

GIP/GLP-1 CO-AGONISTS AND THEIR ASSOCIATED PRODRUGS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No.
5 63/618,011 filed on January 5, 2024, the disclosure of which is expressly incorporated herein.

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
10 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
15 peptide that corresponds to amino acids 72 through 108 of pre-proglucagon.

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
20 secretion, and inhibition of food intake. GLP-1 has been shown to reduce hyperglycemia
(elevated glucose levels) in diabetics. Exendin-4, a peptide from 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
25 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.

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,
30 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. Type 2 diabetes (T2D) patients have impaired incretin responses.

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
5 rapidly by the ubiquitous protease, DPP IV, and therefore, can only be used for short-term metabolic control.

Peptides that constitute balanced, full potency agonists at the GLP-1 and GIP receptors have demonstrated transformative pharmacology in the treatment of metabolic diseases, and most notably reduction in body weight. The most advanced medicine (tirzepatide) is formulated as
10 a subcutaneous 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.

There remains a need for peptides possessing an increased, sustained duration of pharmacological action to minimize frequency in human dosing. Additionally, GIP/GLP-1
15 co-agonists are desired that exhibit reduced peak to trough concentration between 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 GIP/GLP-1 co-agonists exhibiting such desired properties relative to existing medicines such as tirzepatide.

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SUMMARY

As disclosed herein, novel peptides exhibiting agonist activity at both the GLP-1 receptor and the GIP receptor are provided. Peptides having both GIP activity and GLP-1 activity are particularly advantageous for inducing weight loss or preventing weight gain, as
25 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 both the GIP receptor and the GLP-1 receptor.

Tirzepatide is a known GIP/GLP-1 co-agonist peptide that has been approved for
30 medical use in the United States for the treatment of type 2 diabetes and for weight loss. Surprisingly, applicant has discovered that the substitution of the phenylalanine residue at position 6 with an alpha methylated phenylalanine retained activity at the GLP-1 and GPI

receptors and had higher potency at those receptors relative to tirzepatide for both the human and mouse GLP-1 and GPI receptors.

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

5 YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀

(SEQ ID NO: 1) wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

X₇ is Thr or isoacyl-Thr;

10 X₁₃ is Aib;

X₁₅ is Glu or Asp;

X₁₆ is Lys or Aib;

X₁₇ is Thr or Ile;

X₁₉ is Ala or Gln;

15 X₂₀ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C18 fatty acid, a C16-C20 phosphonic fatty acid, a C16-C20 diacid or a C16-C20 alkyl group, optionally via a P20 spacer; and

R₂₀ is COOH or CONH₂, wherein said P20 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

20 integer selected from 1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2. In one embodiment X₁₅ is Glu; X₁₆ is Aib; X₁₇ is Thr; X₁₉ is Ala or Gln, and R₂₀ is CONH₂. In a further embodiment X₁₅ is Asp; X₁₆ is Lys; X₁₇ is Ile; X₁₉ is Gln, and R₂₀ is CONH₂. In an alternative embodiment X₁₅ is Glu; X₁₆ is Lys; X₁₇ is Thr; X₁₉ is Ala, and R₂₀ is CONH₂.

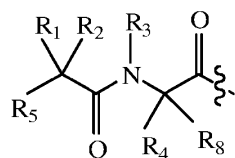
In accordance with one embodiment a prodrug derivative of the GIP/GLP-1 co-agonist peptide of SEQ ID NO: 1 is provided wherein the peptide is modified by the covalent linkage of one or more dipeptides "A-B" to an amine of said peptide, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated 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.

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, 7, 16 and 20, relative to SEQ ID NO: 1.

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 GIP/GLP-1 co-agonist peptide of SEQ ID NO: 1 comprises an isoacyl-Thr at position 7 and dipeptides (A-B) is covalently linked to a primary amine of an amino acid at position 1 and/or position 7 of the peptide of SEQ ID NO: 1, wherein said dipeptide 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 P7 spacer;

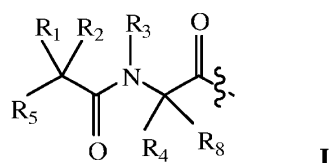
R₂, is H, or C₁-C₄ alkyl;

R₄ and R₈ are independently H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, 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

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 z is 0, k and q are both 2 and p is 1. In one embodiment, the 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 a dipeptides (A-B) via an amide bond to the 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 one embodiment the dipeptide comprises the structure:



wherein

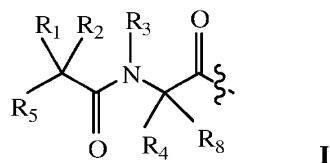
- R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via a spacer;

R_2 , R_4 and R_8 are each H;

R_3 is C_1 - C_6 alkyl; and

- R_5 is NH_2 , wherein the spacer 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.

In one embodiment the dipeptide comprises the structure:



wherein

- 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, optionally via a spacer;

R_2 is H;

R_4 and R_8 are independently D or H;

R_3 is CD_3 , or C_1 - C_6 alkyl; and

- R_5 is NH_2 , wherein the spacer 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 R_4 and R_8 are both D and R_3 is CD_3 .

- 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 a prodrug derivative of a GIP/GLP-1 co-agonist is provided wherein the peptide comprising the sequence of
 5 YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 2)

wherein

X₂ is Aib;

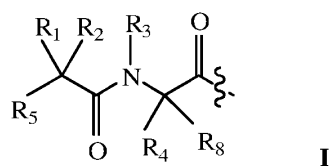
X₆ is alpha methylated Phe;

10 X₁₃ is Aib;

X₂₀ is an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been acylated with a C16-C20 acyl group, a C16-C20 phosphonic fatty acid, or a C16-C20 alkyl group, optionally via a first spacer; and

R₂₀ is COOH or CONH₂, wherein

15 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

20 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 second spacer;

R₂ is H, or C₁-C₄ alkyl;

R₄ 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;

25 R₈ is 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 pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂,

30 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 a second 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 first and second 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, optionally wherein k is 2 and q is 2 and p is 1; or

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 a second spacer;

R_2 is H;

R_4 and R_8 are each D;

R_3 is CD3; and

15 R_5 is NH_2 , wherein said first and second 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, optionally wherein k is 2 and q is 2 and p is 1.

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

20 $YX_2EGTX_6X_7SDYSIX_{13}LDKIAQX_{20}AFVQWLIAGGPSSGAPPPS-R_{20}$ (SEQ ID NO: 3)

wherein

X_2 is Aib;

X_6 is alpha methylated Phe;

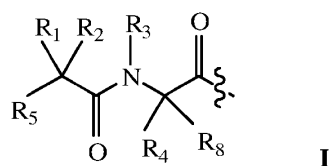
25 X_7 is isoacyl-Thr;

X_{13} is Aib;

X_{20} is an amino acid comprising a $(C_1$ - C_4 alkyl) NH_2 side chain that has been acylated with a C16-C20 acyl group, a C16-C20 phosphonic fatty acid, or a C16-C20 alkyl group, optionally via a first spacer; and

30 R_{20} is $COOH$ or $CONH_2$, wherein

a dipeptide is covalently linked via an amide bond to the amine of isoacyl-Thr at position 7 of the peptide, and optionally at the N-terminal alpha amine, wherein 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 second spacer;

R₂ is H, or C₁-C₄ alkyl;

R₄ 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;

R₈ is 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 pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ 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

I) 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 via a second spacer;

R₂, R₄ and R₈ are each H;

R₃ is C₁-C₄ alkyl; and

R₅ 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) 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 via a second spacer;

R₂ is H;

R₄ and R₈ are each D;

R₃ is CD₃; and

R₅ 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.

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

5 YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3)

wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

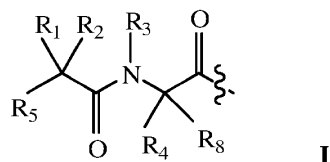
10 X₇ is isoacyl-Thr;

X₁₃ is Aib;

X₂₀ 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 having a molecular weight ranging from about 20k to about 40k; and

15 R₂₀ is COOH or CONH₂, 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:



20 wherein

R₁ 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;

R₂ is H, or C₁-C₄ alkyl;

25 R₄ 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;

R₈ is 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 pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a

30 hydroxypiperidine ring; and

R_5 is NH_2 , wherein said first and second 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, optionally wherein k is 2 and q is 2 and p is 1,

optionally wherein

- 5 I) R_1 comprises a (C1-C4 alkyl) NH_2 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

- 10 R_5 is NH_2 , wherein said first and second 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, optionally wherein k is 2 and q is 2 and p is 1; or

- II) R_1 comprises a (C1-C4 alkyl) NH_2 side chain that has been pegylated,
 optionally via a second spacer, with a straight chain or branched polyethylene chain having a
 15 combined molecular weight ranging from 20k to 40k;

R_2 is H;

R_4 and R_8 are each D;

R_3 is CD_3 ; and

- R_5 is NH_2 , wherein said first and second spacers are independently
 20 $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{alanine-triazole})$, wherein k is 2, and q is 1 or 2, optionally
 wherein k is 2, or $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{cysteine-S-S})$, wherein k is 2, and q is 1 or 2,
 optionally wherein k is 2.

- In accordance with one embodiment said dipeptide is present only at amino acid
 position 7 of SEQ ID NO: 3; in one embodiment the said dipeptide is located only at the N-
 25 terminal alpha amine; and in one embodiment the dipeptide is located at both the N-terminal
 alpha amine and at amino acid position 7 of SEQ ID NO: 3. In one embodiment the
 dipeptides of the GIP/GLP-1 co-agonist peptide, and optionally the amino acid at position 20,
 comprise a branched PEG chain having a molecular weight of 20K or 40K, wherein the
 branched PEG chain comprises 2, 3, 4 or 5 branches. In one embodiment the dipeptides of the
 30 GIP/GLP-1 co-agonist peptide comprise a branched PEG chain, and the amino acid at
 position 20 comprises a straight chain PEG, a C16-C30 fatty acid, a C16-C30 phosphonic
 fatty acid, or a C16-C30 diacid. In one embodiment the branched PEG chain construct
 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 optionally linked together via an amino acid extension of lysine residues. In one embodiment each of the branches of the branched polyethylene chains are covalently linked to the dipeptide(s) of the GIP/GLP-1 co-agonist peptide via a disulfide linkage, wherein the amino acid at position 20 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 20, of the GIP/GLP-1 co-agonist peptide via a disulfide linkage. The disulfide linkages enhance metabolism and clearance of the polyethylene glycol.

In accordance with one embodiment a GIP/GLP-1 co-agonist peptide of SEQ ID NO: 3 is provided wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

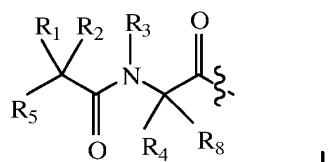
X₇ is isoacyl-Thr;

X₁₃ is Aib;

X₂₀ 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; and

R₂₀ is COOH or CONH₂, 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:



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;

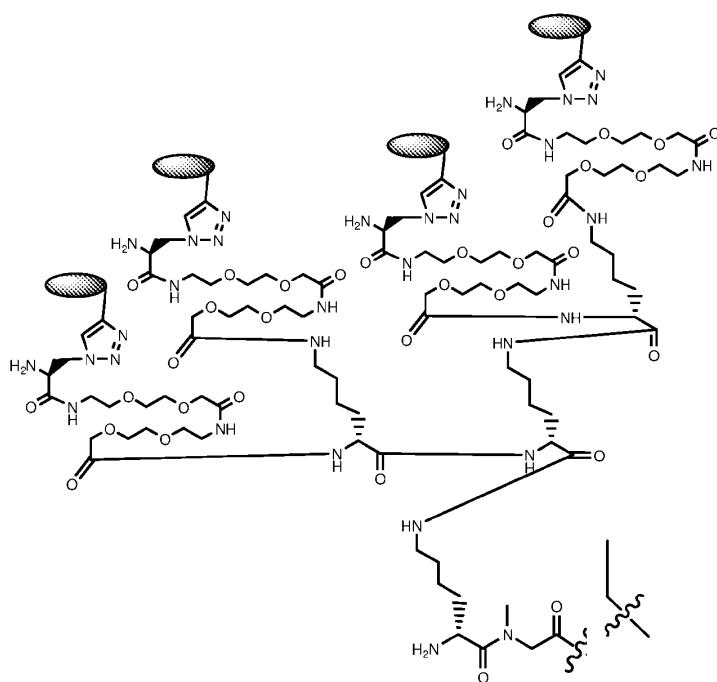
R₂ is H;

R₄ and R₈ are independently D or H;

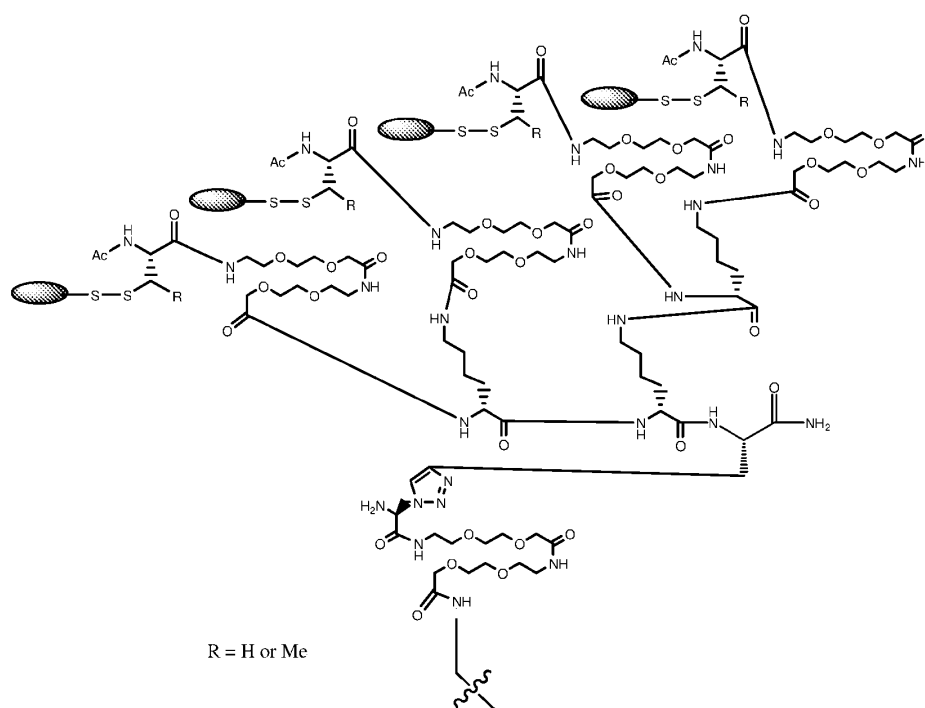
R₃ is C₁-C₄ alkyl or CD₃; and

R_5 is NH_2 , wherein said first and second spacers are independently $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

