glucose, glyceride mixtures of saturated vegetable fatty acids, glycerin, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, self-emulsifying 30 glyceryl monostearate, glyceryl palmitostearate, glycine, glycols, glycofurol, guar gum, heptafluoropropane (HFC), hexadecyltrimethylammonium bromide, high fructose syrup, human serum albumin, hydrocarbons (HC), dilute hydrochloric acid, hydrogenated vegetable oil, type II, hydroxyethyl cellulose, 2-hydroxyethyl- β -cyclodextrin, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, 2-hydroxypropyl- β -

cyclodextrin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, imidurea, indigo carmine, ion exchangers, iron oxides, isopropyl alcohol, isopropyl myristate, isopropyl palmitate, isotonic saline, kaolin, lactic acid, lactitol, lactose, lanolin, lanolin alcohols, anhydrous lanolin, lecithin, magnesium aluminum silicate, magnesium carbonate, normal magnesium carbonate, magnesium carbonate anhydrous, magnesium carbonate hydroxide, magnesium hydroxide, magnesium lauryl sulfate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate, magnesium trisilicate anhydrous, malic acid, malt, maltitol, maltitol solution, maltodextrin, maltol, maltose, mannitol, medium chain triglycerides, meglumine, menthol, methylcellulose, methyl methacrylate, methyl oleate, methylparaben, methylparaben potassium, methylparaben sodium, microcrystalline cellulose and carboxymethylcellulose sodium, mineral oil, light mineral oil, mineral oil and lanolin alcohols, oil, olive oil, monoethanolamine, montmorillonite, octyl gallate, oleic acid, palmitic acid, paraffin, peanut oil, petrolatum, petrolatum and lanolin alcohols, pharmaceutical glaze, phenol, liquified phenol, phenoxyethanol, phenoxypropanol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, polacrilin, polacrilin potassium, poloxamer, polydextrose, polyethylene glycol, polyethylene oxide, polyacrylates, polyethylene-polyoxypropylene-block polymers, polymethacrylates, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene stearates, polyvinyl alcohol, polyvinyl pyrrolidone, potassium alginate, potassium benzoate, potassium bicarbonate, potassium bisulfite, potassium chloride, potassium citrate, potassium citrate anhydrous, potassium hydrogen phosphate, potassium metabisulfite, monobasic potassium phosphate, potassium propionate, potassium sorbate, povidone, propanol, propionic acid, propylene carbonate, propylene glycol, propylene glycol alginate, propyl gallate, propylparaben, propylparaben potassium, propylparaben sodium, protamine sulfate, rapeseed oil, Ringer's solution, saccharin, saccharin ammonium, saccharin calcium, saccharin sodium, safflower oil, saponite, serum proteins, sesame oil, colloidal silica, colloidal silicon dioxide, sodium alginate, sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium bisulfite, sodium chloride, anhydrous sodium citrate, sodium citrate dehydrate, sodium chloride, sodium cyclamate, sodium edentate, sodium dodecyl sulfate, sodium lauryl sulfate, sodium metabisulfite, sodium phosphate, dibasic, sodium phosphate, monobasic, sodium phosphate, tribasic, anhydrous sodium propionate, sodium propionate, sodium sorbate, sodium starch glycolate, sodium stearyl fumarate, sodium sulfite, sorbic acid, sorbitan

esters (sorbitan fatty esters), sorbitol, sorbitol solution 70%, soybean oil, spermaceti wax, starch, corn starch, potato starch, pregelatinized starch, sterilizable maize starch, stearic acid, purified stearic acid, stearyl alcohol, sucrose, sugars, compressible sugar, confectioner's sugar, sugar spheres, invert sugar, *Sugartab*, Sunset Yellow FCF, synthetic

5 paraffin, talc, tartaric acid, tartrazine, tetrafluoroethane (HFC), theobroma oil, thimerosal, titanium dioxide, alpha tocopherol, tocopheryl acetate, alpha tocopheryl acid succinate, beta-tocopherol, delta-tocopherol, gamma-tocopherol, tragacanth, triacetin, tributyl citrate, triethanolamine, triethyl citrate, trimethyl- β -cyclodextrin, trimethyltetradecylammonium bromide, tris buffer, trisodium edentate, vanillin, type I

10 hydrogenated vegetable oil, water, soft water, hard water, carbon dioxide-free water, pyrogen-free water, water for injection, sterile water for inhalation, sterile water for injection, sterile water for irrigation, waxes, anionic emulsifying wax, carnauba wax, cationic emulsifying wax, cetyl ester wax, microcrystalline wax, nonionic emulsifying wax, suppository wax, white wax, yellow wax, white petrolatum, wool fat, xanthan gum,

15 xylitol, zein, zinc propionate, zinc salts, zinc stearate, or any excipient in the *Handbook of Pharmaceutical Excipients*, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety, discloses various

20 components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

25 The pharmaceutical formulations disclosed herein may be designed to be short-acting, fast-releasing, long-acting, or sustained-releasing as described below. The pharmaceutical formulations may also be formulated for immediate release, controlled release or for slow release. The instant compositions may further comprise, for example, micelles or liposomes, or some other encapsulated form, or may be administered in an

30 extended-release form to provide a prolonged storage and/or delivery effect. The disclosed pharmaceutical formulations may be administered according to any regime including, for example, daily (1 time per day, 2 times per day, 3 times per day, 4 times per day, 5 times per day, 6 times per day), every two days, every three days, every four

days, every five days, every six days, weekly, bi-weekly, every three weeks, monthly, or bi-monthly.

In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at least A, wherein A is 0.0001% w/v, 0.001% w/v, 0.01% w/v, 0.1% w/v, 1% w/v, 2% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 40% w/v, 50% w/v, 60% w/v, 70% w/v, 80% w/v, or 90% w/v. In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at most B, wherein B is 90% w/v, 80% w/v, 70% w/v, 60% w/v, 50% w/v, 40% w/v, 30% w/v, 20% w/v, 10% w/v, 5% w/v, 2% w/v, 1% w/v, 0.1% w/v, 0.001% w/v, or 0.0001%. In other embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration range, such as, for example from about A to about B. In some embodiments, A is 0.0001% and B is 90%.

The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at least 5, at least 5.5, at least 6, at least 6.5, at least 7, at least 7.5, at least 8, at least 8.5, at least 9, at least 9.5, at least 10, or at least 10.5 up to and including pH 11, depending on the formulation and route of administration. In certain embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capable of buffering at the desired pH such as, for example, phosphate buffers (e.g. PBS), triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, and others. In certain embodiments, the strength of the buffer is at least 0.5 mM, at least 1 mM, at least 5 mM, at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 120 mM, at least 150 mM, or at least 200 mM. In some embodiments, the strength of the buffer is no more than 300 mM (e.g. at most 200 mM, at most 100 mM, at most 90 mM, at most 80 mM, at most 70 mM, at most 60 mM, at most 50 mM, at most 40 mM, at most 30 mM, at most 20 mM, at most 10 mM, at most 5 mM, at most 1 mM).

In some embodiments the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various containers. In one embodiment aqueous formulations of the GIP/GLP-1 co-agonist, or prodrug derivatives thereof, are prepared at concentrations of about 30mg/ml to about 100mg/ml in dimethyl sulfoxide (DMSO), including about 30% to 100% DMSO, about 50% to 100% DMSO,

about 70% to 100% DMSO, or about 90% to 100% DMSO, with the remainder water. In one embodiment aqueous formulations are prepared in 100% dimethyl sulfoxide. The compounds of the present invention can be used in accordance with some embodiments to prepare pre-formulated solutions ready for injection. In other embodiments the pharmaceutical compositions comprise a lyophilized powder. The pharmaceutical compositions can be further packaged as part of a kit that includes a disposable device for administering the composition to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

5 All therapeutic methods, pharmaceutical compositions, kits and other similar embodiments described herein contemplate that the prodrug compounds include all pharmaceutically acceptable salts thereof.

In some embodiments the kit is provided with a device for administering the prodrug composition to a patient. The kit may further include a variety of containers, e.g., vials, tubes, bottles, and the like. Preferably, the kits will also include instructions for use. In accordance with some embodiments the administering element of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in some embodiments the prodrug composition is prepackaged within the syringe.

15 The present Gcg/GIP/GLP-1 tri-agonists, and the prodrug derivatives thereof, have use in the treatment of diabetes, metabolic syndrome and for reducing weight gain or inducing weight loss. Pharmaceutical compositions comprising the Gcg/GIP/GLP-1 tri-agonists, and the prodrug derivatives thereof, and salts thereof, can be administered by any standard route including by oral or inhalation administration.

The present disclosure also encompasses multimers of the Gcg/GIP/GLP-1 tri-agonist peptides disclosed herein. Two or more of the Gcg/GIP/GLP-1 tri-agonist peptides can be linked together using standard linking agents and procedures known to those skilled in the art. For example, dimers can be formed between two Gcg/GIP/GLP-1 tri-agonist peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the Gcg/GIP/GLP-1 tri-agonist peptides comprising cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues.

Routes of Administration

25 The following discussion on routes of administration is merely provided to illustrate exemplary embodiments and should not be construed as limiting the scope in any way.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the GCG/GIP/GLP-1 co-agonist peptide dissolved in diluents, such as water, saline, DMSO, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water, DMSO and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the Gcg//GIP/GLP-1 co-agonist peptide in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the Gcg/GIP/GLP-1 co-agonist peptide in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

The Gcg/GIP/GLP-1 co-agonist peptide, alone or in combination with other suitable components, can be delivered via pulmonary administration and can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa. In some embodiments, the Gcg/GIP/GLP-1 co-agonist peptide is formulated into a powder blend or into microparticles or nanoparticles. Suitable pulmonary formulations are known in the art. See, e.g., Qian et al., *Int J Pharm* 366: 218-220 (2009); Adjei and Garren, *Pharmaceutical Research*, 7(6): 565-569 (1990); Kawashima et al., *J Controlled Release* 62(1-2): 279-287 (1999); Liu et al., *Pharm Res* 10(2): 228-232 (1993); International Patent Application Publication Nos. WO 2007/133747 and WO 2007/141411.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include
5 suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. The Gcg/GIP/GLP-1 co-agonist peptide can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water,
10 saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2- dimethyl-153-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically
15 acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame,
20 cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic
25 detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for
30 example, alkyl- β -aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof.

The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the Gcg/GIP/GLP-1 co-agonist peptide in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection,

such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactants in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan
5 monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for
10 injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those
15 of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

Additionally, the Gcg/GIP/GLP-1 co-agonist peptide can be made into
20 suppositories for rectal administration by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

25 It will be appreciated by one of skill in the art that, in addition to the above-described pharmaceutical compositions, the Gcg/GIP/GLP-1 co-agonist peptide can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

Dose

30 The compositions of the present disclosures comprising a Gcg/GIP/GLP-1 co-agonist and a peptide as described herein are believed to be useful in methods of treating a disease or medical condition in which GIP and GLP-1 receptor agonism play a role. For purposes of the present disclosures, the amount or dose of the composition of the present disclosure administered should be sufficient to effect, e.g., a therapeutic or

prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the composition of the present disclosures should be sufficient to stimulate cAMP secretion from cells as described herein or sufficient to decrease blood glucose levels, fat levels, food intake levels, or body weight of a mammal, in a period of
5 from about 1 to 4 minutes, 1 to 4 hours or 1 to 4 weeks or longer, e.g., 5 to 20 or more weeks, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular composition of the present disclosure and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

10 Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing the extent to which blood glucose levels or body weight are lowered upon administration of a given dose of the composition of the present disclosures to a mammal among a set of mammals of which is each given a different dose of the composition, could be used to determine a starting dose to be
15 administered to a mammal. The extent to which blood glucose levels or body weight are lowered upon administration of a certain dose can be assayed by methods known in the art.

Typically, the attending physician will decide the dosage of the composition of the present disclosure with which to treat each individual patient, taking into consideration a
20 variety of factors, such as age, body weight, general health, diet, sex, composition of the present disclosure to be administered, route of administration, severity of the condition being treated, and clinical effect to be achieved. The dose of the composition of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular peptide of the
25 present disclosure.

Combinations

The Gcg/GIP/GLP-1 co-agonist peptides disclosed herein may be administered alone or in combination with other therapeutic agents which aim to treat or prevent any of the diseases or medical conditions described herein. For example, the peptides described
30 herein (Gcg/GIP/GLP-1 co-agonists) may be co-administered with (simultaneously or sequentially) an anti-diabetic or anti-obesity agent. Anti-diabetic agents known in the art or under investigation include insulin, leptin, Peptide YY (PYY), Pancreatic Peptide (PP), fibroblast growth factor 21 (FGF21), Y2Y4 receptor agonists, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide

(Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicon); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPAR γ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FB Pase (fructose 1,6-bisphosphatase) inhibitors.

Anti-obesity agents known in the art or under investigation include appetite suppressants, including phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Preludin®), Bontril®, benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin; fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase inhibitors, similar to XENICAL (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

The Gcg/GIP/GLP-1 co-agonist peptide in some embodiments are co-administered with an agent for treatment of non-alcoholic fatty liver disease or NASH. Agents used to treat non-alcoholic fatty liver disease include ursodeoxycholic acid (a.k.a., Actigall, URSO, and Ursodiol), Metformin (Glucophage), rosiglitazone (Avandia), Clofibrate, Gemfibrozil, Polymixin B, and Betaine.

The Gcg/GIP/GLP-1 co-agonist peptide in some embodiments are co-administered with an agent for treatment of a neurodegenerative disease, e.g., Parkinson's Disease. Anti-Parkinson's Disease agents are furthermore known in the art and include, but not limited to, levodopa, carbidopa, anticholinergics, bromocriptine, pramipexole, and ropinirole, amantadine, and rasagiline.

In view of the foregoing, the present disclosures further provide pharmaceutical compositions and kits additionally comprising one of these other therapeutic agents. The additional therapeutic agent may be administered simultaneously or sequentially with the peptide of the present disclosure. In some aspects, the peptide is administered before the additional therapeutic agent, while in other aspects, the peptide is administered after the additional therapeutic agent.

Uses

Based on the information provided for the first time herein, it is contemplated that the compositions (e.g., related pharmaceutical compositions) of the present disclosed peptides are useful for treatment of a disease or medical condition, in which e.g., the lack
5 of activity at the GIP receptor, the GLP-1 receptor, or at both receptors, is a factor in the onset and/or progression of the disease or medical condition. Accordingly, the present disclosures provides a method of treating or preventing a disease or medical condition in a patient, wherein the disease or medical condition is a disease of medical condition in which a lack of GIP receptor activation and/or GLP-1 receptor activation is associated
10 with the onset and/or progression of the disease of medical condition. The method comprises providing to the patient with a composition or conjugate in accordance with any of those described herein in an amount effective to treat or prevent the disease or medical condition.

In some embodiments, the disease or medical condition is metabolic syndrome.
15 Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome or Reaven's syndrome, is a disorder that affects over 50 million Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides,
20 low HDL cholesterol and high LDL cholesterol that enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5) prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor-1 in blood), and (6) pro-inflammatory state (e.g., elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic
25 predisposition.

Metabolic Syndrome is associated with an increased risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with Metabolic Syndrome may progress from an insulin resistant
30 state in its early stages to full blown type II diabetes with further increasing risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, Metabolic Syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced

oxidative stress, and abnormalities in adipocyte-derived hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria
5 for Metabolic Syndrome: (a) abdominal obesity (a waist circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dl or above); (c) HDL cholesterol (40 mg/dl or lower in men and 50 mg/dl or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dl or above).

According to the World Health Organization (WHO), an individual having high insulin
10 levels (an elevated fasting blood glucose or an elevated post meal glucose alone) with at least two of the following criteria meets the criteria for Metabolic Syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m², or a waist measurement over 37 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c)
15 blood pressure of 140/90 or more, or on treatment for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

For purposes herein, if an individual meets the criteria of either or both of the criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment
20 Panel or the WHO, that individual is considered as afflicted with Metabolic Syndrome.

Without being bound to any particular theory, compositions and conjugates described herein are useful for treating Metabolic Syndrome. Accordingly, the invention provides a method of preventing or treating Metabolic Syndrome, or reducing one, two, three or more risk factors thereof, in a subject, comprising providing to the subject a
25 composition described herein in an amount effective to prevent or treat Metabolic Syndrome, or the risk factor thereof.

In some embodiments, the method treats a hyperglycemic medical condition. In certain aspects, the hyperglycemic medical condition is diabetes, diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes, either insulin-dependent or non-insulin-
30 dependent. In some aspects, the method treats the hyperglycemic medical condition by reducing one or more complications of diabetes including nephropathy, retinopathy and vascular disease.

In some aspects, the disease or medical condition is obesity. In some aspects, the obesity is drug-induced obesity. In some aspects, the method treats obesity by preventing

or reducing weight gain or increasing weight loss in the patient. In some aspects, the method treats obesity by reducing appetite, decreasing food intake, lowering the levels of fat in the patient, or decreasing the rate of movement of food through the gastrointestinal system.

5 Because obesity is associated with the onset or progression of other diseases, the methods of treating obesity are further useful in methods of reducing complications associated with obesity including vascular disease (coronary artery disease, stroke, peripheral vascular disease, ischemia reperfusion, etc.), hypertension, onset of diabetes type II, hyperlipidemia and musculoskeletal diseases. The present disclosures
10 accordingly provide methods of treating or preventing these obesity-associated complications.

 In some embodiments, the disease or medical condition is Nonalcoholic fatty liver disease (NAFLD). NAFLD refers to a wide spectrum of liver diseases ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis
15 (irreversible, advanced scarring of the liver). All the stages of NAFLD have in common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes). Simple fatty liver is the abnormal accumulation of a certain type of fat, triglyceride, in the liver cells with no inflammation or scarring. In NASH, the fat accumulation is associated with varying degrees of inflammation (hepatitis) and scarring (fibrosis) of the liver. The
20 inflammatory cells can destroy the liver cells (hepatocellular necrosis). In the terms "steatohepatitis" and "steatonecrosis", steato refers to fatty infiltration, hepatitis refers to inflammation in the liver, and necrosis refers to destroyed liver cells. NASH can ultimately lead to scarring of the liver (fibrosis) and then irreversible, advanced scarring (cirrhosis). Cirrhosis that is caused by NASH is the last and most severe stage in the
25 NAFLD spectrum. (Mendler, Michel, "Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)," ed. Schoenfield, Leslie J., MedicineNet.com, August 29, 2005).

 Alcoholic Liver Disease, or Alcohol-Induced Liver Disease, encompasses three pathologically distinct liver diseases related to or caused by the excessive consumption of
30 alcohol: fatty liver (steatosis), chronic or acute hepatitis, and cirrhosis. Alcoholic hepatitis can range from a mild hepatitis, with abnormal laboratory tests being the only indication of disease, to severe liver dysfunction with complications such as jaundice (yellow skin caused by bilirubin retention), hepatic encephalopathy (neurological dysfunction caused by liver failure), ascites (fluid accumulation in the abdomen),

bleeding esophageal varices (varicose veins in the esophagus), abnormal blood clotting and coma. Histologically, alcoholic hepatitis has a characteristic appearance with ballooning degeneration of hepatocytes, inflammation with neutrophils and sometimes Mallory bodies (abnormal aggregations of cellular intermediate filament proteins).

- 5 Cirrhosis is characterized anatomically by widespread nodules in the liver combined with fibrosis. (Worman, Howard J., "Alcoholic Liver Disease", Columbia University Medical Center website).

Without being bound to any particular theory, the compositions and conjugates described herein are useful for the treatment of Alcoholic Liver Disease, NAFLD, or any
10 stage thereof, including, for example, steatosis, steatohepatitis, hepatitis, hepatic inflammation, NASH, cirrhosis, or complications thereof. Accordingly, the present disclosures provide a method of preventing or treating Alcoholic Liver Disease, NAFLD, or any stage thereof, in a subject comprising providing to a subject a composition described herein in an amount effective to prevent or treat Alcoholic Liver Disease,
15 NAFLD, or the stage thereof. Such treatment methods include reduction in one, two, three or more of the following: liver fat content, incidence or progression of cirrhosis, incidence of hepatocellular carcinoma, signs of inflammation, e.g., abnormal hepatic enzyme levels (e.g., aspartate aminotransferase AST and/or alanine aminotransferase ALT, or LDH), elevated serum ferritin, elevated serum bilirubin, and/or signs of fibrosis,
20 e.g., elevated TGF-beta levels. In certain embodiments, the compositions are used treat patients who have progressed beyond simple fatty liver (steatosis) and exhibit signs of inflammation or hepatitis. Such methods may result, for example, in reduction of AST and/or ALT levels.

GLP-1 and exendin-4 have been shown to have some neuroprotective effect. The
25 present disclosures also provides uses of the compositions described herein in treating neurodegenerative diseases, including but not limited to Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, other demyelination related disorders, senile dementia, subcortical dementia, arteriosclerotic dementia, AIDS-associated dementia, or other dementias, a central nervous system cancer, traumatic brain
30 injury, spinal cord injury, stroke or cerebral ischemia, cerebral vasculitis, epilepsy, Huntington's disease, Tourette's syndrome, Guillain Barre syndrome, Wilson disease, Pick's disease, neuroinflammatory disorders, encephalitis, encephalomyelitis or meningitis of viral, fungal or bacterial origin, or other central nervous system infections, prion diseases, cerebellar ataxias, cerebellar degeneration, spinocerebellar degeneration

syndromes, Friedreich's ataxia, ataxia telangiectasia, spinal dysmyotrophy, progressive supranuclear palsy, dystonia, muscle spasticity, tremor, retinitis pigmentosa, striatonigral degeneration, mitochondrial encephalo-myopathies, neuronal ceroid lipofuscinosis, hepatic encephalopathies, renal encephalopathies, metabolic encephalopathies, toxin-induced encephalopathies, and radiation-induced brain damage.

In some embodiments, the compositions are used in conjunction with parenteral administration of nutrients to non-diabetic patients in a hospital setting, e.g., to patients receiving parenteral nutrition or total parenteral nutrition. Nonlimiting examples include surgery patients, patients in comas, patients with digestive tract illness, or a nonfunctional gastrointestinal tract (e.g. due to surgical removal, blockage or impaired absorptive capacity, Crohn's disease, ulcerative colitis, gastrointestinal tract obstruction, gastrointestinal tract fistula, acute pancreatitis, ischemic bowel, major gastrointestinal surgery, certain congenital gastrointestinal tract anomalies, prolonged diarrhea, or short bowel syndrome due to surgery, patients in shock, and patients undergoing healing processes often receive parenteral administration of carbohydrates along with various combinations of lipids, electrolytes, minerals, vitamins and amino acids. The compositions comprising the Gcg/GIP/GLP-1 co-agonist and as disclosed herein, and the parenteral nutrition composition can be administered at the same time, at different times, before, or after each other, provided that the composition is exerting the desired biological effect at the time that the parenteral nutrition composition is being digested. For example, the parenteral nutrition may be administered, 1, 2 or 3 times per day, while the composition is administered once every other day, three times a week, two times a week, once a week, once every 2 weeks, once every 3 weeks, or once a month.

Kits

The present disclosures further provide kits comprising a Gcg/GIP/GLP-1 co-agonist peptide. Accordingly, in some embodiments, the kit comprises a Gcg/GIP/GLP-1 co-agonist which exhibits at least 0.1% activity of native GIP at the GIP receptor, at least 0.1% activity of native GLP-1 at the GLP-1 receptor and at least 0.1% activity of native glucagon at the Gcg receptor.

In some aspects, the Gcg/GIP/GLP-1 co-agonist is packaged in containers, e.g., vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like. In some embodiments, the Gcg/GIP/GLP-1 co-agonist is provided in the kit as a lyophilized form or in an aqueous solution.

The kits in some embodiments comprise instructions for use. In one embodiment the kit is provided with a device for administering the composition to a patient, e.g., syringe needle, pen device, jet injector or another needle-free injector. In accordance with one embodiment the administering element of the kit is an aerosol dispensing
 5 device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile composition is prepackaged within the syringe.