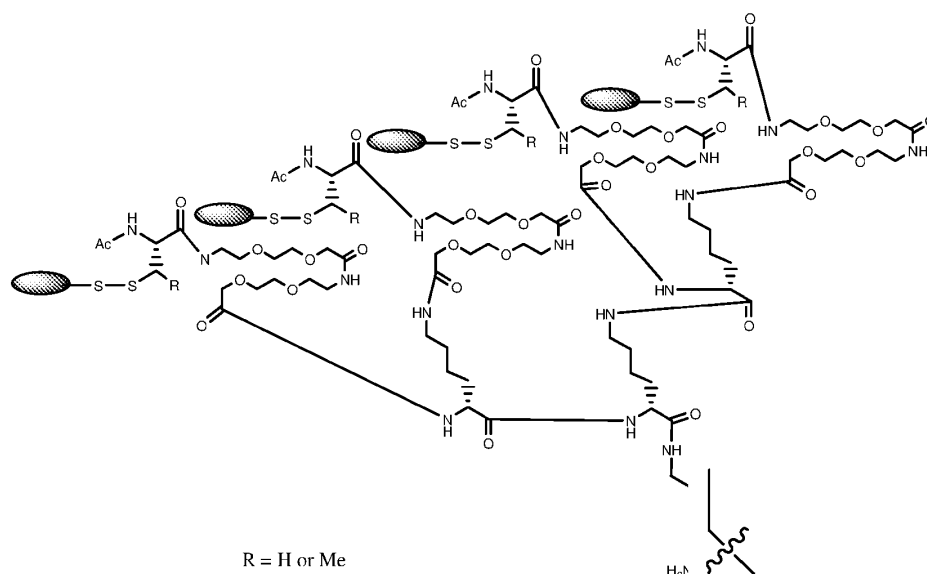
- 5 In accordance with one embodiment R_1 comprises a branched polyethylene chain having a structure of Formula X:



- 10 wherein  represents a 5K or 10K PEG and Ac = acyl group; or having a structure of Formula XI:

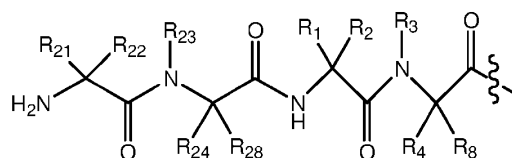


wherein  represents a 5K or 10K PEG and Ac = acyl group; or a structure of Formula XII:



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

- In accordance with one embodiment a prodrug derivative of the GIP/GLP-1 co-agonist peptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 is provided wherein the peptide is modified by the covalent linkage of one or more dipeptides “A-B” and/or sequential dipeptides “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 B (for the dipeptide “A-B”) or D (for the sequential dipeptide “A-B-C-D”) and an amine of the peptide, wherein the side chain of amino acid (A) and (C) is acylated with a C16-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C16-C20 diacid.
- In one embodiment one or more amino acids at position 1 (via the N-terminal alpha amine), 7 (via the alpha amine of the isoacyl-Thr of SEQ ID NO: 3) and/or 20 (via the side chain amine of Lys) are covalently linked via an amide bond to either the dipeptide “A-B” or the sequential dipeptide “A-B-C-D”. In one embodiment the sequential dipeptide “A-B-C-D” 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 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; and

R_{23} is CD₃, C₁-C₆ alkyl, or R_{23} and R_{24} 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 selected from the group
15 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 amino acid at position 1 is linked to the sequential dipeptide
20 "A-B-C-D" via the N-terminal alpha amine, and optionally the amino acids at position 7 (via the alpha amine of the isoacyl-Thr of SEQ ID NO: 3) and/or 20 (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 7 is linked to the sequential dipeptide "A-B-C-D" via the alpha amine of the isoacyl-Thr of SEQ ID NO: 3, and
25 optionally the amino acids at position 1 (via the N-terminal alpha amine) and/or 20 (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: 3)
30 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".

In accordance with one embodiment a GIP/GLP-1 co-agonist peptide of SEQ ID NO: 3 is provided wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

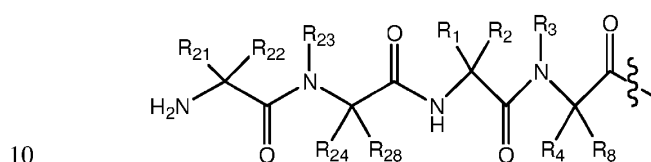
X₇ is isoacyl-Thr;

X₁₃ is Aib;

- 5 X₂₀ 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; and

R₂₀ is COOH or CONH₂, wherein

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



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;

- 15 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;

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

-[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 one embodiment a pharmaceutical composition is provided comprising any of the novel the GIP/GLP-1 co-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 GIP/GLP-1 co-agonist, or prodrug 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 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 aqueous formulations are prepared in 100% dimethyl sulfoxide. 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.

In accordance with one embodiment an improved method of treating diabetes in patients in need thereof is provided. The method comprises the steps of administering a GIP/GLP-1 co-agonist, or prodrug derivatives thereof, of the present disclosure in an amount therapeutically effective to lower blood glucose levels. In one embodiment GIP/GLP-1 co-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.

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 GIP/GLP-1 co-agonist, or prodrug derivatives thereof, of the present disclosure in an amount therapeutically effective reducing weight gain or inducing weight loss in a subject. In one embodiment GIP/GLP-1 co-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, co-agonists, or peptides includes all pharmaceutically acceptable salts or esters thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 Provides the structure of a prodrug analog of tirzepatide (TZP): (Tyr-Aib-Glu-Gly-Thr-(Me-Phe)-Thr*-Ser-Asp-Tyr; SEQ ID NO: 5; showing Thr* (aMe-Phe6, Thr7) as a isoacyl-dipeptide-protected prodrug and its conversion to the active parent peptide (SEQ ID NO: 6) by the intramolecular cleavage of the dipeptide through formation of a diketopiperazine the speed of which is controlled by the chemical nature of the dipeptide, and a subsequent comparatively rapid O to N rearrangement generates the amide bond between residues 6 and 7.

Figs. 2A-2C shows the structure of three different prodrug analogs of the aMe-Phe6 analog of TZP that differ only in the nature of the second amino acid in the dipeptide (circled sections); Fig. 2A: MBX 4209 (SEQ ID NOs: 36 and 37); Fig. 2B: MBX4210 (SEQ ID NOs: 38 and 39); Fig. 2C: MBX 4211 (SEQ ID NOs: 40 and 41), where X = Aib.

Fig. 3A and 3B demonstrate the activity of prodrug derivatives of the aMe-Phe6 analog of TZP. An *in vitro* assay was used to detect the agonist activity of aMe-Phe6-TRP, 4062 (■) (SEQ ID NO: 11), aMe-Phe6-TRP with a dipeptide prodrug element linked i) at the Lys side chain at position 16, 4212 (◆)(SEQ ID NO: 42); ii) at a isoacyl-Thr at position 7, 4210 (▲) (SEQ ID NO: SEQ ID Nos: 38 and 39); and iii) at the N-terminal alpha amine, 4083 (●) (SEQ ID NO: 15) for both the GLP-1 receptor (Fig. 3A) and the GIP receptor (Fig. 3B).

Figs. 4A and 4B show the structure of a isoacyl peptide analog of aMe-Phe6-TRP (YXEGT(alpha-Me)FTSDYSIXLDKIAQK((miniPEG)2-γE-COC18H36CO2H)AFVQWLIAGGPSSGAPPPS-NH₂; MBX 4062 (Fig. 4A; SEQ ID NO: 11)), and a further derivative comprising a dipeptide prodrug element linked to isoacyl-Thr of the isoacyl peptide analog of aMe-Phe6-TRZ (k((miniPEG)2-γE-COC18H36CO2H)(N-Me)G[YXEGT(alpha-Me)F-O]TSDYSIXLDKIAQK((miniPEG)2-γE-COC18H36CO2H)AFVQWLIAGGPSSGAPPPS-NH₂; MBX 4071 (Fig. 4B; SEQ ID NOs: 13 and 14)) wherein X = Aib.

Fig. 5A and 5B provides the results of a single dose of Prodrugs (MBX 1420, MBX 4071, MBX 4127 and MBX 4108 administered subcutaneously to DIO Mice. Fig. 5A shows food intake over a three-day period. Fig. 5B shows the percent body weight reduction. The dose administered was 10nmol/kg. Semaglutide (MBX 1420) was administered as a comparative drug agonist at 1 and 3nmol/kg, respectively.

Fig. 6A and 6B provides the results of three repeat daily doses of prodrugs (MBX 4071, MBX 4127 and MBX 4129 administered subcutaneously to DIO Mice. Fig. 6A shows food intake over a seven-day period. Fig. 6B shows the percent body weight reduction. The doses administered for the prodrugs were 10 and 30nmol/kg, respectively. Semaglutide (MBX 1420) was administered as a comparative drug agonist 3nmol/kg.

Fig. 7 presents the results from ten days daily dosing in DIO-Mice with prodrug MBX 4071 administered subcutaneously at 3 or 10nmol/kg, respectively to DIO Mice. TZP (MBX 4160 (SEQ ID NO: 99)) and the (alpha-Me)-Phe6 TZP analog (MBX 4062) were administered as comparative drug agonists at 1 and 3nmol/kg, respectively. Observations were made through day 14 with dosing beginning on day 0.

Fig. 8 presents the results from ten days daily dosing in DIO-Rats with prodrug MBX 4071 administered subcutaneously at 10 or 30nmol/kg, respectively to DIO Rats. TZP (MBX 4160; SEQ ID NO: 99) and the (alpha-Me)-Phe6 TZP analog (MBX 4062) were administered as comparative drug agonists at 3 and 10nmol/kg, respectively. Observations were made through day 14 with dosing beginning on day 0. The plasma concentration of peptide drug agonists MBX 4060 and MBX 4062 is shown for three representative rats as determined 24 hours following the last administered doses.

Figs. 9A & 9B provides the PK-results of a single dose, SC-Monkey Study with MBX prodrugs (Fig. 9B) and comparative drugs (Semaglutide and TZP; Fig. 9A), where the measured incretin released by the prodrugs is respectively identical to Semaglutide and TZP.

Figs. 10A-10C provides the PK-results of a single dose, SC-Monkey Study with MBX prodrug co-agonist, MBX 4071 (SEQ ID NOs: 13 and 14) respectively administered at 25, 50 or 75 nmol/kg. The concentration of the prodrug (MBX 4071) and the resultant drug (MBX 4062 (SEQ ID NO: 11)) are shown in Fig. 10A. The structure of MBX 4071 (SEQ ID NOs: 13 and 14) is shown in Fig. 10B and the structure of MBX 4062 (SEQ ID NO: 11) is shown in Fig. 10C.

Fig. 11: *In vitro* bioanalysis at the GLP-1 and GIP receptors for a peptide alanine scan of TZP (w/o lipid). Results are reported as absolute potency (EC₅₀, pM), relative potency at each receptor compared to a peptide without substitution (MBX 4051), and direct relative comparison of EC₅₀ at the two receptors. The structure of MBX 4116-4125 is provided in Table 40.

Fig. 12: Compares the *in vitro* bioactivity of various GIP/GLP-1 co-agonists at the human and mouse GIP and GLP-1 receptors. Results are reported as absolute potency (EC₅₀,

pM) at the four receptors. Modifications relative to TZP (MBX 4060; (SEQ ID NO: 99)) are indicated.

Fig. 13: provides comparative results on the body weight lowering in DIO-mice subcutaneously administered daily MBX GIP/GLP-1 co-agonists MBX-4060, 4062, 4189, 1 & 3 nmol/kg respectively for ten days and followed through day 14.

Fig. 14: provides comparative results on food consumption in DIO-mice subcutaneously administered daily MBX GIP/GLP-1 co-agonists MBX-4060, 4062, 4189, 1 & 3 nmol/kg respectively for ten days and followed through day 14.

Fig. 15: is a table comprising the *in vitro* bioactivity of various GIP/GLP-1 co-agonists (MBX 4189, 4190, 4274) at the human GIP and GLP-1 receptors. Results are reported as absolute potency (EC₅₀, pM) at the two receptors, and relative potency to MBX 4060 (TZP; SEQ ID NO: 99). MBX 4274 is an analog of TZP with substitutions with alpha-Me-F6, Glu15, Thr17, Ala19.

Fig. 16A and 16B provides *in vivo* results in DIO-mice comparing the activity of various GIP/GLP-1 co-agonists (MBX 4062, 4189, 4274, 4275, 4280) relative to TZP (MBX 4060) (SEQ ID NO: 99) in relation to body weight reduction (Fig. 16A) and food intake (Fig. 16B). The peptides were administered subcutaneously each day for five days at 3nmol/kg and subsequently for five additional days at 1nmol/kg.

Fig 17: provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study. The peptides MBX 2101, 4062, and three prodrugs 4269, 4271 and 4272 were subcutaneously injected at 50nmol/kg. The peptide concentration over the course of two weeks was assessed by LCMS, and the resultant drug concentration () for the three prodrugs is reported.

Fig. 18: provides the speed of conversion in PBS of prodrug MBX 4083 (SEQ ID NO: 15) to drug MBX 4062 as a function time, through 11 days of analysis by LCMS.

Fig 19: provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study. Two prodrugs MBX 4083 and 4269 were subcutaneously injected at 50nmol/kg. The peptide concentration over the course of two weeks was assessed by LCMS, and the resultant respective drug concentration (MBX 4062 and 4268) for the two prodrugs is reported. The administered prodrugs are displayed in closed symbols and the resultant drugs are in open symbols.

Fig. 20: provides the speed of conversion in PBS of four prodrugs of MBX 4062, MBX 4083, 4232, 4265, 4266) as a function time, through 21 days of analysis by LCMS. The

prodrugs differ by the nature of deuterium substitution in the sarcosine of the prodrug dipeptide that is linked to GIP/GLP-1 co-agonist MBX 4062.

Fig. 21 provides the speed of conversion in PBS of eight prodrugs of MBX 4062 comprising a dipeptide linked at lysine16 (MBX 4206, 4212, 4213, 4215, 4217, 4218, 4220, and 4221) as a function time, through 14 days of analysis by LCMS. The prodrugs differ by the nature of stereochemistry in the dipeptide chemically conjugated to the side-chain amine of the lysine at residue 16, and the nature of the second amino acid of the dipeptide.

Fig. 22 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study. Three prodrugs of MBX 4062 (MBX 4212, 4213 and 4220) were subcutaneously injected at 50nmol/kg. The peptide concentration over the course of two weeks was assessed by LCMS, and the resultant respective drug concentration (MBX 4062) for the two prodrugs is reported. The administered prodrugs are displayed in closed symbols and the resultant drugs are in open symbols. The reported half-life is derived from in vitro analysis as shown in Fig 23.

Fig. 23 provides the speed of conversion in PBS of four prodrugs of MBX 4062 of which two are at the N-terminal amine (MBX 4239 and MBX 4240) and two are at lysine16 (MBX 4241 and 4242) as a function time, through 21 days of analysis by LCMS. The prodrugs differ by the nature of stereochemistry in the dipeptide and the nature of the second amino acid of the dipeptide.

Fig. 24A-24C: provides the structure of various GIP/GLP-1 co-agonists. Fig. 24A represents MBX4223 (SEQ ID NO: 49 and 50) having lipids at residues : 7, 16, 20 linked via a cleavable dipeptide (dKSar) at 7, and 16 (wherein the dK is acylated with (miniPEG)2-γE-COC18H36CO2H); Fig. 24B represents MBX4224 (SEQ ID NO: 51 and 52) having lipids at residues: 1, 7, and 20 linked via a cleavable dipeptide (dKSar) at 1 and 7 (wherein the dK is acylated with (miniPEG)2-γE-COC18H36CO2H); Fig. 24C represents MBX4225 (SEQ ID NO: 53) having lipids at residues: 1, 16, and 20 linked via a cleavable dipeptide (dKSar) at 1 and 16 (wherein the dK is acylated with (miniPEG)2-γE-COC18H36CO2H).

Fig. 25 provides the speed of conversion in PBS for the three MBX 4062 prodrugs shown in Figs. 24A-24C, (MBX 4223, 4224, and 4225) as a function time, through 7 days of analysis by LCMS.

Figs. 26A & 26B provides an analysis of the conversion of a sequential prodrug TZP analog (MBX4234) to the active drug (MBX4062). Fig. 26A is a table providing the conversion over time in PBS of a single sequential prodrug of MBX 4062 (MBX 4234) where the first DKP formation yields MBX 4083 which is followed by a subsequent DKP

formation to form MBX 4062. The analysis was conducted as a function time through 28 days by LCMS. Fig. 26B provides the structure of MBX4234 (SEQ ID NO: 55)).

Fig. 27 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study. Two prodrugs of MBX 4062 (MBX 4083, having the dipeptide dKSar linked to the K at position 20, and 4232, having the dipeptide dKSar linked to the K at position 20) were subcutaneously injected at 50nmol/kg. The peptide concentration over the course of two weeks was assessed by LCMS, and the resultant respective drug concentration (MBX 4062) for the two prodrugs is reported. The administered prodrugs are displayed in closed symbols and the resultant drugs are in open symbols. The reported half-life for MBX 4232 is shown in Fig 20.

Fig. 28 provides a comparative analysis of the PK results of an Inotiv NHP (cynomolgus monkey) Study. Two prodrugs MBX 4083 (single cleavage) and 4291 (sequential cleavage) were subcutaneously injected at 50nmol/kg. The peptide concentration over the course of three weeks was assessed by LCMS, and the resultant respective drug concentration for the two prodrugs is reported. The administered prodrugs are displayed in closed symbols and the resultant drugs are in open symbols.

Fig. 29 is a schematic representation of the conversion of the sequential prodrug MBX4291 to the active peptide MBX4274 (SEQ ID NO: 66).

Fig. 30 provides a comparison of the conversion of two sequential TZP prodrug analogs to the active peptide (MBX4274) wherein the first sequential prodrug analog (MBX4291) is acylated at the first and second dipeptide prodrug elements and the second sequential prodrug analog is acylated only at the second dipeptide prodrug element.

Figs. 31A and 31B provide data from an *in vitro* analysis of the activity of two TZP analogs at the GLP-1 receptor (Fig. 31A) and the GIP receptor (Fig. 31B), wherein the two analogs differ only in the linked acyl group being a fatty diacid (MBX4274) or a phosphonic fatty acid (MBX4347).

Fig. 32 provides analysis of the pharmacokinetics in cynomolgus monkeys of two TZP analogs that differ only in the linked acyl group being a fatty diacid (MBX4274) or a phosphonic fatty acid (MBX4347). The TZP analogs comprising the phosphonic fatty acid has higher retention in cynomolgus monkeys relative to the analog comprising a fatty diacid.

Fig. 33 is a table providing the structure of various sequential dipeptide prodrugs acylated with either a fatty diacid or a phosphonic fatty acid.

Fig. 34 provides data showing the pharmacokinetics of prodrugs having different fatty acids administered to cynomolgus monkeys.

Fig. 35 is a table providing the structure of various sequential dipeptide macromolecular prodrugs with PEG-polymers, wherein dK(4x(OEG-OEG-A(triazole-PEG 5k))Sar designates a D-lysine linked to a branched PEG chain having four separate 5K PEG chains linked via a OEG-OEG-A-triazole linker (see the structure of Formula X).

5 Fig. 36 is a table show the conversion rate for MBX 4329 over time in PBS buffer at 37°C. The prodrug has a half-life of 12.4 days.

Fig. 37 is a graph showing the pharmacokinetics in cynomolgus monkeys of two prodrugs with different PEG-polymers when administered as a single subcutaneous injection at 50nmol/kg, and the measured concentration of the resultant drug MBX 4062 is shown.

10 Fig. 38 is a graph showing the pharmacokinetics in cynomolgus monkeys of prodrugs with time protractors (acylation or pegylation of the dipeptide prodrug element) when administered as a single subcutaneous injection at 50nmol/kg. and the measured concentration of the resultant drug MBX 4274 is shown.

Fig. 39 is a graph comparing the pharmacokinetics in cynomolgus monkeys of
15 prodrugs with straight chain PEG verses branched PEG chains linked to the dipeptide element, and the measured concentration of the resultant drug MBX 4274 is shown. The compounds tested comprise TZP (MBX 4160; SEQ ID NO: 99) modified to comprise amino acid substitution of alpha-Me-F6, Glu15, Thr17, Ala19 and a pegylated dipeptide linked to the N-terminal alpha amine of the TZP peptide, wherein the first amino acid of the dipeptide
20 is a pegylated dLys (at position -1, “dK-1”) and the second amino acid of the dipeptide is an N-methylated glycine (at position 0, “N(Me)G0”). The PEG chains are linked to the dLys at position -1 via a spacer comprising one of the structures disclosed in embodiment 50, wherein (triazole-PEG 20k) represents a single PEG chain of 20 K linked via a triazole comprising linker, (triazole-PEG 40k) represents a single PEG chain of 40 K linked via a
25 triazole comprising linker, (triazole-PEG 5k)4)), represents a branched PEG chain having four linked branches of 5 K linked to dK-1 via a triazole comprising linker, and (triazole-PEG 10k)4)), represents a branched PEG chain having four linked branches of 10 K linked to dK-1 via a triazole comprising linker.

Fig. 40 provides a table of GIP/GLP-1 co-agonists, their compound reference number
30 and corresponding sequence identifiers.

DETAILED DESCRIPTION

DEFINITIONS

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

- 5 The term "about" as used herein means greater or lesser than the value or range of 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
10 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
15 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 carboxyl groups or groups similar thereto.

- Pharmaceutically acceptable base addition salts can be prepared from inorganic and
20 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 amines.

- Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic
25 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 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.

- 30 As used herein, the term "treating" includes alleviation of the symptoms associated with a specific disorder or condition, or the eliminating said symptoms. For 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 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
5 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 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
10 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
15 route such as subcutaneous, intramuscular, intraspinal, or intravenous.

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
20 intended as a relative definition. The term "purified polypeptide" is used herein to 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
25 naturally occurring polynucleotide present in a living animal is not isolated, 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
30 non-naturally occurring amino acids. Non-naturally occurring amino acids 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 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-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;
- 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:
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 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 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.

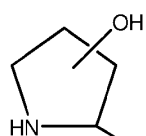
As used herein, a "dimer" is a complex comprising two subunits covalently bound to

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.

- 5 As used herein the term "charged amino acid" refers to an amino acid that 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
10 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.

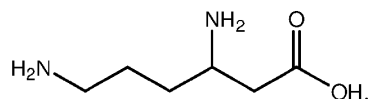
As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety, including for example, a carboxylic acid or sulfonic acid group.

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



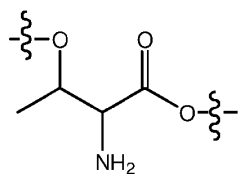
and includes the specific compounds 3- hydroxyproline, 4- hydroxyproline and 5- hydroxyproline and all the allo-stereoisomers.

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

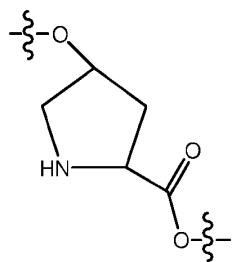


- 20 As used herein the term "isoacyl-peptide" defines a peptide having an ester linked amino acid. A "isoacyl-Thr" is a threonine residue that is linked to another amino acid via an ester bond, rather than a peptide bond, wherein the ester bond is formed using the side chain hydroxyl group of threonine, serine, hydroxyproline or a hydroxylated amino acid.

Accordingly, isoacyl-Thr has the structure:



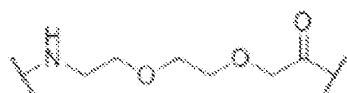
- 25 , and a isoacyl-4-hydroxyproline comprises the structure:



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 an integer ranging from 2-20. 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 (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 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 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 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 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
5 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 receptor.

As used herein, “GIP potency” of a molecule refers to the ratio of the EC50 of the
10 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.

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
15 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 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
20 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 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
25 cyclopentyl.

As used herein, the term “heterocyclic” refers to a cyclic hydrocarbon group 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,
30 morpholine, thiophene, and the like.

As used herein, the term “aryl” refers to a monocyclic or polycyclic aromatic 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.

5 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.

10 ABBREVIATIONS:

D = deuterium, a stable isotope of hydrogen

Lower case k = D-isomer of lysine

Upper case K = L-isomer of lysine

γE = L-isomer of gamma, glutamic acid

15 (miniPEG)₂ = COCH₂OCH₂CH₂OCH₂CH₂NH

COC₁₆H₃₂CO₂H = (C18 diacid)

COC₁₈H₃₂CO₂H = (C20 diacid)

(N-Me)G = sarcosine

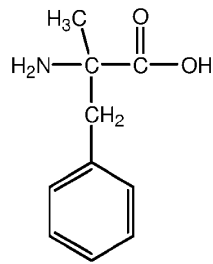
hPip = hydroxypiperidine

20

EMBODIMENTS

Peptides having co-agonist activity at the GIP and GLP-1 receptors have demonstrated transformative pharmacology in the treatment of metabolic diseases, and most notably reduction in body weight. The most advanced medicine (tirzepatide) is formulated as a subcutaneous 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 tirzepatide is provided in Fig. 1.

As disclosed herein, novel peptides exhibiting balanced, full potency agonists at the GLP-1 and GIP receptor are provided. Surprisingly, applicant has discovered that modification of the tirzepatide (TZP) peptide by substitution of the phenylalanine at position 6 with an alpha methyl phenylalanine:



(aMe,Phe⁶ TZP) produces a GIP/GLP-1 receptor co-agonist having higher potency and improved receptor balance relative to tirzepatide (See Table 1). Thus, the tirzepatide can be administered at a lower dosage than tirzepatide to produce a therapeutic effect and potentially lowers the potential for minimize adverse gastrointestinal effects.

Table 1: Tirzepatide vs the aMe,Phe⁶ TZP

	hGLP-1	hGIP	mGLP-1	mGIP
TZP	0.157	0.056	0.170	0.930
aMe,Phe6 TZP	0.088 (1.8x*)	0.026 (2.2x*)	0.110 (1.6x*)	0.120 (7.7x*)

* fold increase in potency at the relevant receptor compared to TZP

In accordance with one embodiment a GIP/GLP-1 receptor co-agonist is provided, wherein the peptide comprises the sequence of
YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀
(SEQ ID NO: 1), or a peptide that differs from SEQ ID NO: 1 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions,
wherein
X₂ is an α,α-disubstituted amino acid, optionally Aib;
X₆ is alpha methylated Phe
X₇ is Thr or isoacyl-Thr;
X₁₃ is Tyr or an α,α-disubstituted amino acid, optionally Aib;
X₁₅ is Glu or Asp;
X₁₆ is Aib, Orn or Lys;
X₁₇ is Thr, Val or Ile;
X₁₉ is Ala or Gln;

X_{20} 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, a C16-C30 diacid, or a C16-C30 alkyl group, optionally via a spacer; and

R_{20} is COOH or CONH₂, 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.