In some embodiments, the kit comprises a pharmaceutically acceptable carrier, such as any of those described herein.
 10 The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

Exemplary Embodiments

In accordance with embodiment 1, a peptide having Gcg/GLP-1/GIP tri-agonist
 15 activity is provided, wherein the peptide comprises the sequence of

$X_1X_2X_3\text{GT}X_6X_7\text{SDYSIX}_{13}\text{L}X_{15}X_{16}X_{17}X_{18}\text{Q}X_{20}\text{AFVQWLLEGGPSSGAPPPS}-$
 R_{20} (SEQ ID NO: 1),

$X_1X_2X_3\text{GTFTSDYSIX}_{13}\text{LDK}X_{17}X_{18}\text{Q}X_{20}\text{AFVQWLLEGGPSSGAPPPS}-$
 R_{20} (SEQ ID NO: 3),

20 $X_1X_2X_3\text{GT}X_6\text{TSDYSIX}_{13}\text{L}X_{15}X_{16}X_{17}X_{18}\text{Q}X_{20}\text{AFX}_{23}X_{24}X_{25}\text{LLEGGPSSGAPPPS}$
 $-\text{R}_{20}$ (SEQ ID NO: 4); or

$X_1X_2X_3\text{GT}X_6X_7\text{SD}X_{10}\text{SIX}_{13}\text{L}X_{15}X_{16}X_{17}X_{18}\text{Q}X_{20}\text{AFVQWLL}X_{28}\text{GGPSSGAPPPS}$
 $-\text{R}_{20}$ (SEQ ID NO: 6), wherein

- X_1 is Tyr or His;
- 25 X_2 is an α,α -disubstituted amino acid, optionally Aib;
- X_3 is Gln or His;
- X_6 is an alpha methylated amino acid or Phe;
- X_7 is Thr or (α -Me)Thr or isoacyl-Thr;
- X_{10} is Tyr or is an amino acid comprising a (C1-C8 alkyl) NH_2 side chain
- 30 that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P10 spacer,
- X_{13} is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe, or Aib;
- X_{15} is Glu or Asp;

X₁₆ is Aib, Orn, Lys, optionally wherein Lys is acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P16 spacer;

5 X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17 spacer, optionally wherein X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid, optionally via a P17 spacer;

X₁₈ is Ala or Tyr;

X₂₀ is an α,α -disubstituted amino acid, optionally Aib;

10 X₂₃ is Ile or Val;

X₂₄ is Glu, Asp or Gln;

X₂₅ is Tyr or Trp;

X₂₈ is Glu or Asp; and

15 R₂₀ is COOH or CONH₂, wherein said P10, P16 and P17 spacer is independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

20 In accordance with embodiment 2, the peptide of embodiment 1 is provided, wherein the peptide comprises the sequence of

X₁X₂X₃GTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀ (SEQ ID NO: 1),

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3), or

25 X₁X₂X₃GTX₆X₇SDX₁₀SIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLX₂₈GGPSSGAPPPS-R₂₀ (SEQ ID NO: 6), wherein

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

30 X₆ is Phe;

X₇ is Thr or (α -Me)Thr;

X₁₀ is Tyr;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe or Aib;

X₁₅ is Glu or Asp;

X₁₆ is Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid, optionally via a P16 spacer;

X₁₈ is Ala or Tyr;

X₂₀ is Aib;

X₂₈ is Glu or Asp; and

R₂₀ is CONH₂, wherein said P17 spacer is independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 3 the peptide of embodiment 1 or 2 is provided wherein the peptide comprises the sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 3) or

X₁X₂X₃GTX₆X₇SDX₁₀SIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLX₂₈GGPSSGAPPPS
-R₂₀ (SEQ ID NO: 6),

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln;

X₆ is Phe;

X₇ is Thr;

X₁₀ is Tyr;

X₁₃ is (aMe)Leu;

X₁₅ is Glu or Asp;

X₁₆ is Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid, via a P17 spacer;

X₁₈ is Ala or Tyr;

X₂₀ is Aib;

X₂₈ is Glu or Asp; and

R₂₀ is CONH₂, wherein said P17 spacer is independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are
 5 independently an integer selected from the range of 0-4.

In accordance with embodiment 4, the peptide of embodiment 3 is provided wherein the peptide comprises the sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
 (SEQ ID NO: 3)

10 X₁ is Tyr; X₂ is Aib; X₃ is Gln; X₁₃ is (aMe)Leu;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid, via a P17 spacer;

X₁₈ is Ala; X₂₀ is Aib; and

15 R₂₀ is CONH₂, wherein said P17 spacer is independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

20 In accordance with embodiment 5, the peptide of any one of embodiments 1-4 is provided wherein wherein X₁₇ is Lys or Orn, wherein the side chain of the Lys or Orn is linked via an amide bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂.

In accordance with embodiment 6, the peptide of embodiment 1 or 2 is provided,
 25 wherein X₁₆ is Lys, acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid or a C16-C30 diacid via a P16 spacer, optionally wherein X₁₆ is K(eK-eK-γECOC₁₈H₃₆CO₂H).

In accordance with embodiment 7, the peptide of embodiment 1 or 2 is provided, wherein X₁₆ is Lys.

30 In accordance with embodiment 8, the peptide of embodiment 1 is provided, wherein X₁₀ is Lys, acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid or a C16-C30 diacid via a P10 spacer.

In accordance with embodiment 9, the peptide of embodiment 1 is provided, wherein X₁₀ is Tyr.

In accordance with embodiment 10, the peptide of embodiment 1 is provided, wherein

X₆ is an alpha methylated Phe; and

X₇ is isoacyl-Thr.

5 In accordance with embodiment 11, the peptide of any one of embodiments 1-10 is provided, wherein R₂₀ is CONH₂.

In accordance with embodiment 12, the peptide of any one of embodiments 1-3 is provided, wherein X₂ and X₂₀ are both Aib.

10 In accordance with embodiment 13, a peptide having Gcg/GLP-1/GIP tri-agonist activity is provided, wherein the peptide comprises the sequence of

X₁X₂X₃GTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPS-R₂₀ (SEQ ID NO: 1)

X₁ is Tyr or His;

X₂ is a Aib;

15 X₃ is Gln or His;

X₆ is an alpha methylated Phe or Phe;

X₇ is Thr or (α-Me)Thr or isoacyl-Thr;

X₁₃ is Tyr, (αMe)Leu, (αMe)Ile, (αMe)Met, (αMe)nLeu, (aMe)Phe or Aib;

20 X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17 spacer, optionally wherein X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid;

X₁₈ is Ala or Tyr;

X₂₀ is Aib; and

30 R₂₀ is COOH or CONH₂, wherein said P17 spacer is independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 14, the peptide of embodiment 13 is provided, wherein R₂₀ is CONH₂.

In accordance with embodiment 15, the peptide of embodiment 13 or 14 is provided, wherein X₂ and X₂₀ are both Aib.

5 In accordance with embodiment 16, the peptide of any one of embodiments 13-15 is provided, wherein X₁₃ is (αMe)Leu.

In accordance with embodiment 17, the peptide of any one of embodiments 13-16 is provided, wherein X₁₆ is Lys.

10 In accordance with embodiment 18, the peptide of any one of embodiments 13-17 is provided, wherein

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid.

In accordance with embodiment 19, the peptide of embodiment 18 is provided, wherein X₁₇ is Lys or Orn, wherein the side chain of the Lys or Orn is linked via an amide
15 bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂.

In accordance with embodiment 20, the peptide of any one of embodiments 13-19 is provided wherein

X₆ is an alpha methylated Phe; and

20 X₇ is isoacyl-Thr.

In accordance with embodiment 21, the peptide of any one of embodiments 13-19 is provided, wherein X₆ is Phe and X₇ is Thr or (α-Me)Thr.

In accordance with embodiment 22, the peptide of embodiment 21 is provided wherein X₇ is Thr.

25 In accordance with embodiment 23, a peptide having Gcg/GLP-1/GIP tri-agonist activity is provided, wherein the peptide comprises the sequence of

X₁X₂X₃GTFX₇SDYSIX₁₃LX₁₅X₁₆X₁₇AQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀
(SEQ ID NO: 5), or

30 X₁X₂X₃GTFTSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 2)

wherein

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

X₇ is Thr or (α-Me)T;

X₁₃ is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe or Aib;

X₁₅ is Glu or Asp;

5 X₁₆ is Aib, Orn or Lys;

X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17 spacer;

X₁₈ is Ala or Tyr;

10 X₂₀ is Aib; and

R₂₀ is COOH or CONH₂, wherein said P17 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

15 In accordance with embodiment 24, the peptide of embodiment 23 is provided, wherein X₁₇ is an amino acid comprising a (C3-C4 alkyl)NH₂ side chain that has been acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via said P17 spacer.

20 In accordance with embodiment 25, the peptide of embodiment 23 or 24 is provided, wherein R₂₀ is CONH₂.

In accordance with embodiment 26, the peptide of any one of embodiments 23-25 is provided, wherein X₁₃ is (αMe)Leu.

25 In accordance with embodiment 27, the peptide of any one of embodiments 23-25 is provided, wherein X₁₃ is Leu.

In accordance with embodiment 28, the peptide of any one of embodiments 23-27 is provided, wherein X₁₅ is Asp;

30 In accordance with embodiment 29, the peptide of any one of embodiments 23-28 is provided, wherein X₁₆ is Lys.

In accordance with embodiment 30, the peptide of any one of embodiments 23-29 is provided, wherein

X₁₇ is an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been acylated with a C16-C30 diacid or a C16-C20 phosphonic fatty acid.

In accordance with embodiment 31, the peptide of embodiment 30 is provided, wherein X₁₇ is Lys or Orn, wherein the side chain of the Lys or Orn is linked via an amide
5 bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂.

In accordance with embodiment 32, the peptide of embodiment 23 is provided, wherein

X₁₃ is (aMe)Leu;
10 X₁₅ is Asp;
X₁₆ is Lys and

X₁₇ is an acylated Lys, wherein the acyl group of the acylated Lys is a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid or a C16-C20 diacid, optionally linked via said P17 spacer to the Lys side chain, optionally wherein R₂₀ is CONH₂.

15 In accordance with embodiment 33, the peptide of any one of embodiments 23-26 and 32 is provided wherein said P17 spacer comprises the structure:

-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4.

In accordance with embodiment 34, the peptide of embodiment 33 is provided
20 wherein the P17 spacer comprises the structure:

-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-
wherein k is 2, p is 1 and q is 1 or 2.

In accordance with embodiment 35, the peptide of any one of embodiments 1-34 is provided wherein X₁₇ is Lys, wherein the lysine side chain is linked via an amide bond
25 to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂.

In accordance with embodiment 36, the peptide of any one of embodiments 1-35 is provided wherein R₂₀ is CONH₂.

In accordance with embodiment 37, the peptide of any one of embodiments 1-36
30 is provided wherein the peptide comprises the sequence:

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 3), wherein

I)

X₁ is Tyr;

X_2 and X_{20} are both Aib;

X_3 is Gln;

X_{13} is (aMe)Leu;

X_{17} is Lys, wherein the lysine side chain is linked via an amide bond to -

5 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-C16-C20 diacid}$, wherein k is 2, and p and q are independently an integer selected from 1 or 2;

X_{18} is Ala; and

R_{20} is CONH_2 , or

II)

10 X_1 is His;

X_2 and X_{20} are both Aib;

X_3 is His;

X_{13} is (aMe)Leu;

X_{17} is Lys, wherein the lysine side chain is linked via an amide bond to -

15 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-C16-C20 diacid}$, wherein k is 2, and p and q are independently an integer selected from 1 or 2;

X_{18} is Tyr; and

R_{20} is CONH_2 , or

III)

20 X_1 is Tyr;

X_2 and X_{20} are both Aib;

X_3 is Gln;

X_{13} is (aMe)Leu;

X_{17} is Lys, wherein the lysine side chain is linked via an amide bond to -

25 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-C16-C20 diacid}$, wherein k is 2, and p and q are independently an integer selected from 1 or 2;

X_{18} is Tyr; and

R_{20} is CONH_2 .

30 In accordance with embodiment 38, the peptide of any one of embodiments 1-37 is provided wherein

X_{17} is an acylated Lys wherein the lysine side chain is linked via an amide bond to $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$.

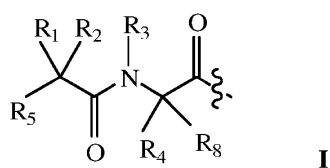
In accordance with embodiment 39, a prodrug derivative of the peptide of any one of embodiments 1-38 is provided wherein said peptide is modified by the covalent

linkage of one or more dipeptides (A-B) to an amine of said peptide, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated amino acid linked to said peptide through an amide bond between a carboxyl moiety of B and an amine of the peptide (optionally a primary amine, e.g. the N-terminal alpha amine), wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid.

In accordance with embodiment 40, the prodrug derivative of the peptide of claim 34 is provided wherein said dipeptides (A-B) are covalently linked to said peptide at one or more amino acids located at positions independently selected from positions 1, 7 and 16 relative to SEQ ID NO: 1.

In accordance with embodiment 41, the prodrug derivative of the peptide of claim 34 is provided wherein said dipeptide (A-B) is covalently linked to the N-terminal alpha amine of said peptide.

In accordance with embodiment 42 the prodrug derivative of any one of embodiments 39-41 is provided wherein said dipeptide (A-B) comprises the structure:



wherein

(I) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl, with the proviso that when A-B is linked to the alpha amino group on the N-terminus of Q and (i) both R₁ and R₂ are H, and (ii) R₃ is methyl, then Q is not F⁷GLP-1(8-37); or

(II) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is selected from the group consisting of CH₃, CH₂(C₁-C₁₀ alkyl), CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃ alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl), and CH₂(C₀-C₁₂ alkyl)(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(III) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is

a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

5 R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)), or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

10 R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

15 R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(IV) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂,
20 (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

25 R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

30 R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(V) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂,

(C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(VI) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃ or C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of D, CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(VII) R₁ is (C₁-C₄ alkyl)NH₂, optionally (C₄ alkyl)NH₂, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂, R₈ are both H;

R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂., optionally wherein R₄ and R₃ together with the atoms to which they are attached form a hydroxy substituted pyrrolidine ring or a piperidine ring; or

VIII) R₁ is (C₁-C₄ alkyl)NH₂, optionally (C₄ alkyl)NH₂, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂ is H;

R₄ and R₈ are both D;

R₃ is CD₃; and

R₅ is NH₂; or

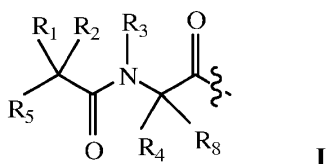
IX) R₁ is (C₁-C₄ alkyl)NH₂, optionally (C₄ alkyl)NH₂, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂, R₄ and R₈ are each H;

R₃ is CH₃; and

R₅ is NH₂, optionally wherein the "A" amino acid of the dipeptide A-B is in the D-stereoisomer configuration.

In accordance with embodiment 43, the prodrug derivative of any one of the peptides of embodiments 39-42 is provided wherein said peptide comprises a isoacyl-Thr at position 7 and a P7 dipeptide is covalently linked via an amide bond to the alpha amine of said isoacyl-Thr at position 7, said P7 dipeptide comprising the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P7 spacer;

5 R₂, is H or C₁-C₄ alkyl;

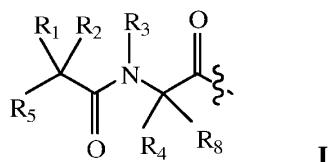
R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₈ is H, D or C₁-C₄ alkyl;

10 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said P7 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 44, the prodrug derivative of any one of the peptides of embodiments 39-43 is provided wherein said P7 dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

25 R₂ is H;

R₄ and R₈ are each H or D;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein said P7 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0, 1 or 2.

30 In accordance with embodiment 45, the prodrug derivative of any one of the peptides of embodiments 39-44 is provided wherein said P7 spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

In accordance with embodiment 46, the prodrug derivative of any one of the peptides of embodiments 39-45 is provided wherein

- 5 R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 diacid covalently linked to said side chain, via a -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-spacer;

R₂ is H;

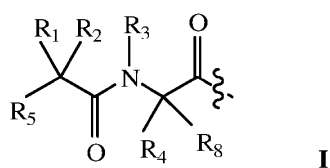
R₄ and R₈ are each D;

- 10 R₃ is CD₃; and

R₅ is NH₂.

In accordance with embodiment 47, the prodrug derivative of any one of the peptides of embodiments 39-46 is provided wherein a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide

- 15 comprising the structure:



wherein

- R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;
- 20

R₂ is H or C₁-C₄ alkyl;

R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₈ is H, D or C₁-C₄ alkyl;

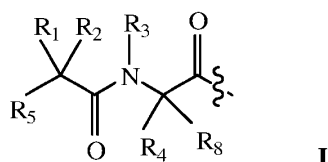
- 25 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

- R₅ is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z
- 30

is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 48, the prodrug derivative of any one of the peptides of embodiments 39 to 47 is provided wherein said P1 dipeptide comprises the

5 structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain,

10 optionally via said P1 spacer;

R₂ is H;

R₄ and R₈ are each H or D;

R₃ is CD₃ or C₁-C₆ alkyl; and

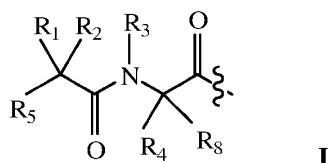
R₅ is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q- (gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein R₄ and R₈ are both D and R₃ is CD₃.

In accordance with embodiment 49, the prodrug derivative of any one of the peptides of embodiments 39-48 is provided wherein said P1 spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-

20 C20 diacid.

In accordance with embodiment 50, the prodrug derivative of any one of the peptides of embodiments 39-49 is provided wherein said P1 dipeptide comprises the structure:



25 wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 diacid covalently linked to said side chain, via a -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid) spacer;

R₂ is H;

5 R₄ and R₈ are each independently H or D;

R₃ is CH₃ or CD₃; and

R₅ is NH₂ with the optional proviso that when either R₄ or R₈ is D, R₃ is CH₃.

10 In accordance with embodiment 51, the prodrug derivative of embodiment 50 is provided wherein

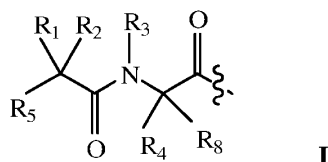
R₁ comprises -(C₄ alkyl)NH-(COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or -(C₄ alkyl)NH-(COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂;

R₂ R₄ and R₈ are each H;

R₃ is CH₃; and

15 R₅ is NH₂.

In accordance with embodiment 52 the prodrug derivative of any one of embodiments 39-51 is provided wherein a P16 dipeptide is covalently linked via an amide bond to side chain amine of an amino acid located at position 16 relative to SEQ ID NO: 1, wherein said P16 dipeptide comprising the structure:



20

wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P16 spacer;

25 R₂ is H, or C₁-C₄ alkyl;

R₄ and R₈ are independently H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

30

R_5 is NH_2 , wherein said P16 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid) $_z$ -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 53, a prodrug derivative of a peptide having Gcg/GLP-1/GIP tri-agonist activity is provided, wherein the peptide comprises the sequence of

$X_1X_2X_3GTFTSDYSIX_{13}LDKX_{17}X_{18}QX_{20}AFVQWLLEGGPSSGAPPPS-R_{20}$
(SEQ ID NO: 3)

wherein

R_{20} is COOH or CONH $_2$, optionally R_{20} is CONH $_2$

X_1 is Tyr or His;

X_2 is Aib;

X_3 is Gln or His;

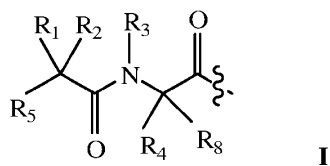
X_{13} is (aMe)Leu;

X_{17} is an amino acid comprising a (C1-C4 alkyl)NH-[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ NH] $_q$ -(gamma glutamic acid) $_p$ -C16-C20 diacid side chain or a(C1-C4 alkyl)NH-[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ NH] $_q$ -(gamma glutamic acid) $_p$ -C16-C20 phosphonic fatty acid side chain, wherein k is 2, and p and q are independently an integer selected from 1 or 2, optionally wherein p is 1 and k and q are both 2;

X_{18} is Ala or Tyr and

X_{20} is Aib, further wherein

a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of (C1-C4 alkyl)NH $_2$, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R_2 , is H;

R_4 and R_8 are both H or both D:

R₃ is CD₃, or C₁-C₆ alkyl, with the optional proviso that when R₄ and R₈ are both D, then R₃ is C₁-C₆ alkyl and when R₄ and R₈ are both H, R₃ is CD₃; and

R₅ is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, with the proviso that w.

In accordance with embodiment 54, the prodrug derivative of embodiment 52 or 10 53 is provided wherein said P1 spacer is $-\text{[COCH}_2\text{(OCH}_2\text{CH}_2\text{)}_2\text{-NH]}-$ (gamma glutamic acid), and the acyl group is a C18-C20 diacid or a C18-C20 phosphonic fatty acid.

In accordance with embodiment 55, the prodrug derivative of any one of embodiments 39-54 is provided wherein R₂₀ is CONH₂.

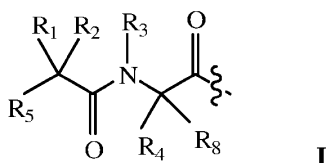
In accordance with embodiment 56, the prodrug derivative of any one of
 15 embodiments 39 to 55 is provided wherein X₁₇ is Lys, wherein the lysine side chain is
 linked via an amide bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or
 (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂.

In accordance with embodiment 57, the prodrug derivative of any one of
embodiments 39 to 56 is provided wherein R₁ comprises (C4 alkyl)NH-
20 (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H.

In accordance with embodiment 58, the prodrug derivative of any one of embodiments 39 to 57 is provided wherein R₄ and R₈ are both D.

In accordance with embodiment 59, the prodrug derivative of any one of embodiments 39 to 58 is provided wherein R₃ is CD₃.

25 In accordance with embodiment 60 the prodrug derivative of any one of
embodiments 39 to 59 is provided wherein said peptide comprises a P16 dipeptide
covalently linked via an amide bond to the side chain amine of the amino acid at position
16, said P16 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of $(C_1-C_4 \text{ alkyl})NH_2$, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P16 spacer;

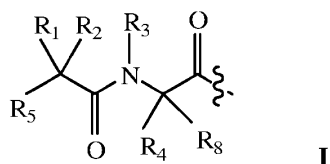
R_2 is H, or C_1-C_4 alkyl;

5 R_4 and R_8 are independently H, D, C_1-C_4 alkyl, C_1-C_4 alkenyl, $-(CH_2)_nCH_2OH$ or $(C_1-C_4 \text{ alkyl})phenyl$, wherein n is an integer selected from 0-2;

R_3 is CD_3 , C_1-C_6 alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

10 R_5 is NH_2 , wherein said P16 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

15 In accordance with embodiment 61 the prodrug derivative of embodiment 60 is provided wherein said P16 dipeptide comprises the structure:



wherein

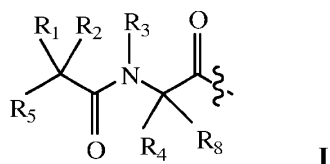
20 R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

R_2 , R_4 and R_8 are each H;

R_3 is C_1-C_6 alkyl; and

25 R_5 is NH_2 , wherein said P16 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 62 the prodrug derivative of embodiment 60 or 61 is provided wherein said P16 dipeptide comprises the structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

5 R₂ is H;

R₄ and R₈ are each D or H;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein said P16 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally

10 wherein R₄ and R₈ are each D and/or R₃ is CD₃.

In accordance with embodiment 63 the prodrug derivative of any one of embodiments 60-62 is provided wherein said P16 spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

15 In accordance with embodiment 64, the prodrug derivative of any one of the peptides of embodiments 39-63 is provided wherein the first amino acid of said P1, P7 or P16 dipeptide (A-B) is an amino acid in the D-configuration.

In accordance with embodiment 65, a prodrug derivative of a peptide having Gcg/GLP-1/GIP tri-agonist activity is provided, wherein the peptide comprises the

20 sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 3)

wherein

R₂₀ is CONH₂

25 X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

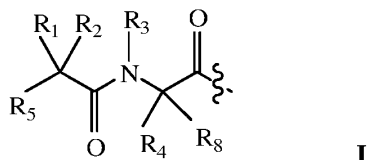
X₁₃ is (aMe)Leu;

30 X₁₇ is an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been pegylated, optionally via a first spacer, with a straight chain or branched polyethylene glycol chain, said polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;;

X₁₈ is Ala or Tyr and

X₂₀ is Aib, further wherein

a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide comprising the structure:



wherein

5 R_1 comprises a (C1-C4 alkyl)NH₂ side chain that has been pegylated, optionally via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R_2 is H, or C₁-C₄ alkyl;

10 R_4 is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R_8 is H, D, or C₁-C₄ alkyl;

R_3 is CD₃, C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

15 R_5 is NH₂, wherein said first and second spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1,

optionally wherein

20 I) R_1 comprises a (C1-C4 alkyl)NH₂ side chain that has been pegylated, optionally via a first spacer, with a straight chain or branched polyethylene chain having a combined molecular weight ranging from 20k to 40k;

R_2 , R_4 and R_8 are each H;

R_3 is C₁-C₄ alkyl; and

25 R_5 is NH₂, wherein said first and second spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1; or

II) R_1 comprises a (C1-C4 alkyl)NH₂ side chain that has been pegylated, optionally via a second spacer, with a straight chain or branched polyethylene chain having a combined molecular weight ranging from 20k to 40k;

30 R_2 is H;

R_4 and R_8 are each D;

R₃ is CH₃ or CD₃; and

R₅ is NH₂, wherein said first and second spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2,

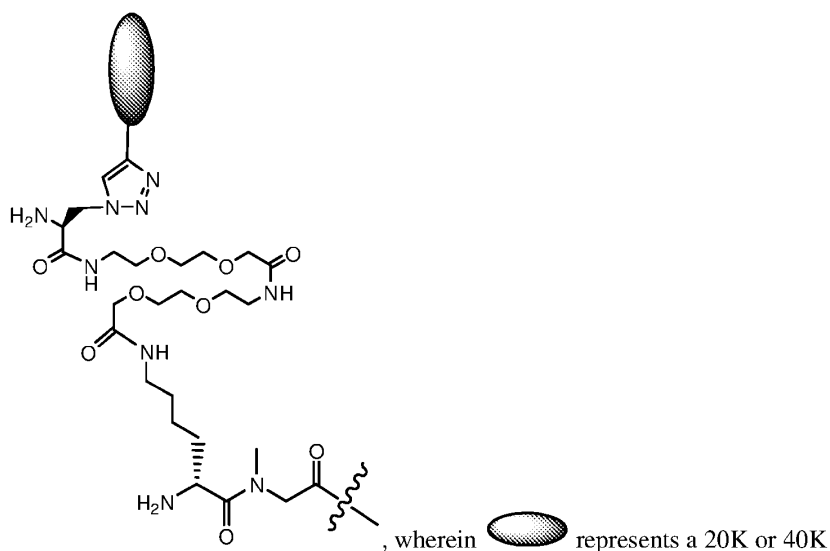
optionally wherein k is 2, or $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is

5 2, and q is 1 or 2, optionally wherein k is 2.