In accordance with one embodiment the peptide of SEQ ID NO: 1 is provided wherein X_2 and X_{13} are both Aib, optionally wherein X_{20} is an acylated Lys, wherein the acyl group of the acylated Lys is a C16-C24 acid, a C16-C24 phosphonic fatty acid, or a C16-C24 diacid, optionally linked via a spacer to the Lys side chain, wherein the 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 one embodiment a GIP/GLP-1 receptor co-agonist is provided, wherein the peptide comprises the sequence of

YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS- R_{20} (SEQ ID NO: 1), or a peptide that differs from SEQ ID NO: 1 by 1 or 2 amino acid substitutions, optionally wherein the amino acid substitutions are conservative amino acid substitutions, wherein

X_2 is Aib;
 X_6 is alpha methylated Phe;
 X_7 is Thr or isoacyl-Thr;
 X_{13} is Tyr or Aib;
 X_{15} is Glu or Asp;
 X_{16} is Aib, Orn or Lys;
 X_{17} is Thr, Val or Ile;
 X_{19} is Ala or Gln;

X_{20} is an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group, a C16-C30 phosphonic fatty acid, or a C16-C30 alkyl group, optionally via a spacer; and R_{20} is COOH or CONH₂, wherein the 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 X₂₀ is Lys having a C16 to C20 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: -

- 5 [COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-, wherein k is 2, and p and q are independently an integer selected from 1 or 2. In one embodiment the acylated amino acid is Lys wherein the lysine side chain is linked via an amide bond to (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H. In accordance with one embodiment X₇ is isoacyl-Thr, wherein “isoacyl-Thr” is intended to encompass both stereoisomer forms. In accordance with one
10 embodiment a GIP/GLP-1 receptor co-agonist is provided, wherein the peptide comprises the sequence of

YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀

(SEQ ID NO: 1), or a peptide that differs from SEQ ID NO: 1 by 1 or 2 amino acid substitutions, optionally wherein the amino acid substitutions are conservative amino acid

- 15 substitutions

wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

X₇ is Thr or isoacyl-Thr;

- 20 X₁₃ is Aib;

X₁₅ is Glu or Asp;

X₁₆ is Lys;

X₁₇ is Thr or Ile;

X₁₉ is Ala or Gln, and X₂₀ is an amino acid comprising a (C1-C4 alkyl)NH₂

- 25 side chain that has been acylated with a C16-C30 acyl group, a C16-C30 phosphonic fatty acid, or a C16-C30 alkyl group, optionally via a spacer; and R₂₀ is COOH or CONH₂, wherein the 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)_kNH]_q-(gamma glutamic acid)_p-, wherein z is 0 or 1, k is an integer
30 selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein X₂₀ is Lys having a C16 to C20 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: -[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-, wherein k is 2, and p and q are independently an integer selected from 1 or 2. In one embodiment the

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

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

5 $\text{YX}_2\text{EGTX}_6\text{TSDY}\text{SIX}_{13}\text{LX}_{15}\text{X}_{16}\text{X}_{17}\text{AX}_{19}\text{X}_{20}\text{AFVQWLIAGGPSSGAPPPS-R}_{20}$ (SEQ
 ID NO: 4)

wherein

I) X_2 is Aib;

X_6 is alpha methylated Phe;

10 X_{13} is Aib;

X_{15} is Asp;

X_{16} is Lys;

X_{17} is Ile; and

X_{19} is Gln; or

15 II) X_2 is Aib;

X_6 is alpha methylated Phe;

X_{13} is Aib;

X_{15} is Glu;

X_{16} is Lys;

20 X_{17} is Thr;

X_{19} is Ala; or

III) X_2 is Aib;

X_6 is alpha methylated Phe;

X_{13} is Aib;

25 X_{15} is Glu;

X_{16} is Aib;

X_{17} is Thr;

X_{19} is Ala or Gln; and

30 X_{20} is an amino acid comprising a (C1-C4 alkyl) NH_2 side chain that has been acylated
 with a C16-C30 acyl group or a C16-C30 alkyl group, optionally via a spacer; and R_{20} is
 COOH or CONH₂, wherein the 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 X_{20} is Lys having a C16 to C20 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 independently an integer selected from 1 or 2. In one embodiment the

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

The present disclosure also encompasses prodrug derivative of the novel GIP/GLP-1 receptor co-agonists disclosed herein. In one embodiment a prodrug derivative of any of the novel GIP/GLP-1 receptor co-agonists disclosed herein is provided wherein a dipeptide

10 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 GIP/GLP-1 receptor co-agonists of SEQ ID NO: 1 at a 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

15 chemically cleave under physiological conditions to release the dipeptide (forming a diketopiperazine) and the active GIP/GLP-1 receptor co-agonist. In one embodiment a dipeptide prodrug element is covalently linked one or more amino acids at one or more positions selected from position 1, 7 and 16 of the GIP/GLP-1 receptor co-agonist of SEQ ID NO: 1. In some embodiments the dipeptide prodrug element is linked to the N-terminal alpha

20 amine of the GIP/GLP-1 receptor co-agonist of SEQ ID NO: 1. Subsequent removal of the dipeptide, under physiological conditions and in the absence of enzymatic activity, restores full activity to the GIP/GLP-1 receptor co-agonist.

In some embodiments a prodrug 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 amino acid linked to

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

30 is at least about 8 days up to 21 days.

In some embodiments A and B are selected to inhibit enzymatic cleavage of the A-B dipeptide from the GIP/GLP-1 receptor co-agonist by enzymes found in mammalian serum. In some embodiments A and/or B are selected such that the cleavage of A-B from the GIP/GLP-1 receptor co-agonist in PBS under physiological conditions, is not more than

twofold the cleavage half-life of A-B from the GIP/GLP-1 receptor co-agonist in a solution comprising a DPP-IV protease (i.e., cleavage of A-B from the GIP/GLP-1 receptor co-agonist 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 GIP/GLP-1 receptor co-agonists 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 oral and inhalation. However, any of the standard routes of administration including intramuscular injection, can be used to administer the GIP/GLP-1 receptor co-agonist peptides to a patient.

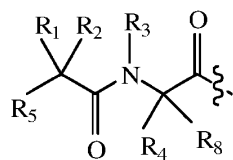
Peptides having both GIP activity and GLP-1 activity are particularly advantageous for inducing weight loss or preventing weight gain, as well as for treating metabolic syndrome, hyperglycemia, and diabetes. Thus, in one aspect, the invention provides methods for inducing weight loss or preventing weight gain, which involve administering to a patient in need thereof an effective amount of a peptide, that exhibits activity at both the GIP receptor and the GLP-1 receptor, optionally in the form of a prodrug.

In accordance with one embodiment a isoacyl peptide derivative of SEQ ID NO: 1 or SEQ ID NO: 2 is provided wherein 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 an isoacyl-threonine (SEQ ID NO: 3), 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, and the amino acid at

position 6 is an alpha methylated amino acid, optionally wherein the amino acid at position 6 is alpha methyl phenylalanine.

In accordance with one embodiment a prodrug derivative of any of the GIP/GLP-1 receptor co-agonist disclosed herein is provided. In one embodiment a GIP/GLP-1 receptor co-agonist peptides 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 or SEQ ID NO: 2. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1 and 7 of SEQ ID NO: 1 or SEQ ID NO: 3. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1 and 16 of SEQ ID NO: 1 or SEQ ID NO: 2. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 7 and 16 of SEQ ID NO: 1 or SEQ ID NO: 3. In one embodiment a dipeptide prodrug element is covalently linked to an amino acid at position 1, 7 and 16 of SEQ ID NO: 1 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. In one embodiment, the dipeptide linked to the amino acid at position 7 is linked to the alpha amine of a isoacyl-Thr of a isoacyl peptide comprising SEQ ID NO: 1 or SEQ ID NO: 3. In one embodiment, the dipeptide linked to the amino acid at position 16 is linked to the side chain ε-amino group of a lysine residue of SEQ ID NO: 1 or SEQ ID NO: 2.

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



I

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,
 5 (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;

10 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
 15 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,
 20 (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;

25 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
 30 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,

5 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,
 10 (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
 15 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
 20 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;

25 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

(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,
 30 (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;

5 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
10 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,
15 (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;

20 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;

25 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
30 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

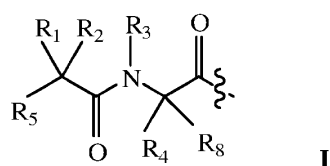
- alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the
- 5 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
- 10 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;
- 15 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 lLys or dLys, acylated with a C₁₆-C₂₀ fatty acid, a C₁₆-C₂₀ phosphonic fatty acid, or a C₁₆-C₂₀ diacid;
- 20 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
- 25 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 lLys or dLys, acylated with a C₁₆-C₂₀ fatty acid, a C₁₆-C₂₀ phosphonic fatty acid, or a C₁₆-C₂₀ diacid;
- R₂ is H;
- 30 R₄ and R₈ are both D;
- R₃ is CD₃; and
- R₅ is NH₂; or
- IX) R₁ is (C₁-C₄ alkyl)NH₂, 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

R_5 is NH_2 .

- In accordance with one embodiment a prodrug derivative of SEQ ID NO: 1 or
 5 SEQ ID NO: 3 is provided wherein the alpha amine group of the isoacyl-Thr at position 7 is linked to a P7 dipeptide via an amide bond, wherein the P7 dipeptide comprising the structure:



wherein

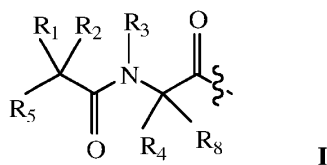
- 10 R_1 comprises a side chain of $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$, wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P7 spacer;

R_2 , and R_8 are independently H, or $\text{C}_1\text{-C}_4$ alkyl;

- 15 R_4 is H, $\text{C}_1\text{-C}_4$ alkyl or $\text{C}_1\text{-C}_4$ alkenyl, $\text{C}_1\text{-C}_4$ alkenyl, $-(\text{CH}_2)_n\text{CH}_2\text{OH}$ or $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{phenyl}$, wherein n is an integer selected from 0-2;

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

- 20 R_5 is NH_2 , 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 $(\text{gamma glutamic acid})_z\text{-[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, 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. In a further embodiment, the prodrug derivative of SEQ ID NO: 1 or SEQ ID NO: 3 is provided wherein said P7 dipeptide comprises the structure:



wherein

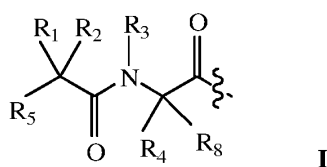
R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

R_2 , R_4 and R_8 are each H;

5 R_3 is C_1 - C_6 alkyl; and

R_5 is NH_2 , wherein the P7 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (γ glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1. IN a further embodiment the prodrug derivative of SEQ ID NO: 1 or SEQ ID NO: 3 is provided wherein the P7 spacer is $-[COCH_2(OCH_2CH_2)_2-NH]-(\gamma \text{ glutamic acid})-$, and the acyl group is a C18-C20 diacid.

In one embodiment the P7 dipeptide comprises the structure:



wherein

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, optionally via said P1 spacer;

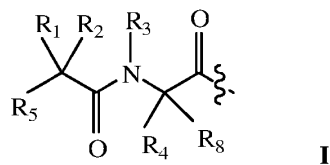
R_2 is H;

R_4 and R_8 are both D;

R_3 is CD_3 ; and

20 R_5 is NH_2 , wherein said P1 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (γ glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1. In a further embodiment the P1 spacer is $-[COCH_2(OCH_2CH_2)_2-NH]-(\gamma \text{ glutamic acid})-$, and the acyl group is a C18-C20 diacid.

In accordance with one embodiment a prodrug derivative of SEQ ID NO: 1 or SEQ ID NO: 2 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

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 P1 spacer;

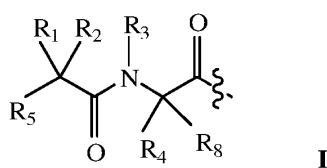
R_2 , and R_8 are independently H, or C_1-C_4 alkyl;

5 R_4 is H, C_1-C_4 alkyl, C_1-C_4 alkenyl, 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 C_1-C_6 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

10 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 $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, 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 one embodiment the P1 dipeptide comprises the structure:



wherein

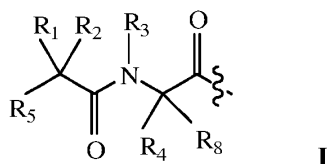
20 R_1 comprises a side chain of $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

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 P1 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 a further embodiment the P1 spacer is $-[COCH_2(OCH_2CH_2)_2-NH]-(\text{gamma glutamic acid})_p$, and the acyl group is a C18-C20 diacid.

In one embodiment the P1 dipeptide comprises the structure:



wherein

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, optionally via said P1 spacer;

5 R_2 is H;

R_4 and R_8 are both D;

R_3 is CD_3 ; and

R_5 is NH_2 , wherein said P1 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (gamma glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1. In a further embodiment
10 the P1 spacer is $-[COCH_2(OCH_2CH_2)_2-NH]-(\text{gamma glutamic acid})_-$, 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 comprising the sequence of

YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS- R_{20} (SEQ ID NO:

15 2)

wherein

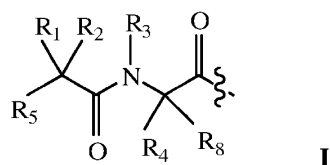
R_{20} is COOH or CONH₂;

X_2 is Aib;

X_6 is alpha methylated Phe;

20 X_{13} is Aib;

X_{20} is Lys having a C16 to C18 fatty acid, a C16-C18 phosphonic fatty acid, or a C16 to C20 diacid linked to the lysine side chain via a P20 spacer comprising the structure: $-[COCH_2(OCH_2CH_2)_kNH]_q-(\text{gamma glutamic acid})_p-$ wherein k is 2, and p and q are independently an integer selected from 1 or 2, further wherein a P1 dipeptide is covalently
25 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 $(C_4 \text{ alkyl})NH_2$, having a C18-C20 fatty acid, a
30 C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain via a P1 spacer;

R₂ is H;

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;

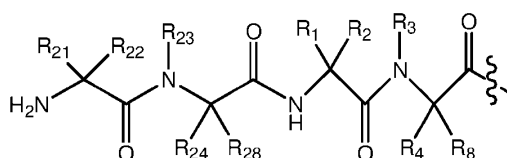
R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said P1 spacer and P20 spacer 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 a further embodiment X₂₀ is Lys acylated via its side chain with

(COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H, and R₂₀ is CONH₂, optionally wherein R₄ and R₈ are each D and R₃ is CD₃ or R₄ and R₈ are each H and R₃ is C₁-C₆ alkyl, or R₄ and R₈ are each H and R₃ is CH₃. In a further embodiment X₂₀ is Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆(PO₃H₂), and R₂₀ is CONH₂, optionally wherein R₄ and R₈ are each D and R₃ is CD₃ or R₄ and R₈ are each H and R₃ is C₁-C₆ alkyl, or R₄ and R₈ are each H and R₃ is CH₃.

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 one embodiment, in any of the prodrug derivative disclosed herein, the prodrug A-B is provided wherein R₃ and R₄ together with the atoms to which they are attached form a piperidine ring.

In accordance with one embodiment a prodrug derivative is provided wherein a sequential dipeptide structure of



is covalently linked via an amide bond to said peptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 at one or more of amino acid positions 1, 7, or 16, wherein

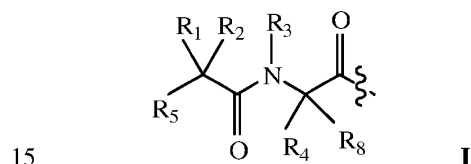
R₁ is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

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;

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

5 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 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 peptide of SEQ ID 1 or SEQ ID NO: 2, optionally with a dipeptide having the structure of



covalently linked to the amino acid at position 16 and/or at position 7 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 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, or C₁-C₄ alkyl;

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_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;

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.

Acylation and alkylation

5 The GIP/GLP-1 co-agonists of the present invention are acylated or alkylated at position 20 and at the first amino acid of the dipeptide prodrug element of the prodrug derivative of the GIP/GLP-1 co-agonists. Acylation or alkylation can increase the half-life of the GIP/GLP-1 co-agonists peptides in circulation. Acylation or alkylation can advantageously delay the onset of action and/or extend the duration of action at the GIP
10 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 GIP/GLP-1 co-agonists peptide may be maintained after acylation. 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
15 increased as compared to that of the unacylated version of the glucagon peptides.

 In some embodiments, the GIP/GLP-1 co-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 GIP/GLP-1 co-agonists peptide and the acyl group or alkyl group. In some
20 embodiments, the acyl group is a fatty acid or diacid, or salt thereof, e.g. a C4 to C30 fatty acid/diacid, a C8 to C24 fatty acid/diacid, cholic acid, a C4 to C30 alkyl, a C8 to C24 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
25 consisting of: Trp, Glu, Asp, Cys and a spacer comprising $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_m\text{COOH}$, 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 GIP/GLP-1 co-agonists peptides may comprise two acyl groups or two alkyl groups, or a combination thereof.

30 The acyl group of the acylated GIP/GLP-1 co-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 a 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. In some embodiments, the acyl group is a C8 to C20 phosphonic fatty acid, e.g., a C18 phosphonic fatty acid or a C20 phosphonic fatty acid.

Addition modifications can be made to the co-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 GIP/GLP-1 co-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 GIP/GLP-1 co-agonist peptides, or prodrug derivatives thereof, as disclosed herein, in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions may comprise the GIP/GLP-1 co-agonist peptides, or prodrug derivatives thereof, as the sole pharmaceutically active component, or the GIP/GLP-1 co-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 Polypeptide), MP1, PYY, MC-4, NK2, amylin, calcitonin, leptin.

In accordance with some embodiments a pharmaceutical composition is provided comprising any of the novel GIP/GLP-1 co-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 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
 5 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 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
 10 propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, 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,
 15 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, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents,
 20 tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or 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,
 25 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, butylparaben sodium, calcium
 30 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, 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, chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC) chlorophenoxyethanol, chloroxylenol, 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, dextrates, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl 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, 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 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,
- 5 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,
- 10 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,
- 15 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,
- 20 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 paraffin, talc,
- 25 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,
- 30 trisodium edentate, vanillin, type I 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, 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*
5 *Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety, discloses various 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.
10 Supplementary active ingredients also can be incorporated into the compositions.

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,
15 or some other encapsulated form, or may be administered in an 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,
20 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
25 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
30 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 6.5, 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.

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.

The present GIP/GLP-1 co-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 GIP/GLP-1 co-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 GIP/GLP-1 co-agonist peptides disclosed herein. Two or more of the GIP/GLP-1 co-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 GIP/GLP-1 co-agonist peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the GIP/GLP-1 co-agonist peptides comprising cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues.

Routes of Administration

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 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 GIP/GLP-1 co-agonist peptide in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the 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 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 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 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 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, 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-1,3-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 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, 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 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 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 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 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 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 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 GIP/GLP-1 co-agonist peptide can be made into 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.

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

Dose

5 The compositions of the present disclosures comprising a 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
10 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 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
15 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.

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
20 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 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.

25 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 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
30 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 present disclosure.

Combinations

The 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 herein (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, amylin agonists, calcitonin agonists and NK2 agonists, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicron); 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 FBPase (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 (Prelu-2®, 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 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 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.

10 *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 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 provide 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 with the onset and/or progression of the disease of medical condition. The method comprises providing to the patient 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. 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, 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 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 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,

5 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)).

10 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 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
15 more); and (e) fasting blood glucose (110 mg/dl or above). According to the World Health Organization (WHO), an individual having high insulin 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
20 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c) 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
25 set forth by the 2001 National Cholesterol Education Program Adult Treatment 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
30 risk factors thereof, in a subject, comprising providing to the subject a 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-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.

5 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.

10 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 accordingly provides
15 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 (irreversible, advanced scarring of the liver). All the stages of NAFLD have in common the accumulation
20 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 inflammatory cells can destroy the liver cells (hepatocellular necrosis). In the terms "steatohepatitis" and "steatonecrosis", steato
25 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 NAFLD spectrum. (Mendler, Michel, "Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)," ed. Schoenfield,
30 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 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). 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 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, 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, 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 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 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, Friedreichs ataxia, ataxia telangiectasia, spinal dysmyotrophy, progressive supranuclear palsy, dystonia, muscle spasticity, tremor, retinitis pigmentosa, striatonigral degeneration, mitochondrial encephalomyopathies, neuronal ceroid lipofuscinosis, hepatic encephalopathies, renal encephalopathies, 5 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 10 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, 15 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 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 20 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

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

In some aspects, the GIP/GLP-1 co-agonist is packaged in containers, e.g., vials, 30 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 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 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.

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 GIP/GLP-1 co-agonist activity is provided, said peptide comprising the sequence of

YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀
(SEQ ID NO: 1),

YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 2) or

YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3), or a peptide that differs from SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative substitutions, optionally wherein the peptide differs from SEQ ID NO: 1 or SEQ ID NO: 3 by 1, 2 or 3 conservative amino acid substitutions

wherein

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

X₆ is alpha methylated Phe;

X₇ is Thr or isoacyl-Thr;

X₁₃ is Tyr or an α,α -disubstituted amino acid, optionally Aib;

X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is Thr, Val or Ile;

X₁₉ is Ala or Gln;

X_{20} is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C18 fatty acid, a C16-C20 phosphonic fatty acid, a C16-C20 diacid or a C16-C20 alkyl group, optionally via a P20 spacer; and

R_{20} is COOH or CONH₂, wherein said P20 spacer is selected from the group
 5 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, 1 or 2, 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 2 the peptide of embodiment 1 is provided wherein
 10 X_{20} 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 P20 spacer; and

R_{20} is CONH₂.

In accordance with embodiment 3 the peptide of embodiment 1 or 2 is provided
 15 wherein X_{20} 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 P20 spacer to the Lys side chain.

In accordance with embodiment 4 the peptide of any one of embodiments 1-3 is provided wherein said P20 spacer comprises the structure: (gamma glutamic acid)_z-
 20 [COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p or -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p-; wherein z is 0 or 1, k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4.

In accordance with embodiment 5 the peptide of any one of embodiments 1-4 is provided wherein X_{20} is Lys having a C16 to C20 fatty acid, a C16-C20 phosphonic fatty
 25 acid, or a C16 to C20 diacid linked to the lysine side chain via a P20 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.

In accordance with embodiment 6 the peptide of any one of embodiments 1-5 is
 30 provided wherein the acylated amino acid at position 20 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-COC₁₈H₃₆(PO₃H₂).

In accordance with embodiment 7 the peptide of any one of embodiments 1-6 is provided wherein the α,α -disubstituted amino acid at X₁₆ is Aib.

In accordance with embodiment 8 the peptide of any one of embodiments 1-6 is provided wherein

- 5 X₂ is Aib;
 X₆ is alpha methylated Phe;
 X₇ is Thr or isoacyl-Thr;
 X₁₃ is Aib;
 X₁₅ is Glu or Asp;
10 X₁₆ is Lys or Aib;
 X₁₇ is Thr or Ile; and
 X₁₉ is Ala or Gln, and
 R₂₀ is CONH₂.

- 15 In accordance with embodiment 9 the peptide of any one of embodiments 1-6 is provided wherein

 X₂ is Aib; X₆ is alpha methylated Phe; X₇ is Thr or isoacyl-Thr; X₁₃ is Aib; X₁₅ is Asp; X₁₆ is Lys; X₁₇ is Ile; X₁₉ is Gln, and
 R₂₀ is CONH₂.

- 20 In accordance with embodiment 10 the peptide of any one of embodiments 1-6 is provided wherein

 X₂ is Aib; X₆ is alpha methylated Phe; X₇ is Thr or isoacyl-Thr; X₁₃ is Aib; X₁₅ is Glu; X₁₆ is Lys; X₁₇ is Thr; X₁₉ is Ala or Gln, and
 R₂₀ is CONH₂.

- 25 In accordance with embodiment 11 the peptide of any one of embodiments 1-6 is provided wherein

 X₂ is Aib; X₆ is alpha methylated Phe; X₇ is Thr or isoacyl-Thr; X₁₃ is Aib; X₁₅ is Glu; X₁₆ is Aib; X₁₇ is Thr; X₁₉ is Ala or Gln, and R₂₀ is CONH₂; or
 X₂ is Aib; X₆ is alpha methylated Phe; X₇ is Thr; X₁₃ is Aib; X₁₅ is Glu; X₁₆ is Lys; X₁₇ is Thr; X₁₉ is Ala and R₂₀ is CONH₂.

- 30 In accordance with embodiment 12 the peptide of any one of embodiments 1-6 is provided wherein

 X₂ is Aib; X₆ is alpha methylated Phe; X₇ is Thr or isoacyl-Thr; X₁₃ is Aib; X₁₅ is Glu or Asp; X₁₆ is Lys; X₁₇ is Thr or Ile; X₁₉ is Ala or Gln, and R₂₀ is CONH₂.

In accordance with embodiment 13 the peptide of any one of embodiments 1-12 is provided wherein

X₁₅ is Asp;

X₁₇ is Ile; and

5 X₁₉ is Gln.

In accordance with embodiment 14 the peptide of any one of embodiments 1-12 is provided wherein

X₁₅ is Glu;

X₁₇ is Thr; and

10 X₁₉ is Ala.

In accordance with embodiment 15 the peptide of any one of embodiments 1-14 is provided wherein R₂₀ is CONH₂.

In accordance with embodiment 16 a prodrug derivative of the peptide of any one of embodiments 1-15 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, wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C30 fatty acid, C16-C30 phosphonic fatty acid, or a C16-C30 diacid.

20 In accordance with embodiment 17 a prodrug derivative of embodiment 16 is provided wherein the GIP/GLP-1 co-agonist peptide is modified by the covalent linkage of two or more dipeptides (A-B) via an amide bond to

i) the amine of isoacyl-Thr at position 7 of the peptide;

ii) the N-terminal alpha amine;

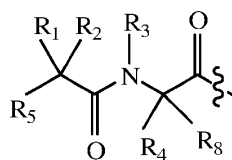
25 iii) the side chain of the amino acid at position 16;

iv) i) and ii);

v) i) and iii)

vi) i), ii) and iii).

30 In accordance with embodiment 18 a prodrug derivative of embodiment 16 or 17 is provided wherein the dipeptide comprises 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 spacer;

5 R_2 , is H, or C_1-C_4 alkyl;

R_4 and R_8 are independently H, D, C_1-C_4 alkyl, C_1-C_4 alkenyl, C_1-C_4 alkenyl, 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;

10 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, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

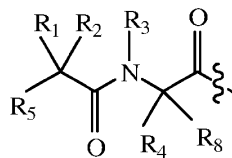
R_5 is NH_2 , wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein z is 0 or 1, k is an integer selected from the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

In accordance with embodiment 19 a prodrug derivative of any one of embodiments 16-18 is provided wherein the GIP/GLP-1 co-agonist peptide comprises the covalent linkage of a dipeptides (A-B) via an amide bond to the 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 embodiment 20 a prodrug derivative of any one of embodiments 16-19 is provided wherein

X_6 is alpha methylated Phe;

25 X_7 is isoacyl-Thr; and a dipeptide (A-B) is covalently linked to a primary amine of the isoacyl-Thr at position 7 (a "P7 dipeptide") and optionally to N-terminal alpha amine at position 1 (a "P1 dipeptide"), wherein said dipeptide comprises 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 P7 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, C_1-C_4 alkenyl, 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;

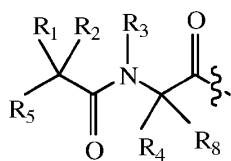
10 R_3 is CD_3 , C_1-C_6 alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

15 R_5 is NH_2 , 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 $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, 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.