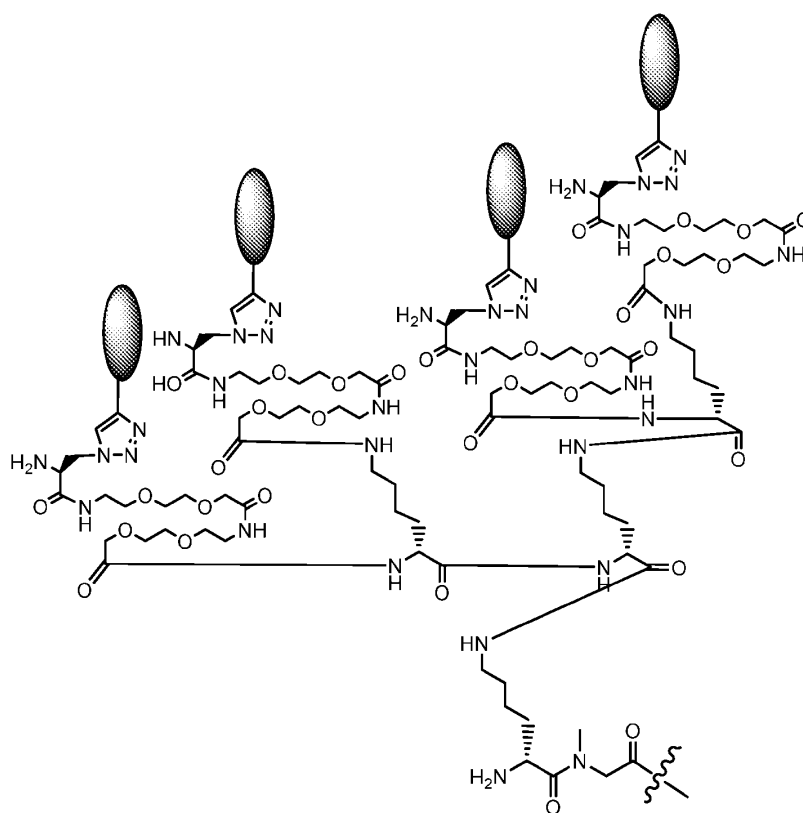
In accordance with embodiment 66, a peptide of embodiment 65 is provided wherein the first amino acid of said dipeptide is covalently linked to a branched polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a spacer comprising

10 $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2,
optionally wherein k is 2.

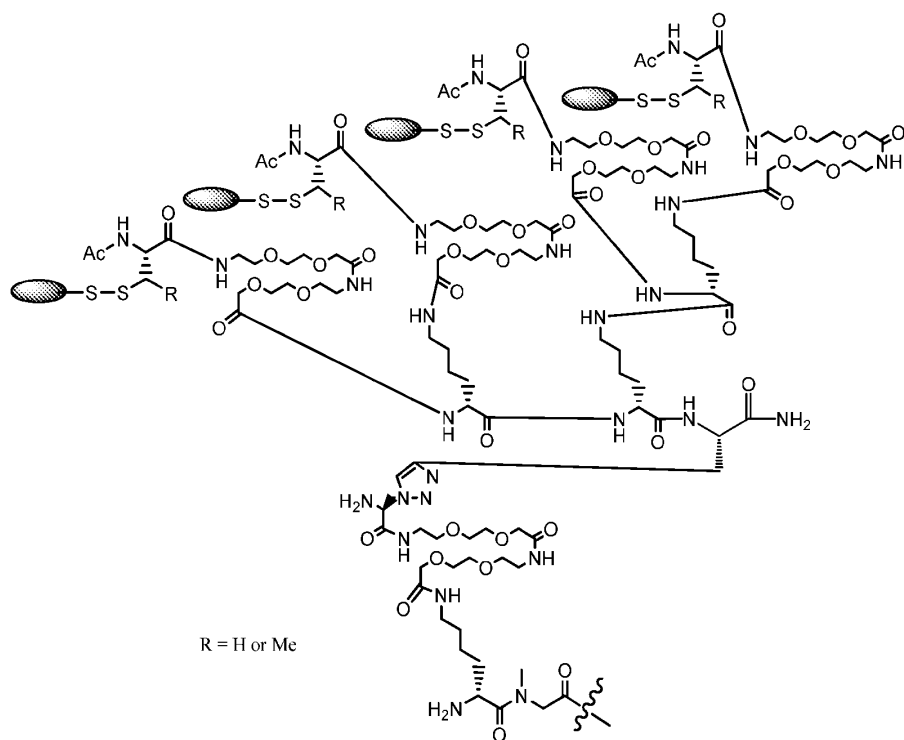
In accordance with embodiment 67, a peptide of embodiment 65 or 66 is provided wherein the first amino acid of the dipeptide comprises a structure selected from:



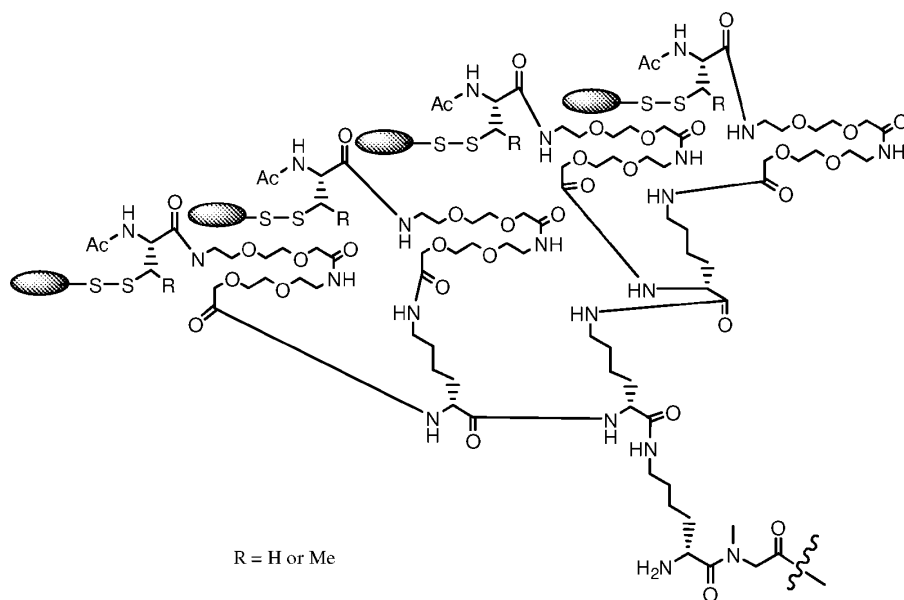
15 PEG,



wherein  represents a 5K or 10K PEG,

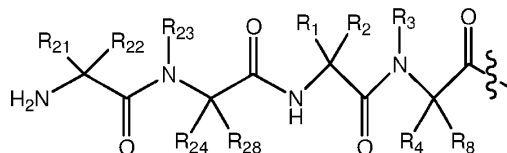


wherein  represents a 5K or 10K PEG and Ac = acyl group, or



wherein  represents a 5K or 10K PEG and Ac = acyl group.

In accordance with embodiment 68, the prodrug derivative of any one of the peptides of embodiments 39 to 67 is provided wherein any one or more of the P1, P7 or P16 dimers comprises a sequential dipeptide structure of



5

covalently linked via an amide bond to said peptide,
wherein

R₁ comprises (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid,
a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R₂₁ is (C₁-C₄ alkyl)NH₂ or comprises (C₁-C₄ alkyl)NH₂ covalently linked
to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid,
optionally via a DD2 spacer;

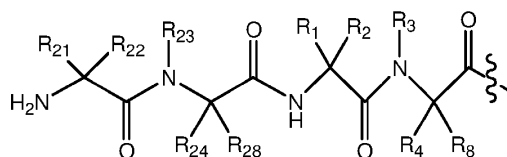
R₂, and R₂₂, are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D or C₁-C₄ alkyl;

R₃ is C₁-C₆ alkyl, CD₃ or R₃ and R₄ together with the atoms to which they
are attached form a 3,4-dehydropyrrolidine, or a substituted or unsubstituted pyrrolidine,
hydroxypyridine or piperidine ring; and

R₂₃ is C₁-C₆ alkyl, CD₃ or R₂₃ and R₂₄ together with the atoms to which
they are attached form a 3,4-dehydropyrrolidine, or a substituted or unsubstituted
pyrrolidine, hydroxypyridine or piperidine ring, wherein said DD1 and DD2 spacers are
independently selected from the group consisting of a gamma glutamic acid, a gamma
glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-
[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer
selected from the range of 2-4 and q and p are independently an integer selected from the
range of 0-4.

In accordance with embodiment 69, the prodrug derivative of the peptide of
embodiment 68 is provided wherein said sequential dipeptide comprises the structure:



wherein

R₁ comprises (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

5 R₂₁ is C₁-C₄ alkyl)NH₂ or comprises (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

R₂, and R₂₂ are each H;

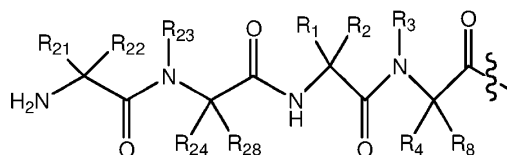
R₄, R₈, R₂₄ and R₂₈ are independently H or D;

10 R₃ and R₂₃ are independently C₁-C₆ alkyl or CD₃; and

wherein said DD1 and DD2 spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 70, the prodrug derivative of any one of the peptides of embodiments 68-69 is provided wherein said sequential dipeptide comprises the structure:



linked to the N-terminal alpha amine of said peptide wherein

20 R₁ is comprises (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R₂₁ is C₁-C₄ alkyl)NH₂ or comprises (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

R₂, and R₂₂ are each H;

25 R₄, R₈, R₂₄ and R₂₈ are each independently H or D;

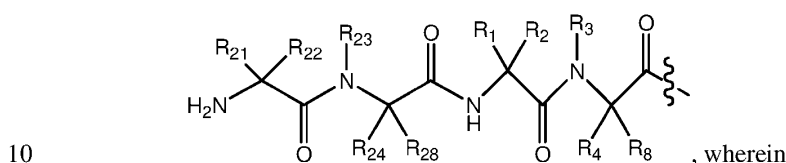
R₃ and R₂₃ are each independently CH₃ or CD₃; and

said DD1 spacer and DD2 spacer are independently - [COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 0, 1 or 2 and p is

0, 1 or 2, optionally wherein k is 2 and q is 1 and p is 1, optionally wherein R₄, R₈, R₂₄ and R₂₈ are each H and R₃ and R₂₃ are each CH₃.

In accordance with embodiment 71, the prodrug derivative of any one of the peptides of embodiments 68-78 is provided wherein said DD1 spacer and DD2 spacer are each -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid), and the acyl group is a C18-C20 diacid.

In accordance with embodiment 72 the prodrug derivative of any one of embodiments 39-71 is provided wherein any one or more of the P1, P7 or P16 dimers comprises a sequential dipeptide structure (A-B-C-D) of



R₁ comprises a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

15 R₂₁ comprises (C₁-C₄ alkyl)NH₂ or a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

20 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said second and third spacers are independently

25 -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

In accordance with embodiment 73 the prodrug derivative of embodiment 72 is provided wherein R₂₁ comprises a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, 30 wherein the pegylation at R₂ and R₂₁ is the same or is different.

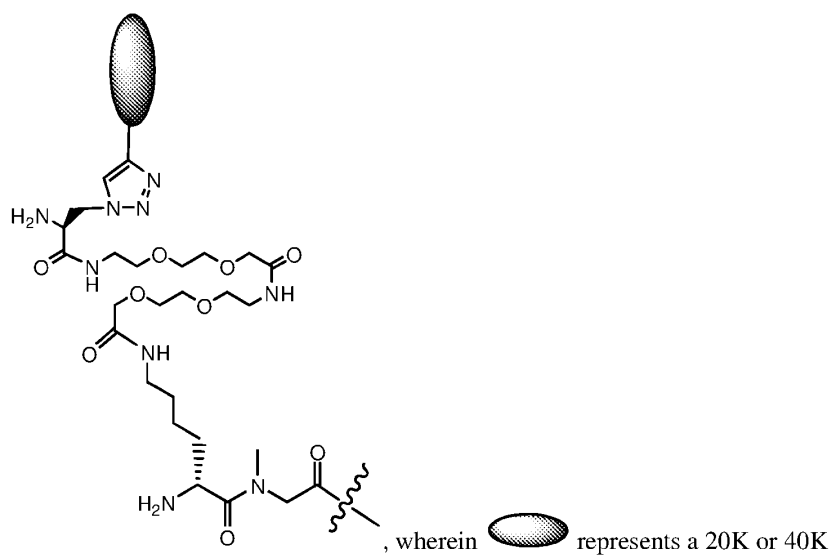
In accordance with embodiment 74 the prodrug derivative of embodiment 72 or 73 is provided wherein said sequential dipeptide structure (A-B-C-D) is covalently linked to

- 5 i) the amino acid at position 1, relative to SEQ ID NO: 1, optionally linked to the N-terminal alpha amine;
- ii) the amino acid at position 7, relative to SEQ ID NO: 1;
- iii) the amino acid at position 16, relative to SEQ ID NO: 1; or
- iv) the amino acid at position 1 and 7, relative to SEQ ID NO: 1.

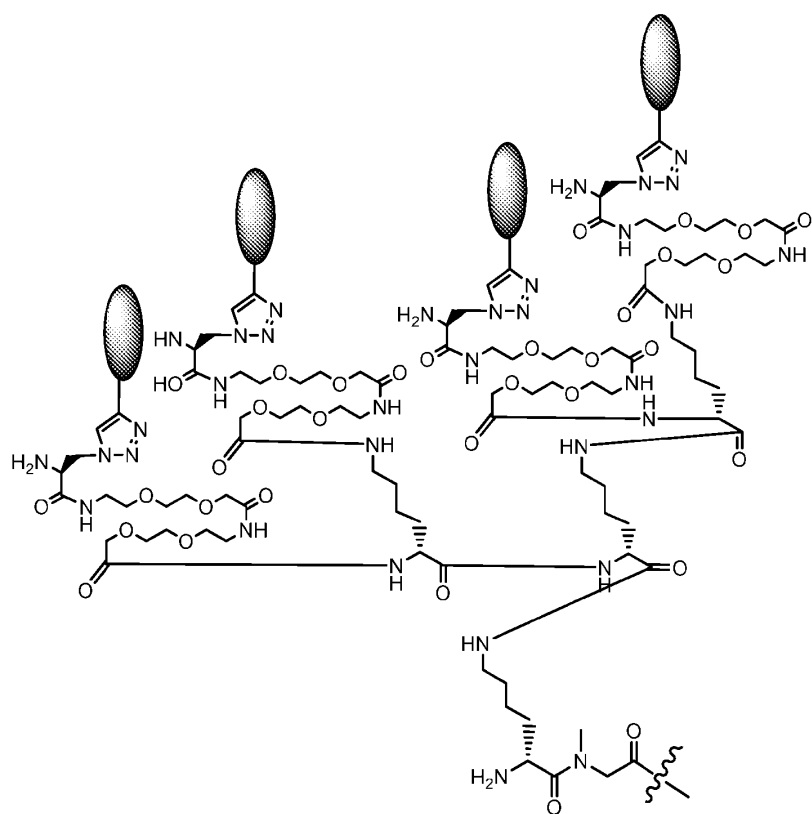
10 In accordance with embodiment 75 the prodrug derivative of any one of embodiments 68-74 is provided wherein R₁ and optionally R₂₁ comprises a branched PEG chain construct comprising

- i) two PEG branches of 10K each
- ii) two PEG branches of 20K each;
- 15 iii) four PEG branches of 5K each; or
- iv) four PEG branches of 10K each.

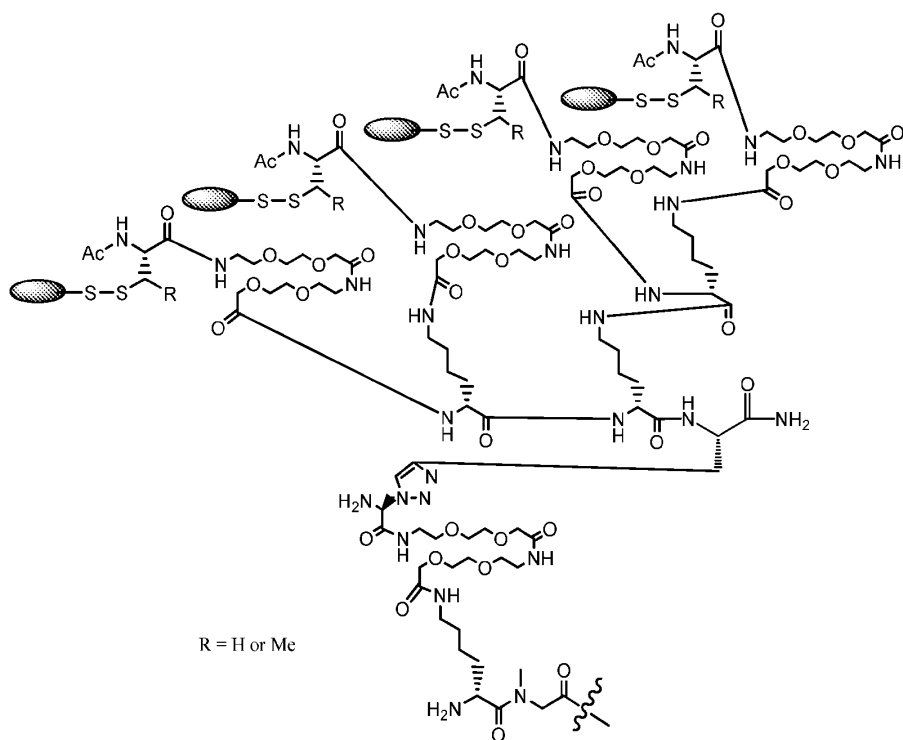
In accordance with embodiment 76 the prodrug isoacyl peptide derivative of any one of embodiments 68-75 is provided wherein the first amino acid (A) and optionally the
20 third amino acid (C) of said sequential dipeptide element (A-B-C-D) comprises a structure selected from the group consisting of:



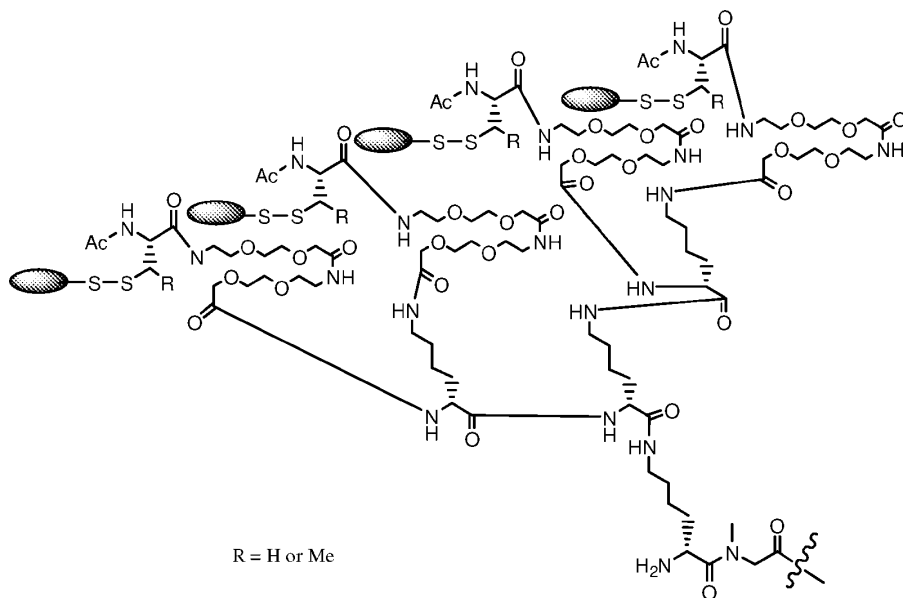
PEG,



wherein  represents a 5K or 10K PEG,



wherein  represents a 5K or 10K PEG and Ac = acyl group and



wherein  represents a 5K or 10K PEG and Ac = acyl group.

In accordance with embodiment 77 the prodrug derivative of any one of
embodiments 39-76 is provided wherein the first amino acid of said P1, P7, or P16
dipeptide (A-B) or sequential dipeptide (A-B-C-D) is an amino acid in the D-stereoisomer
configuration, optionally wherein the amino acid is dLys, optionally wherein the third
5 amino acid of said sequential dipeptide (A-B-C-D) is an amino acid in the D-stereoisomer
configuration, optionally wherein the amino acid is dLys.

In accordance with embodiment 78, a pharmaceutical composition is provided
comprising the peptide of any one of claims 1 to 77 and a pharmaceutically acceptable
10 carrier.

In accordance with embodiment 79 a kit comprising a pharmaceutical composition
of embodiment 68 and a device for administering said pharmaceutical composition to the
patient is provided.

In accordance with embodiment 80, the kit of embodiment 79 is provided,
15 wherein the device comprises a syringe comprising the pharmaceutical composition.

In accordance with embodiment 81, a method of reducing weight gain or inducing
weight loss is provided, comprising administering to a patient in need thereof a
pharmaceutical composition of embodiment 78 in an effective amount to reduce weight
gain or induce weight loss.

20 In accordance with embodiment 82, a method of treating diabetes is provided,
comprising administering to a patient in need thereof a pharmaceutical composition of
embodiment 78 in an amount effective to lower blood glucose levels.

EXAMPLES

25 **Synthesis of a Tri-agonists:**

MBX 4301 – a representative triagonist drug candidate

The incretin peptide analogs were assembled on a 0.1 mmol Chem matrix rink
amide resin using an ABI-433A peptide synthesizer and Fmoc/Oxyma/DIC coupling
protocols. Fmoc-Lys(Mtt)-OH and Fmoc-Alpha-Me-Leu-OH were coupled manually,
30 respectively at amino acid residue 17 and amino acid residue 13. Boc-Tyr(tBu)-OH was
used at the first amino acid position. The Mtt side chain of Lys¹⁷ was deprotected and the
resultant free amine was used for additional side-chain extension. This amine was
coupled sequentially with two repeat additions of Fmoc-NH-PEG2-CH₂COOH and
Fmoc-Glu-OtBu, followed by (tert-Butoxy)-20-oxoicosanoic acid. The peptide was

chemically removed from the synthetic resin by treatment with a TFA solution containing 5% TIS, 3% H₂O at room temperature with gentle agitation for 2 hours. The resin was removed by filtration, and the peptide precipitated by addition of cold ether (50 ml). The peptide precipitate was collected by centrifugation and washed with cold ether (3 x 50 ml).

5 The crude peptide mixture was subjected to purification by preparative reverse-phase HPLC column (Kinetex® 5 µm C8 100 Å LC Column 250 X 21.2 mm, 25-60 % aqueous ACN (0.1% TFA), at a flow rate of 15 mL/min). The pure peptide was assessed by analytical LCMS and pooled fractions were lyophilized to provide the final product as a white fluffy solid.

10

MBX 4083 – N terminal prodrug

Similar to synthesis of MBX4062, Fmoc-Tyr(tBu)-OH, Fmoc-Sar-OH and Boc-dLys(Fmoc)-OH were coupled successively to N-terminal of Aib². After deprotection of Mtt at side chain of K²⁰ and Fmoc at side chain of dK⁻¹, two identical fatty acid linkers
15 were introduced as previously described in synthesis of MBX 4062.

MBX 4071 – Thr⁷ isoacyl prodrug

Fmoc-Thr-OH was used for Thr⁷. Fmoc-Sar-OH and Boc-dK(Fmoc)-OH were added. Two fatty acid linkers were simultaneously coupled after deprotection of Mtt
20 group and Fmoc group. Fmoc-aMePhe was coupled to the free hydroxyl group of T7 with DIC/DMAP. Further extension of peptide chain as was reported in preparation of MBX 4062 completed the synthesis.

MBX 4206 – Lys¹⁶ prodrug

25 The amino acid building block Fmoc-Lys(Alloc)-OH was utilized for Lys¹⁶. After completion of the peptide backbone assembly the Alloc group was removed in the presence of Pd(PPh₃)₄/Ph₃SiH. Deprotection of side chain of Lys²⁰ afforded two free amino groups, which were ready for fatty acid modification. Two identical fatty acids with linkers were introduced as previously described in synthesis of MBX 4062.

30

MBX 4225 – a double prodrug

Same as employed in synthesis of MBX4206, Fmoc-Lys(Alloc)-OH was used for Lys¹⁶. After deprotection of N-terminal of Tyr¹ and side chain of Lys¹⁶, Fmoc-Sar-OH and Boc-dK(Fmoc)-OH were introduced. HFIP/DCM followed by piperidine/DMF

provided three free amine groups. Three identical fatty acids with linkers were introduced as previously described in synthesis of MBX 4062.

MBX 4291 – a sequential prodrug

5 Boc-dK(Fmoc)-OH, Fmoc-dLys(Mtt)-OH, and Fmoc-Lys(Mtt)-OH were used for dLys⁻³, dLys⁻¹ and Lys²⁰. HFIP/DCM Treatment followed by piperidine/DMF treatment provided three free amine groups, to which three identical fatty acids with linkers were introduced as previously described in synthesis of MBX 4062.