In accordance with embodiment 21 a prodrug derivative of embodiments 20 is provided wherein the peptide is modified by the covalent linkage of a dipeptides (A-B) via an amide bond to the 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.

20 In accordance with embodiment 22 a prodrug derivative of any one of embodiments 16-18 is provided wherein said dipeptides (A-B) are covalently linked to said peptide at two or more amino acids located at positions independently selected from positions 1, 7, and 16, relative to SEQ ID NO: 1.

25 In accordance with embodiment 23 the prodrug derivative of embodiment 16 or 17 is provided wherein said dipeptide (A-B) is covalently linked to one or more amino acids located at positions 1, 7 and/or 16 of said peptide, wherein said dipeptide (A-B) comprises the structure:



I

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,
 5 (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;

5 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,

10 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,
15 (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 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
25 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,

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

(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 a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the

5 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;

10 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

(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

15 atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the

20 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;

25

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;

30

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) R_1 and R_2 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) R_7 ,
 5 (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 R_1 and R_2 together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R_1 and R_2 together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R_3 is C₁-C₁₈ alkyl;

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

15 R_8 is H;

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 C₁-C₄ alkyl; and,

20 R_7 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) R_1 is (C₁-C₄ alkyl)NH₂, 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_8 are both H;

25 R_4 and R_3 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

R_5 is NH₂, 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; or

30 (VIII) R_1 is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C₁₆-C₂₀ fatty acid, a C₁₆-C₂₀ phosphonic fatty acid, or a C₁₆-C₂₀ diacid;

R_2 is H;

R_4 and R_8 are both D;

R_3 is CD₃; and

R₅ is NH₂; or

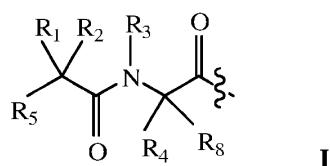
IX) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂, R₄ and R₈ are each H;

5 R₃ is CH₃; and

R₅ is NH₂.

In accordance with embodiment 24 the prodrug derivative of any one of embodiments 16-23 is provided wherein said P7 dipeptide comprises the structure:



10 wherein

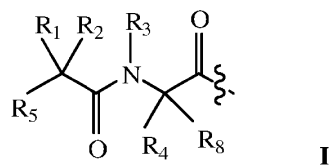
R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

R₂, R₄ and R₈ are each H;

15 R₃ is 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 or 1, optionally wherein R₃ is CH₃.

20 In accordance with embodiment 25 the prodrug derivative of any one of embodiments 15-16 is provided wherein said P7 dipeptide comprises the structure:



wherein

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

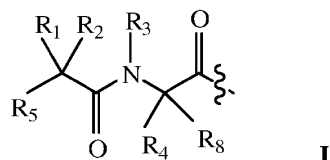
R₂ is H;

R₄ and R₈ are each D;

R₃ is CD₃; and

R_5 is NH_2 , wherein said P7 spacer comprises $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q-$ (gamma glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 26 the prodrug derivative of any one of embodiments 16-25 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 comprising the structure:



wherein

R_1 comprises a side chain of $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$, 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;

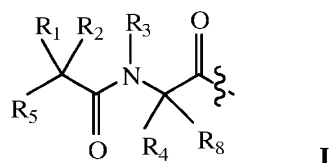
R_2 is H, or $\text{C}_1\text{-C}_4$ alkyl;

R_4 and R_8 are independently H, D, $\text{C}_1\text{-C}_4$ alkyl, $\text{C}_1\text{-C}_4$ alkenyl, $\text{C}_1\text{-C}_4$ alkenyl, $-(\text{CH}_2)_n\text{CH}_2\text{OH}$ or $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{phenyl}$, wherein n is an integer selected from 0-2;

R_3 is CD_3 , $\text{C}_1\text{-C}_6$ 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

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.

In accordance with embodiment 27 the prodrug derivative of any one of embodiments 16-26 is provided wherein said P1 dipeptide comprises 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, optionally via said P1 spacer;

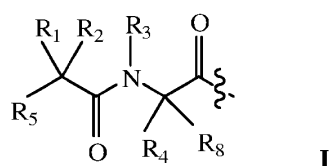
R_2 , R_4 and R_8 are each H;

R_3 is C_1 - C_6 alkyl; and

R_5 is NH_2 , wherein said P1 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (gamma glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein R_3 is

5 CH_3 .

In accordance with embodiment 28 the prodrug derivative of any one of embodiments 16-27 is provided wherein said P1 dipeptide comprises the structure:



wherein

10 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, optionally via said P1 spacer;

R_2 is H;

R_4 and R_8 are each D;

15 R_3 is CD_3 ; and

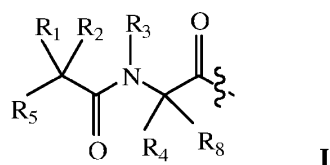
R_5 is NH_2 , wherein said P7 spacer comprises $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (gamma glutamic acid) $_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 29 the prodrug derivative of any one of embodiments 26-28 is provided wherein said P1 spacer is

20 $-[COCH_2(OCH_2CH_2)_2-NH]-(\text{gamma glutamic acid})_n$, and the acyl group is a C18-C20 diacid.

In accordance with embodiment 30 the prodrug derivative of any one of embodiments 16-29 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

25 P16 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 P16 spacer;

R₂ is H, or C₁-C₄ alkyl;

5 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

10 R₅ is NH₂, 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-[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 31 a prodrug derivative of a GIP/GLP-1 co-agonist is provided wherein the peptide comprises the sequence of

YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀

(SEQ ID NO: 1), or

YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID

20 NO: 3),

wherein

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

X₆ is alpha methylated Phe;

X₇ is Thr or isoacyl-Thr;

25 X₁₃ is Tyr or an α,α-disubstituted amino acid, optionally Aib;

X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is Thr, Val or Ile;

X₁₉ is Ala or Gln;

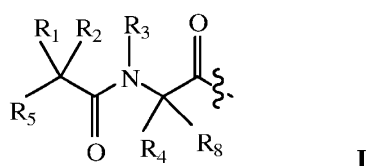
30 X₂₀ is an amino acid comprising a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, optionally via a first spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k; and

R₂₀ is CONH₂, wherein

a dipeptide is covalently linked via an amide bond to

- i) the amine of isoacyl-Thr at position 7 of the peptide;
- ii) the N-terminal alpha amine;
- iii) the side chain of the amino acid at position 16;
- iv) i) and ii);
- v) i) and iii)
- vi) i), ii) and iii),

wherein the dipeptide comprises the structure:



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;
- R_4 is H, D, C1-C4 alkyl, C1-C4 alkenyl, C1-C4 alkynyl, $-(CH_2)_nCH_2OH$ 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
- R_5 is NH₂, wherein said first and second 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, optionally wherein k is 2 and q is 2 and p is 1, optionally wherein
- 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 $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, 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;

R_2 is H;

5 R_4 and R_8 are each D;

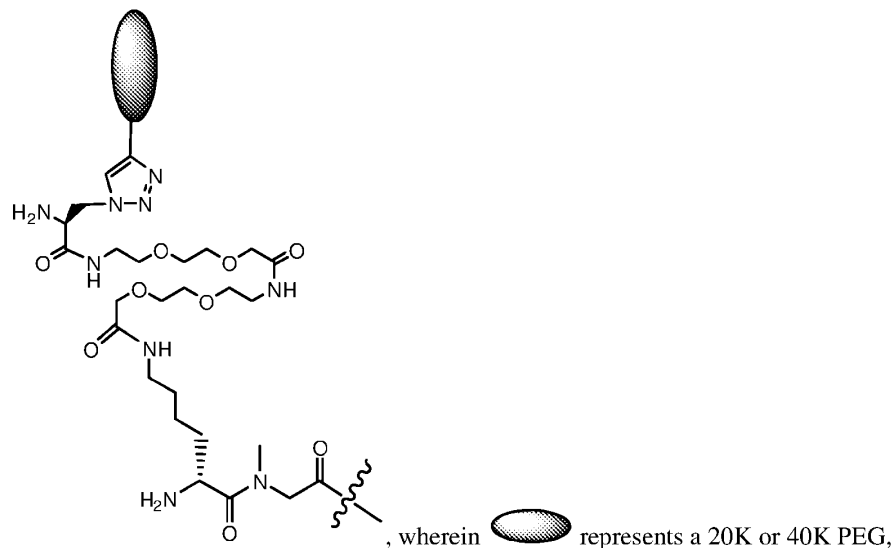
R_3 is CD₃; and

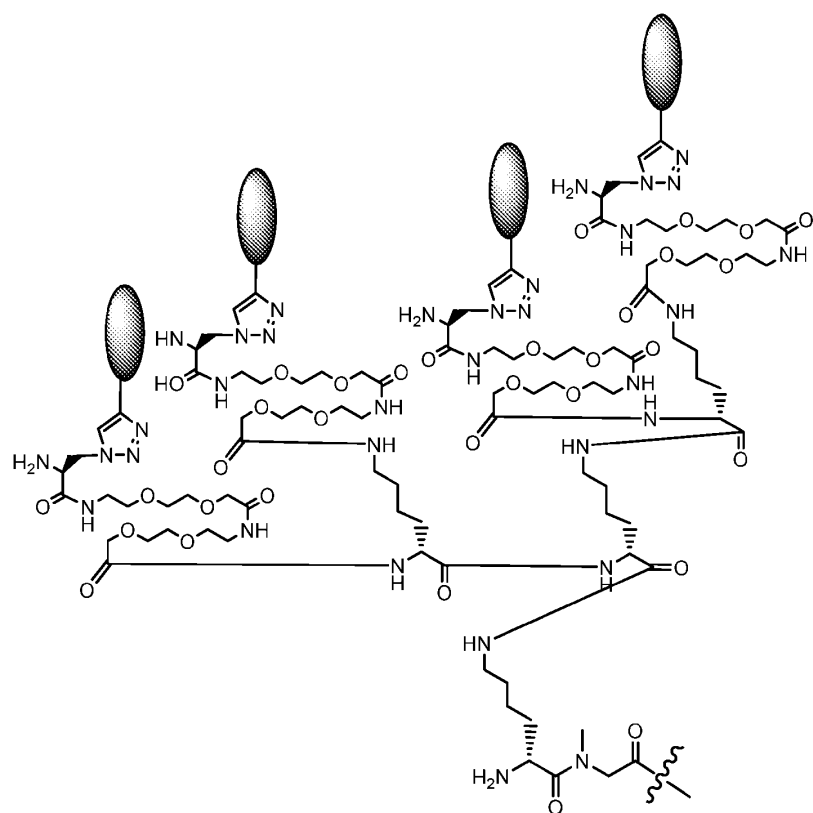
R_5 is NH₂, wherein said first and second spacers are independently -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1
10 or 2, optionally wherein k is 2.

In accordance with embodiment 32, a peptide of embodiment 31 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

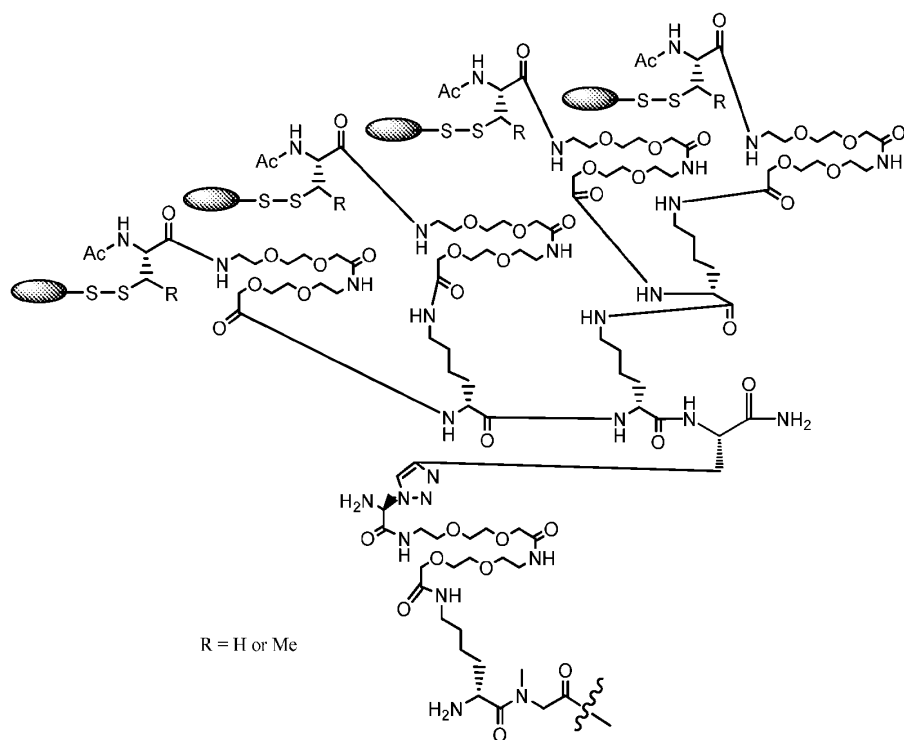
15 -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

In accordance with embodiment 33, a peptide of embodiment 31 or 32 is provided wherein the first amino acid of the dipeptide comprises a structure selected from:

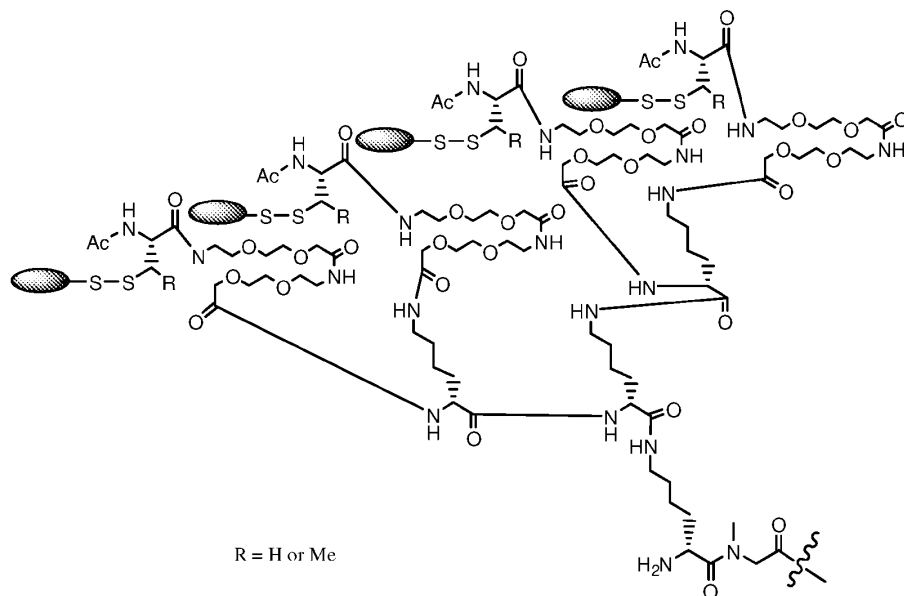




wherein  represents a 5K or 10K PEG,



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



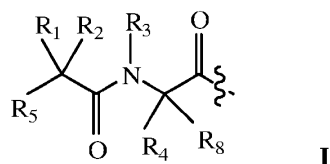
wherein  represents a 5K or 10K PEG and Ac = acyl group.