MBX 4359 – phosphonic fatty acid

10 Similar to synthesis of MBX4301, 19-(Bis(benzyloxy)phosphoryl)nonadecanoic acid was used instead of 20-(tert-Butoxy)-20-oxoicosanoic acid. The cleavage was carried out in a TFA solution containing 5% 12 N HCl, 4 % TIS, and 1% anisole at room temperature with gentle agitation for 3 hours.

15 **Assessment of cleavage half-life**

Peptide prodrugs were dissolved in PBS buffer and adjusted to obtain a pH of 7.4. The resulting solution was incubated at 37 °C. Aliquots were taken at designed time points and analyzed by LC-MS. The analysis was performed using an Agilent 1260 Infinity instrument with Phenomenex Kinetex C8 2.6μ 100A (75×4.6 mm) column. Flow rate of 1mL/min and a gradient of 10% - 80% acetonitrile in water, with 0.1% trifluoroacetic acid over 10 min. Data was collected using absorption at 214 nm. Positive mode MS data were obtained with an Agilent 6120 Quadrupole LC/MS. The concentration of prodrug and drug were determined by their relative peak areas. The first order dissociation rate constant of the prodrug was determined by plotting the logarithm of the concentration of the prodrug at various time points. The slope of this plot provides the rate constant 'k'. The half-life of the cleavage was calculated based upon the formula $t_{1/2} = 0.693/k$.

Luciferase-Based Reporter Assay for GLP-1, Glucagon and GIP cAMP

30 **Agonism**

The ability of each peptide analog or prodrug to induce cAMP was measured in a firefly luciferase-based reporter assay. The cAMP production that is induced is directly proportional to the peptide binding and activation of the specific single overexpressed receptor. HEK293 cells co-transfected with the human or mouse GLP-1, GIP or glucagon

receptors and a luciferase gene linked to a cAMP inducible responsive element were employed for the bioassay.

The cells were serum-deprived by culturing 16 hours in Dulbecco Minimum Essential Medium (Gibco, Life Technologies, Grand Island, NY) supplemented with 0.3% FetalClone III (HyClone, Logan, UT) and then incubated with serial dilutions of the peptide analogs or prodrugs for 5 hours at 37° C., 5% CO₂ in 96-well “Costar 3610” Assay plates (Corning, Kennebunk, ME). At the end of the incubation, 50 µL of Steady-Lite Plus luminescence substrate reagent (PerkinElmer, Waltham, MA) were added to each well.

The plate was shaken briefly at 600 rpm, incubated for four minutes and light output was measured on an EnSpire Alpha Multi-mode Plate Reader (PerkinElmer, Waltham, MA). The effective 50% concentrations (EC₅₀) were calculated using Origin 2019b software (OriginLab, Northampton, MA), and the effective concentration 50 (EC₅₀) was determined by sigmoidal fitting. Potency was determined by comparative analysis of relative EC₅₀ values. Each experiment was repeated at least three times with each sample assayed in duplicate.

***In Vivo* Mouse Pharmacology**

Peptide-based drug and prodrug candidates were tested for their *in vivo* effects in diet induced obese mice (DIO strain C57B16). Groups of eight mice with initial body weight that varied at start of treatment in the 55-65g range were subcutaneously injected with vehicle or test peptides at a specified dose that varied between 1-30 nmol/kg. The mice varied in age from 5 to 12 months and had been on a high fat diet for approximately two months prior to treatment. Body weights and food consumption were typically measured each morning and no less frequently than every other day in repeat dose experimental protocols.

C57Bl/6 mice were obtained from Jackson Laboratories. The mice were single- or group-housed as was approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Cincinnati on a 12:12 h light-dark cycle at 22 °C with free access to food and water.

***In Vivo* Rat Pharmacology**

Peptide-based drug and prodrug candidates were tested for their *in vivo* effects in diet induced obese rats (DIO Sprague Dawley strain). Groups of six rats with initial body

weight that varied at start of treatment in the 600-700g range were subcutaneously injected with vehicle or test peptides at a specified dose that varied between 3-30 nmol/kg. The mice varied in age from 6 to 12 months and had been on a high fat diet for approximately two months prior to treatment. Body weights and food consumption were typically measured each morning and no less frequently than every other day in repeat dose experimental protocols.

The SD rats were obtained from Harlan Laboratories. They were singly housed as approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Cincinnati on a 12:12 h light-dark cycle at 22 °C with free access to food and water.

Assessment of Pharmacokinetics: Measuring Plasma Peptide Concentration by LCMS

Following peptide administration blood was collected on EDTA-coated tubes from 4 mice, 4 rats or two cynomolgus monkeys at specified times. The injected dose volume for mice was 8ml/kg, for rats 0.8ml/kg and for monkeys was 0.3 mL/kg. Individual dose volumes were calculated based on the animals' most recently recorded body weight. Blood samples were maintained at $5 \pm 3^{\circ}\text{C}$ (wet ice) and centrifuged at $5 \pm 3^{\circ}\text{C}$ within 1 hour of the collection of each blood sample. The resultant plasma was transferred to a tube and then stored under conditions set to maintain $-75 \pm 15^{\circ}\text{C}$ until analysis.

Standard curve samples were prepared by serial dilution with species specific plasma on the day of analysis. Aliquots (40 μl) of standard curve and study samples were transferred to a 96-well plate and mixed with 160 μl of Methanol:Acetonitrile (ACN) (1:1, v/v) internal standard solution. After 10 minutes of centrifugation, supernatants were diluted 2-fold with acidified (0.1% formic acid) ACN:Water (3:1, v/v) and analyzed by LC-MS/MS.

A Shimadzu CBM-20A Nexera UPLC system and a CTC PAL autosampler comprised the front end of the LC-MS/MS system. Chromatography was based on an Accurcore C8 Column, 2.6 μm , 2.1 mm X 30 mm (Thermo 17226-032130) and a binary gradient program of 0.1% formic acid (aq) and 0.1% formic acid in ACN. Mobile phase solvent A consisted of micro filtered water:formic acid (1000:1 v/v), and solvent B consisted of ACN:formic acid (1000:1). The flow rate was 0.8 ml/min., the column temperature was ambient, and the injection volume was 5 μl . The two needles rinses

were ACN:Water (25:75, v/v) and ACN:Isopropanol:Acetone in 0.1% formic acid (5:4:1, v/v/v). The gradient cycle started at 15% B (concentration) with a linear increase to 65% B in 0.75 minute. The column was cleaned with 98% B for 0.25 min. and returned to initial %B during acquisition time of 1.20 minute. The total cycle time for each injection, 5 including re-equilibration of initial % B, was approximately 3 minutes. The first 0.3 min. of each run was diverted to waste.

Mass spectrometric data were generated using Analyst software controlling a Sciex API 6500 plus triple-quadrupole mass spectrometer (Model 5060743-J) in positive ionization mode.

10

What is claimed is:

1. A peptide having Gcg/GLP-1/GIP tri-agonist activity, said peptide comprising the sequence of

5 $X_1X_2X_3\text{GTX}_6X_7\text{SDYSIX}_{13}\text{LX}_{15}X_{16}X_{17}X_{18}\text{QX}_{20}\text{AFVQWLLEGGPSSGAPPPS}-$
 R_{20} (SEQ ID NO: 1);

$X_1X_2X_3\text{GTX}_6\text{TSDYSIX}_{13}\text{LX}_{15}X_{16}X_{17}X_{18}\text{QX}_{20}\text{AFX}_{23}X_{24}X_{25}\text{LLEGGPSSGAPPPS}$
 $-\text{R}_{20}$ (SEQ ID NO: 4),

10 $X_1X_2X_3\text{GTFTSDYSIX}_{13}\text{LX}_{15}X_{16}X_{17}X_{18}\text{QX}_{20}\text{AFVQWLLEGGPSSGAPPPS}-\text{R}_{20}$
 (SEQ ID NO: 2)

 wherein

X_1 is Tyr or His;

X_2 is an α,α -disubstituted amino acid, optionally Aib;

X_3 is Gln or His;

15 X_6 is Phe or alpha methylated Phe;

X_7 is Thr or (α -Me)Thr;

X_{13} is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe or Aib;

X_{15} is Glu or Asp;

X_{16} is Aib, Orn or Lys;

20 X_{17} is an amino acid comprising a (C1-C8 alkyl) NH_2 side chain that has
 been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17
 spacer;

X_{18} is Ala or Tyr;

X_{20} is an α,α -disubstituted amino acid, optionally Aib; and

25 R_{20} is COOH or CONH₂, wherein said P17 spacer is selected from the
 group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid
 dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic
 acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are
 independently an integer selected from the range of 0-4.

30
2. The peptide of claim 1, wherein

X_2 and X_{20} are both Aib;

X_{13} is (aMe)Leu;

X_{15} is Asp;

X₁₆ is Lys; and

X₁₇ is an acylated Lys or dLys, wherein side chain of the Lys or dLys is covalently linked to a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via said P17 spacer.

5

3. The peptide of claim 2, wherein said peptide comprises the sequence of SEQ ID NO: 2 and said P17 spacer comprises the structure:

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH]}_q\text{-(gamma glutamic acid)}_p\text{-}$; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4.

10

4. The peptide of claim 3, wherein X₁₇ is Lys having a C16 to C18 fatty acid, a C16-C20 phosphonic fatty acid, or a C16 to C20 diacid linked to the lysine side chain via a P17 spacer comprising the structure:

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH]}_q\text{-(gamma glutamic acid)}_p\text{-}$

wherein k is 2, and p and q are independently an integer selected from 1 or 2,

15 optionally wherein k and q are each 2 and p is 1.

5. The peptide of claim 4, wherein X₁₇ is Lys, wherein the lysine side chain is linked via an amide bond to $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$ or $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$.

6. The peptide of any one of claims 1 to 5, wherein R₂₀ is CONH₂.

20

7. The peptide of any one of 1 to 6 wherein the peptide comprises the sequence:

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 3)

wherein

25

I)

X₁ is Tyr;

X₂ and X₂₀ are both Aib;

X₃ is Gln;

X₁₃ is (aMe)Leu;

X₁₇ is Lys, wherein the lysine side chain is linked via an amide bond to -
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid or -
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic fatty acid,
 wherein k is 2, and p and q are independently an integer selected from 1 or 2;

5 X₁₈ is Ala; and
 R₂₀ is CONH₂, or

II)

X₁ is His;
 X₂ and X₂₀ are both Aib;
 10 X₃ is His;
 X₁₃ is (aMe)Leu;
 X₁₇ is Lys, wherein the lysine side chain is linked via an amide bond to -
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid or -
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic fatty acid,
 15 wherein k is 2, and p and q are independently an integer selected from 1 or 2;

X₁₈ is Tyr; and
 R₂₀ is CONH₂, or

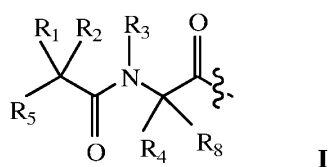
III)

X₁ is Tyr;
 20 X₂ and X₂₀ are both Aib;
 X₃ is Gln;
 X₁₃ is (aMe)Leu;
 X₁₇ is Lys, wherein the lysine side chain is linked via an amide bond to -
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid or -
 25 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic fatty acid,
 wherein k is 2, and p and q are independently an integer selected from 1 or 2;
 X₁₈ is Tyr; and
 R₂₀ is CONH₂.

30 8. The peptide of any one of claims 1-7 wherein
 X₁₇ is an acylated Lys wherein the lysine side chain is linked via an amide
 bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H.

9. A prodrug derivative of the peptide of any one of claims 1-8 wherein said peptide is modified by the covalent linkage of one or more dipeptides (A-B) to an amine of said peptide, wherein A is an amino acid and B is an N-alkylated amino acid linked to said peptide through an amide bond between a carboxyl moiety of B and an amine of the peptide, wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid.

10. The prodrug derivative of claim 9 wherein one or more dipeptides are covalently linked via an amide bond to a primary amine of an amino acid selected from the Lys at position 16, the amine of isoacyl-Thr at position 7 of the peptide, and the N-terminal alpha amine, wherein said dipeptides comprise the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a spacer;

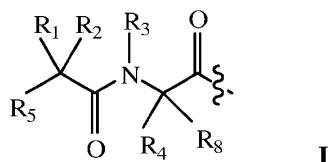
R₂ is H, or C₁-C₄ alkyl;

R₄ and R₈ are independently H, D, C₁-C₄ alkyl or -(CH₂)_nCH₂OH, wherein n is an integer selected from 0-2;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein z is 0, k and q are both is 2 and p is 2.