In accordance with embodiment 34 a prodrug derivative of a peptide having GIP/GLP-1 co-agonist activity is provided, wherein said peptide comprises the sequence of YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 2)

- 5 wherein
 X₂ is Aib;
 X₆ is alpha methylated Phe;
 X₁₃ is Aib;
 X₂₀ is Lys having a C16 to C18 fatty acid, a C16-C18 phosphonic fatty acid,
 10 or a C16 to C20 diacid linked to the lysine side chain via a P20 spacer comprising the structure:

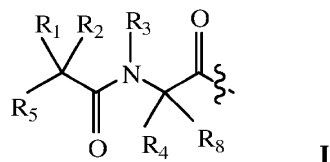


- wherein k is 2, and p and q are independently an integer selected from 1 or 2, further
 wherein
 15 a P1 dipeptide is covalently linked via an amide bond to the N-terminal alpha
 amine of said peptide, said P1 dipeptide comprising the structure:



- wherein
 R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a
 20 C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain via
 a P1 spacer;
 R₂ is H;
 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;
 25 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 P1 spacer and P20 spacer 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
 30 1, optionally wherein R₄ and R₈ are each H, and R₃ is CH₃ or CH₂CH₃, or R₄ and R₈ are each
 D and R₃ is CD₃.

In accordance with embodiment 35 the prodrug derivative of any one of embodiments 16-34 is provided wherein X_{16} is Lys, modified by the covalent linkage of a P16 dipeptide (A-B) to the side chain amine via an amide bond, wherein said P16 dipeptide comprises the structure:



5

wherein

R_1 comprises a side chain of $(C_1-C_4 \text{ alkyl})NH_2$, wherein a C16-C30 fatty acid, a C18-C20 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P16 spacer;

10 R_2 is H, or C₁-C₄ alkyl;

R_4 and R_8 are independently H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, C₁-C₄ alkenyl, - $(CH_2)_nCH_2OH$ or $(C_1-C_4 \text{ alkyl})phenyl$, wherein n is an integer selected from 0-2;

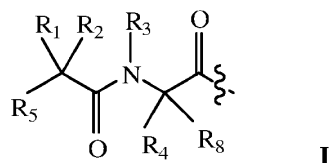
R_3 is CD_3 , C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a

15 hydroxypiperidine ring; and

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

20 from the range of 0-4.

In accordance with embodiment 36 the prodrug derivative of embodiment 35 is provided wherein said P16 dipeptide comprises the structure:



wherein

25 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, optionally via said P16 spacer;

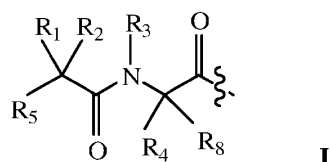
R_2 , R_4 and R_8 are each H;

R_3 is C₁-C₆ alkyl; and

R_5 is NH₂, wherein said P16 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 37 the prodrug derivative of any one of embodiments

5 35-36 is provided wherein said 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,

10 optionally via said P16 spacer;

R_2 is H;

R_4 and R_8 are each D;

R_3 is CD₃; and

R_5 is NH₂, wherein said P16 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, k is 2, q is 1 or 2 and p is 0 or 1.

15

In accordance with embodiment 38 the prodrug derivative of any one of embodiments 35 to 37 is provided wherein said P16 spacer is

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

20 In accordance with embodiment 39 the prodrug derivative of any one of embodiments 16-38 is provided wherein the first amino acid of said P1, P7, or P16 dipeptide (A-B) is an amino acid in the D-configuration, optionally wherein the amino acid is dLys.

In accordance with embodiment 40 the prodrug derivative of any one of embodiments 16-39 is provided wherein

25 R_3 and R_4 together with the atoms to which they are attached form a piperidine ring.

In accordance with embodiment 41 the prodrug derivative of any one of embodiments 16-40 is provided wherein said peptide comprising the sequence of

YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO:

2), or

30 or

YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID

NO: 3),

wherein

X₂ is Aib;

5 X₆ is alpha methylated Phe;

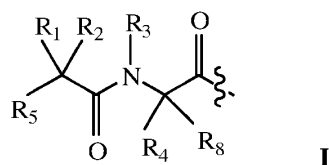
X₇ is Thr or isoacyl-Thr;

X₁₃ is Aib;

X₂₀ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group, a C16-C30 phosphonic fatty acid, or a C16-C30 alkyl group, optionally via a P20 spacer; and

R₂₀ is COOH or CONH₂, 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:



15 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 via a P1 spacer;

R₂ is H;

20 R₄ and R₈ are each D;

R₃ is CD₃; and

R₅ is NH₂, wherein said P1 spacer and P20 spacer 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

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 via a P1 spacer;

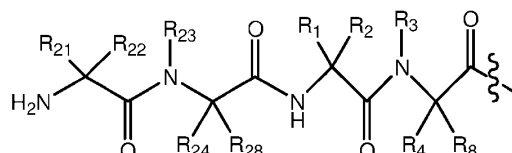
R₂ R₄ and R₈ are each H;

R₃ is CH₃; and

30 R₅ is NH₂, wherein said P1 spacer and P20 spacer are independently

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

In accordance with embodiment 42 the prodrug derivative of any one of embodiments 16-41 is provided wherein any one or more of the P1, P7 or P16 dimers comprises a sequential dipeptide structure of



covalently linked via an amide bond to said peptide,
wherein

R_1 comprises $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$ 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} is $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$ or comprises $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$ covalently linked to a C16-C30 fatty acid, C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

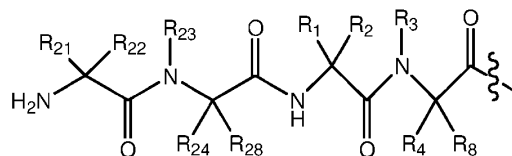
R_2 , and R_{22} are each H;

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

R_3 and R_{23} are independently $\text{C}_1\text{-C}_6$ alkyl or CD_3 ;

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

In accordance with embodiment 43 the prodrug derivative of embodiment 42 is provided wherein said sequential dipeptide comprises the structure:



wherein

R_1 comprises $(\text{C}_1\text{-C}_4 \text{ alkyl})\text{NH}_2$ covalently linked to a C16-C30 fatty acid, a C18-C20 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, C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

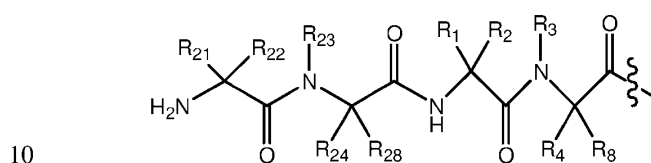
R_2 , R_4 , R_8 , R_{22} , R_{24} and R_{28} are each H;

R_3 and R_{23} are independently C_1-C_6 alkyl; and

5 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, optionally wherein R_3 and R_{23} are each CH_3 .

In accordance with embodiment 44 the prodrug derivative of embodiment 42 is provided wherein said sequential dipeptide comprises the structure:



wherein

R_1 comprises $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a DD1 spacer;

15 R_{21} comprises $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C20 fatty acid, C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a DD2 spacer;

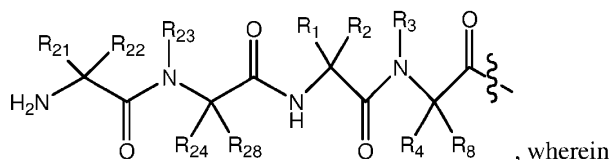
R_2 , and R_{22} are each H;

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

20 R_3 and R_{23} are each CD_3 ; wherein 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 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 embodiment 45 the prodrug derivative of any one of embodiments 42-44 is provided wherein said DD1 spacer and DD2 spacer are each $-[COCH_2(OCH_2CH_2)_2-NH]-(\text{gamma glutamic acid})$, and the acyl group is a C18-C20 diacid.

25 In accordance with embodiment 46 the prodrug derivative of any one of embodiments 16-41 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;

5 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;

10 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

15 -[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 47 the prodrug derivative of embodiment 46 is provided wherein R₂₁ comprises a (C₁-C₄ alkyl)NH₂ side chain that has been pegylated, 20 wherein the pegylation at R₂ and R₂₁ is the same or is different.

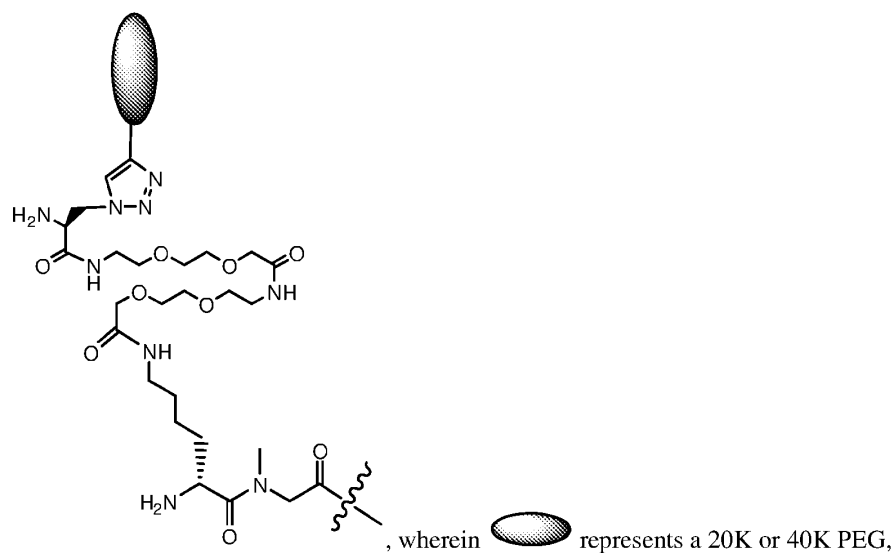
In accordance with embodiment 48 the prodrug derivative of embodiment 46 or 47 is provided wherein said sequential dipeptide structure (A-B-C-D) is covalently linked to

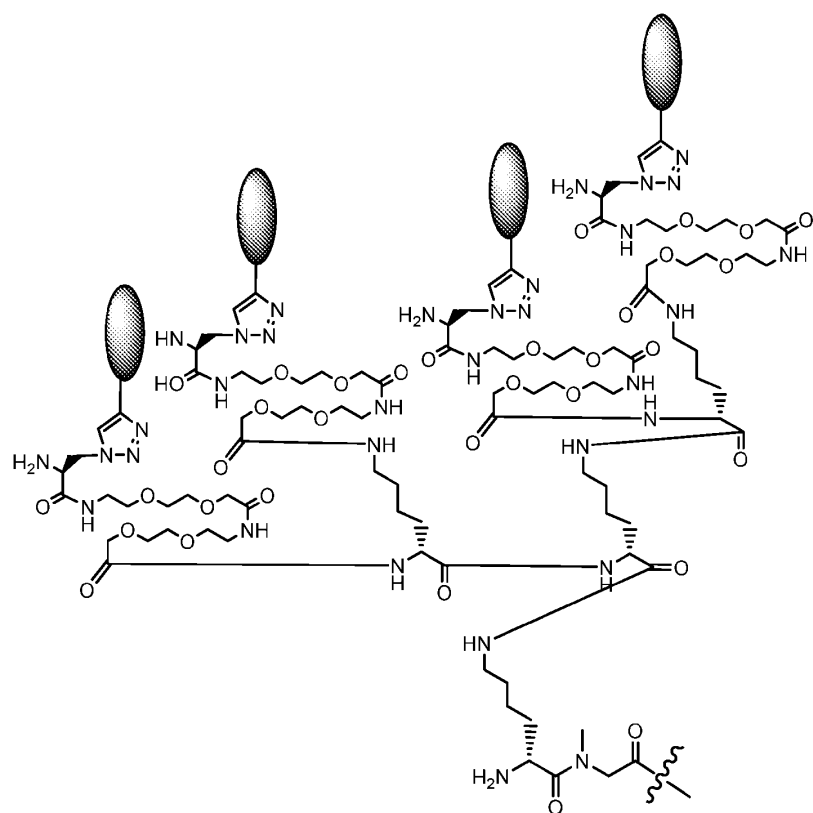
- i) the amino acid at position 1, relative to SEQ ID NO: 1;
- ii) the amino acid at position 7, relative to SEQ ID NO: 1;
- 25 iii) the amino acid at position 16, relative to SEQ ID NO: 1; or
- iv) the amino acid at position 1 and 7, relative to SEQ ID NO: 1.