11. The prodrug derivative of the peptide of claim 9 or 10 wherein a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide comprising the structure:



5 wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R₂, is H or C₁-C₄ alkyl;

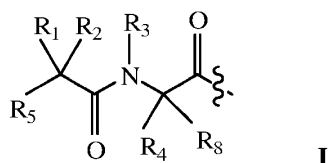
10 R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₈ is H, D or C₁-C₄ alkyl;

15 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

20 R₅ is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

12. The prodrug derivative of the peptide of claim 11 wherein said P1 dipeptide comprises the structure:



25 wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

R₂ is H;

R₄ and R₈ are each H or D;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein said P1 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-
 5 (gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

13. The prodrug derivative of the peptide of claim 12 wherein said P1 spacer
 is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-
 10 C20 diacid or a C18-C20 phosphonic fatty acid.

14. The prodrug derivative of the peptide of claim 11 wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 diacid or a
 C18-C20 phosphonic fatty acid covalently linked to said side chain, via a -

15 [COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid).spacer;

R₂ is H;

R₄ and R₈ are each D;

R₃ is CH₃ or CD₃; and

R₅ is NH₂.

20

15. A prodrug derivative of a peptide having Gcg/GLP-1/GIP tri-agonist
 activity, said peptide comprising the sequence of

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
 (SEQ ID NO: 3)

25 wherein

X₁ is Tyr or His;

X₂ is Aib;

X₃ is Gln or His;

X₁₃ is (aMe)Leu;

30

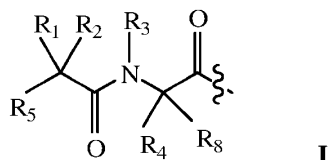
X₁₇ is an amino acid comprising a (C1-C8 alkyl)NH-
 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid side chain or a (C1-
 C8 alkyl)NH-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic
 fatty acid, wherein k is 2, and p and q are independently an integer selected from 1 or 2;

X₁₈ is Ala or Tyr and

X_{20} is Aib,

R_{20} is COOH or CONH₂, further wherein

a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

R_2 is H;

R_4 and R_8 are independently H or D;

R_3 is CD₃, or C₁-C₆ alkyl; and

R_5 is NH₂, wherein said P1 spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally with the proviso that when R_3 is C₁-C₆ alkyl, both R_4 and R_8 are D.

16. The prodrug derivative of the peptide of claim 15 wherein said P1 spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid or a C18-C20 phosphonic fatty acid.

17. The prodrug derivative of the peptide of any one of claims 9-21 wherein the first amino acid of said P1, P7 or P16 dipeptide (A-B) is an amino acid in the D-configuration.

18. The prodrug derivative of the peptide of claim 15

X₁X₂X₃GTFTSDYSIX₁₃LDKX₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3)

wherein

X_1 is Tyr;

X_2 is Aib;

X_3 is Gln;

5 X_{13} is (aMe)Leu;

X_{17} is an amino acid comprising a (C1-C8 alkyl)NH-

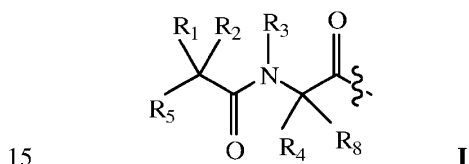
[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid side chain or a (C1-C8 alkyl)NH-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic fatty acid, wherein k is 2, and p and q are independently an integer selected from 1 or 2;

10 X_{18} is Ala or Tyr and

X_{20} is Aib,

R_{20} is CONH₂; and further wherein

a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha amine of said peptide, said P1 dipeptide comprising the structure:



wherein

R_1 comprises comprising a (C1-C8 alkyl)NH-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 diacid side chain or a (C1-C8 alkyl)NH-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-C16-C20 phosphonic fatty acid,

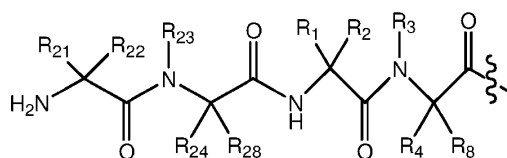
20 wherein k is 2, and p and q are independently an integer selected from 1 or 2;

R_2 , R_4 and R_8 are each H;

R_3 is CH₃; and

R_5 is NH₂,

25 19. The prodrug derivative of the peptide of any one of claims 9-18 wherein any one or more of the P1, P7 or P16 dipeptides comprises a sequential dipeptide structure of



covalently linked via an amide bond to said peptide,

wherein

R_1 comprises $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid,
5 a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} is $(C_1-C_4 \text{ alkyl})NH_2$ or $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

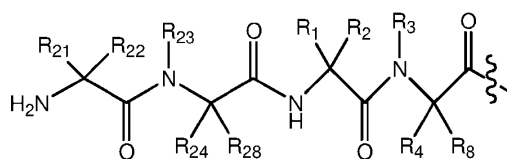
R_2 , and R_{22} , are independently H, or C₁-C₄ alkyl;

10 R_4 , R_8 , R_{24} and R_{28} are independently H, D or C₁-C₄ alkyl;

R_3 is C₁-C₆ alkyl, CD₃ or R_3 and R_4 together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R_{23} is C₁-C₆ alkyl, CD₃ or R_{23} and R_{24} together with the atoms to which
15 they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said DD1 and DD2 spacers are independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are
20 independently an integer selected from the range of 0-4.

20. The prodrug derivative of the peptide of claim 19 wherein said sequential dipeptide comprises the structure:



25 wherein

R_1 comprises $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} comprises $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

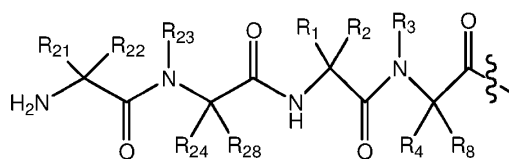
30 R_2 , and R_{22} are each H;

R_4 , R_8 , R_{24} and R_{28} are independently H or D;

R_3 and R_{23} are independently C_1 - C_6 alkyl or CD_3 ; and
 wherein said DD1 and DD2 spacers are independently
 $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein k is 2, q is 1 or 2
 and p is 0 or 1.

5

21. The prodrug derivative of the peptide of claim 19 wherein said sequential dipeptide comprises the structure:



linked to the N-terminal alpha amine of said peptide wherein

10

R_1 comprises $(C_1$ - C_4 alkyl) NH_2 covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} comprises $(C_1$ - C_4 alkyl) NH_2 covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

R_2 , and R_{22} are each H;

15

R_4 , R_8 , R_{24} and R_{28} are D;

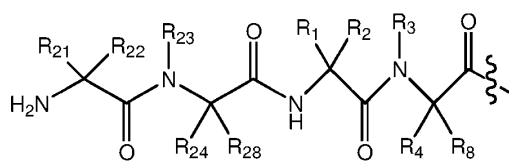
R_3 and R_{23} are each independently CH_3 or CD_3 ; and

said DD1 spacer and DD2 spacer are independently -

$[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein k is 2, q is 0, 1 or 2 and p is 0, 1 or 2, optionally wherein k is 2 and q is 1 and p is 1.

20

22. The prodrug derivative of the peptide of claim 2194 wherein said sequential dipeptide comprises the structure:



linked to the N-terminal alpha amine of said peptide wherein

R_1 comprises $(C_1$ - C_4 alkyl) NH_2 covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

25

R_{21} comprises $(C_1$ - C_4 alkyl) NH_2 covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

R_2 , R_4 , R_8 , R_{22} , R_{24} and R_{28} are each H;

R₃ and R₂₃ are independently C₁-C₆ alkyl, optionally R₃ and R₂₃ are each CH₃; and

said DD1 spacer and DD2 spacer are independently -
[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 0, 1 or 2 and p is
5 0, 1 or 2, optionally wherein k is 2 and q is 1 and p is 1, optionally wherein R₃ and R₂₃ are
each CH₃.

23. The prodrug derivative of the peptide of any one of claims 19 to 22
wherein said DD1 spacer and DD2 spacer are each -[COCH₂(OCH₂CH₂)₂-NH]-(gamma
10 glutamic acid)-, and the acyl group is a C₁₈-C₂₀ diacid.

24. The prodrug derivative of the peptide of claim 22 wherein
R₁ comprises -(C₄ alkyl)NH-(COCH₂(OCH₂CH₂)₂NH)₂-γE-
COC₁₈H₃₆CO₂H or -(C₄ alkyl)NH- (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂;;
15 R₂₁ comprises -(C₄ alkyl)NH-(COCH₂(OCH₂CH₂)₂NH)₂-γE-
COC₁₈H₃₆CO₂H or -(C₄ alkyl)NH- (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂;;
R₂, R₄, R₈, R₂₂ R₂₄ and R₂₈ are each H;
R₃ and R₂₃ are each CH₃.

20 25. A prodrug derivative of a peptide having Gcg/GLP-1/GIP tri-agonist
activity, said prodrug derivative comprising the sequence of

X₁X₂X₃GTX₆TSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFX₂₃X₂₄X₂₅LLEGGPSSGAPPPS
-R₂₀ (SEQ ID NO: 4),

X₁X₂X₃GTFX₇SDYSIX₁₃LX₁₅X₁₆X₁₇AQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀
25 (SEQ ID NO: 5),

X₁X₂X₃GTFTSDYSIX₁₃LX₁₅X₁₆X₁₇X₁₈QX₂₀AFVQWLLEGGPSSGAPPPS-R₂₀
(SEQ ID NO: 2)

wherein

30 X₁ is Tyr or His;
X₂ is an α,α-disubstituted amino acid, optionally Aib;
X₃ is Gln or His;
X₆ is Phe;
X₇ is Thr or (α-Me)Thr;

X_{13} is Tyr, (aMe)Leu, (aMe)Ile, (aMe)Met, (aMe)nLeu, (aMe)Phe or Aib;

X_{15} is Glu or Asp;

X_{16} is Aib, Orn or Lys;

X_{17} is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has
 5 been acylated with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a P17
 spacer;

X_{18} is Ala or Tyr;

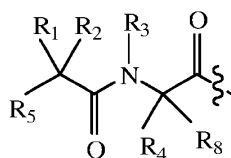
X_{20} is an α,α -disubstituted amino acid, optionally Aib; and

R_{20} is COOH or CONH₂, wherein said P17 spacer is selected from the
 10 group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic
 acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma
 glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and
 q and p are independently an integer selected from the range of 0-4 wherein

i) a dipeptide is covalently linked via an amide bond to the amine of
 15 isoacyl-Thr at position 7 of the peptide, or

ii) a dipeptide is covalently linked via an amide bond to the N-terminal
 alpha amine, or

iii) both i) and ii), wherein the dipeptide comprises the structure:



I

20 wherein

R_1 comprises a (C1-C4 alkyl)NH₂ side chain that has been pegylated,
 optionally via a second spacer, with a straight chain or branched polyethylene glycol
 chain having a molecular weight ranging from about 20k to about 40k;

R_2 is H, or C1-C4 alkyl;

25 R_4 is H, D, C1-C4 alkyl, C1-C4 alkenyl, C1-C4 alkenyl, -(CH₂)_nCH₂OH or
 (C1-C4 alkyl)phenyl, wherein n is an integer selected from 0-2;

R_8 is H, D, or C1-C4 alkyl;

R_3 is CD₃, C1-C6 alkyl, or R_3 and R_4 together with the atoms to which they
 are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a
 30 piperidine or a hydroxypiperidine ring; and

R_5 is NH₂, wherein said first and second spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1.

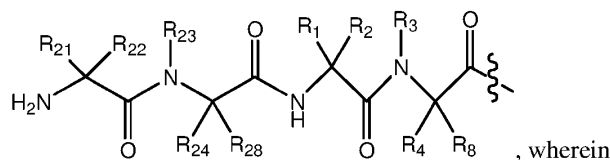
26. The prodrug derivative of claim 25 wherein said dipeptide is linked to the
5 N-terminal alpha amine of the peptide.

27. The prodrug derivative of any of claims 9-18 wherein the

i) the dipeptide linked to the amine of isoacyl-Thr at position 7 of the peptide, or

10 ii) the dipeptide linked to the N-terminal alpha amine, or

iii) the dipeptide of i) or ii), wherein the dipeptide is a sequential dipeptide “A-B-C-D” comprising the structure of



R₁ comprises a (C₄ alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂₁ comprises (C4 alkyl)NH₂ or a (C4 alkyl)NH₂ side chain that has been pegylated, via a third spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

20 R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

25 R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said second and third spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2,
optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is
2, and q is 1 or 2, optionally wherein k is 2.

28. A pharmaceutical composition comprising the peptide of any one of claims 1 to 27 and a pharmaceutically acceptable carrier.

29. A kit comprising a pharmaceutical composition of claim 28 and a device
5 for administering said pharmaceutical composition to the patient.

30. The kit of claim 29, wherein the device comprises a syringe comprising the pharmaceutical composition.

10 31. A method of reducing weight gain or inducing weight loss, comprising administering to a patient in need thereof a pharmaceutical composition of claim 28 in an amount effective to reduce weight gain or induce weight loss.

15 32. A method of treating diabetes, comprising administering to a patient in need thereof a pharmaceutical composition of claim 28 in an amount effective to lower blood glucose levels.