In accordance with embodiment 49 the prodrug derivative of any one of embodiments 46-48 is provided wherein R₁ and optionally R₂₁ comprises a branched PEG chain construct 30 comprising

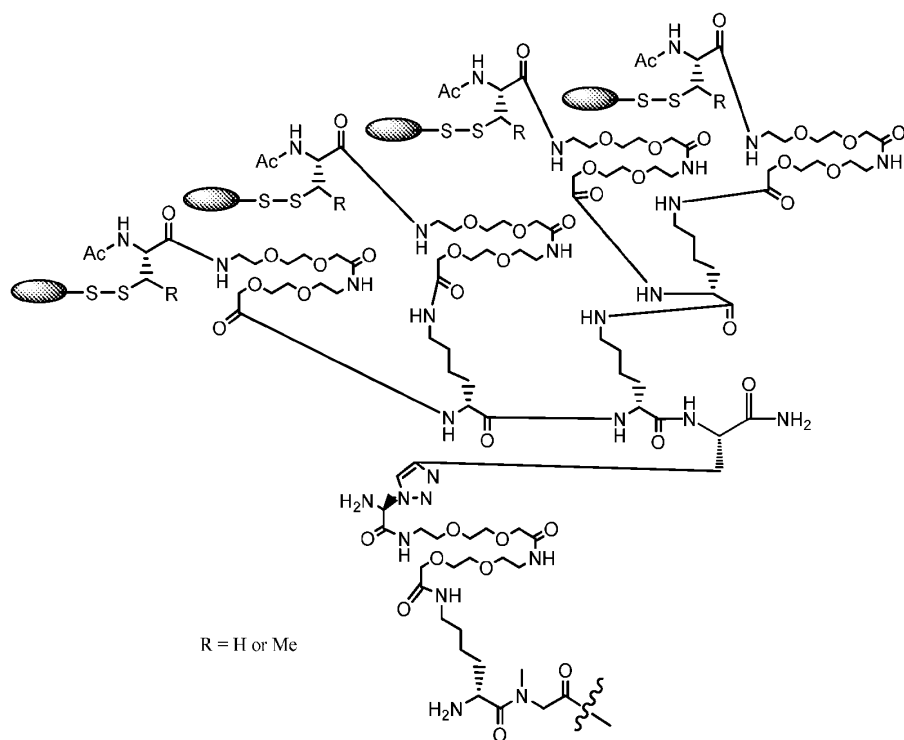
- i) two PEG branches of 10K each
- ii) two PEG branches of 20K each;
- iii) four PEG branches of 5K each; or
- iv) four PEG branches of 10K each.

In accordance with embodiment 50 a prodrug derivative of any one of the peptides of embodiments 46-49 is provided wherein the first amino acid (A) and optionally the third amino acid (C) of said sequential dipeptide element (A-B-C-D) comprises a structure selected from the group consisting of:

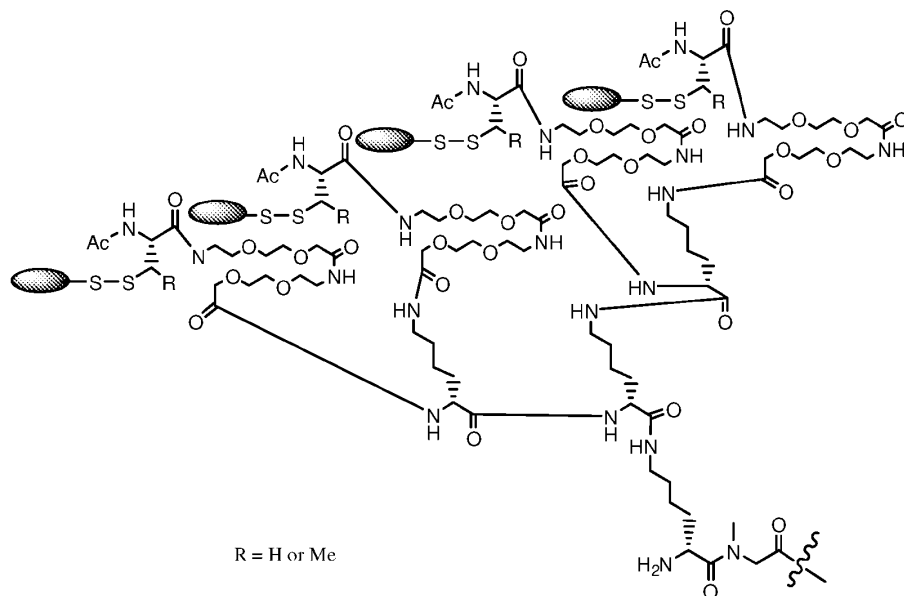




wherein  represents a 5K or 10K PEG,



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



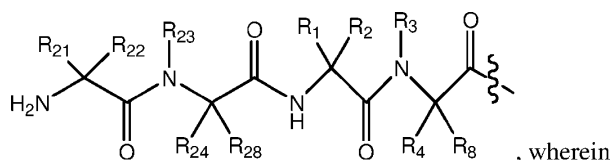
wherein  represents a 5K or 10K PEG and Ac = acyl group.

In accordance with embodiment 51 the prodrug derivative of any one of embodiments 16-50 is provided wherein the first amino acid and optionally the third 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-configuration, optionally wherein said amino acid in the D-configuration is dLys.

5 In accordance with embodiment 52 a prodrug derivative of the peptide of embodiment 11-15 is provided, wherein

X_{20} is Lys, wherein the lysine side chain is linked via an amide bond to $(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}CO_2H$ or $(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}PO_3H_2$; and

10 a sequential dipeptide is linked to the N-terminal alpha amine, wherein said a sequential dipeptide "A-B-C-D" comprises the structure of



R_1 and R_{21} independently comprises $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}CO_2H$ or $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}PO_3H_2$;

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

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

R_4 , R_8 , R_{24} and R_{28} are independently H or D, optionally wherein the first and third amino acid of the sequential dipeptide are in the D-stereoisomer configuration.

20 In accordance with embodiment 53, the prodrug derivative of embodiment 52 is provided wherein

R_1 and R_{21} each comprises $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}CO_2H$ or each comprises $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}PO_3H_2$;

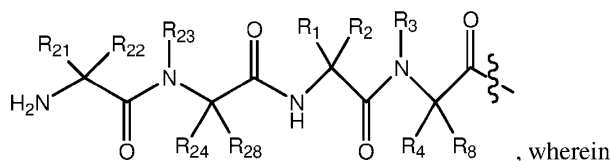
R_2 , R_4 , R_8 , R_{22} , R_{24} and R_{28} are all H; and

R_3 and R_{23} are each CH_3 .

25 In accordance with embodiment 54 a prodrug derivative of the peptide of embodiment 11-15 is provided, wherein

X_{20} is Lys, wherein the lysine side chain is linked via an amide bond to $(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}CO_2H$ or $(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}PO_3H_2$; and

30 a sequential dipeptide is linked to the N-terminal alpha amine, wherein said a sequential dipeptide "A-B-C-D" comprises the structure of



R_1 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;

5 R_{21} 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;

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 CD₃, or CH₃; and

R_{23} is CD₃, or CH₃, 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, optionally wherein the first and third amino acid of the sequential dipeptide are in the D-stereoisomer configuration.

In accordance with embodiment 55 a pharmaceutical composition is provided comprising the peptide of any one of embodiments 1 to 53 and a pharmaceutically acceptable carrier or excipient.

20 In accordance with embodiment 56 a kit is provided comprising a pharmaceutical composition of embodiment 55 and a device for administering said pharmaceutical composition to the patient, optionally wherein the device comprises a syringe comprising the pharmaceutical composition.

25 In accordance with embodiment 57 a method of reducing weight gain or inducing weight loss in a subject is provided, wherein a peptide of any one of embodiments 1-15, or a prodrug derivative of any one of embodiments 16-54, or a pharmaceutical composition of embodiment 55 is administered to a subject in need thereof, in an amount effective to reduce weight gain or induce weight loss. In one embodiment the pharmaceutical composition is administered by inhalation. In one embodiment the pharmaceutical composition is administered orally.

30 In accordance with embodiment 58 a method of diabetes in a subject is provided, wherein a peptide of any one of embodiments 1-15, or a prodrug derivative of any one of

embodiments 16-54, or a pharmaceutical composition of embodiment 54 is administered to a patient in need thereof in an amount effective to lower blood glucose levels.

EXAMPLES

5 **Synthesis of Various GIP/GLP-1 Co-agonists**

In the following synthesis protocols, a superscript number indicates the amino acid position in the synthesized peptide.

MBX 4062 and 4274 – a drug with aMePhe

- MBX4062 was assembled on 0.1 mmol Rink amide ChemMatrix® resin using an
- 10 ABI-433A peptide synthesizer and Fmoc/Oxyma/DIC coupling protocols. Fmoc-Lys(Mtt)-OH was used for K²⁰. Fmoc-aMePhe-OH was used for aMeF⁶. Boc-Tyr(tBu)-OH was employed to introduce the N-terminal Tyr. Mtt group was removed with HFIP/DCM and the resultant free amine was used for additional extension. This amine was coupled sequentially with two repeat additions of Fmoc-NH-PEG₂-CH₂COOH, followed by Fmoc-Glu-OtBu, and
- 15 finally 20-(tert-Butoxy)-20-oxoicosanoic acid. The peptide was chemically removed from the synthetic resin by treatment with a TFA solution containing 2.5% TIS, 2.5% 2-mercaptoethanol, 2.5 % anisole, and 2.5% 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. The peptide precipitate was collected by centrifugation and washed with cold ether.
- 20 The impure peptide was subjected to purification by preparative reverse-phase HPLC column (Kinetex® 5 µm C8 100 Å LC Column 250 X 21.2 mm, 10-50 % 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.

25 **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 dK¹, two identical fatty acid linkers were introduced as previously described in synthesis of MBX 4062.

30

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 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

5 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.

10

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

MBX 4234 and 4291 – a sequential prodrug

 Boc-dK(Fmoc)-OH, Fmoc-dLys(Mtt)-OH, and Fmoc-Lys(Mtt)-OH were used for
20 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 4347 – phosphonic fatty acid

25 Similar to synthesis of MBX4062, 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.

30 **MBX 4329** – PEGylated prodrug

 After deprotection of Boc-dLys(Fmoc)-OH at N-terminal, two repeat additions of Fmoc-NH-PEG₂-CH₂COOH, followed by Boc-Dap(N₃)-OH were coupled sequentially. The peptide was cleaved and purified after modification at side chain of K²⁰. The resulting peptide with azide and PEG-alkyne were taken up into 1: 1 MeOH/H₂O solution. The in situ

generated Cu(I) from copper sulfate and ascorbic acid was added to the mixture, which was subjected to purification after completion of Click Chemistry.

MBX 4353 – branched PEGylated prodrug with disulfide bond linkage

5 To the side chain of Boc-dLys at the N-terminal of a dipeptide prodrug unit acylated to sarcosine, Fmoc-Lys(Fmoc)-OH was coupled sequentially to the side chain free amine to generate four amine groups in a branched manner. The initial lysine coupling generated two amines to which the subsequent lysine coupling generates four amines after treatment with 20 % piperidine/DMF. Each of these four amine groups were coupled sequentially with two
10 repeat additions of Fmoc-NH-PEG₂-CH₂COOH, followed by Fmoc-Cys(Trt)-OH, and finally acetic anhydride. The side chain of K²⁰ was modified as mentioned before. Upon cleavage from resin and purification, the peptide with four free thiol groups was obtained. Thiolysis with an excess amount of activated PEG 10k-SSPy followed by prep HPLC afforded the branched 4 x PEG 10k prodrug with a disulfide bond.

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
20 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
25 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 $t_{1/2} = 0.693/k$.

30 **Luciferase-Based Reporter Assay for GLP-1 and GIP cAMP 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 or GIP 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
5 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
10 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.

15

***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
20 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.

C57B1/6 mice were obtained from Jackson Laboratories. The mice were single- or
25 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**

30 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 or 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 rats 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 single 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, 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.

What is claimed is:

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

YX₂EGTX₆X₇SDYSIX₁₃LX₁₅X₁₆X₁₇AX₁₉X₂₀AFVQWLIAGGPSSGAPPPS-R₂₀

(SEQ ID NO: 1) wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

X₇ is Thr or isoacyl-Thr;

X₁₃ is Tyr or an α,α -disubstituted amino acid, optionally Aib;

X₁₅ is Glu or Asp;

X₁₆ is Aib, Orn or Lys;

X₁₇ is Thr, Val or Ile;

X₁₉ is Ala or Gln;

X₂₀ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group, a C16-C30 phosphonic fatty acid, or a C16-C30 alkyl group, optionally via a P20 spacer; and

R₂₀ is COOH or CONH₂, wherein said P20 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.

2. The peptide of claim 1, wherein X₂₀ 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 a P20 spacer.

3. The peptide of claim 1 or 2, wherein X₂₀ 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 P20 spacer to the Lys side chain.

4. The peptide of claim 3, wherein said P20 spacer comprises the structure:

(gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-; wherein z is 0 or 1, k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4.

5. The peptide of claim 3 or 4, wherein X₂₀ 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 P20 spacer comprising the structure:-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p- wherein k is 2, and p and q are independently an integer selected from 1 or 2.

6. The peptide of claim 2, wherein X₂₀ 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.

7. The peptide of any one of claims 1 to 6, wherein the α,α-disubstituted amino acid at X₂ and X₁₃ is Aib.

8. The peptide of any one of 1 to 7 wherein

X₂ is Aib;
 X₆ is alpha methylated Phe;
 X₁₃ is Aib;
 X₁₅ is Glu or Asp;
 X₁₆ is Lys;
 X₁₇ is Thr or Ile;
 X₁₉ is Ala or Gln.

9. The peptide of claim 8 wherein

X₇ is Thr;
 X₁₅ is Asp;
 X₁₇ is Ile;
 X₁₉ is Gln.

10. The peptide of claim 8 wherein

X₇ is Thr;
 X₁₅ is Glu;

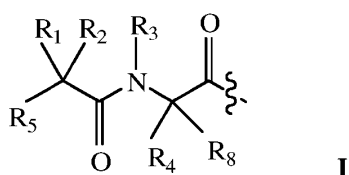
X₁₇ is Thr;

X₁₉ is Ala.

11. The peptide of any one of claims 1-10 wherein R₂₀ is CONH₂.

12. A prodrug derivative of the peptide of any one of claims 1-11 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, 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.

13. The prodrug derivative of claim 12 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;

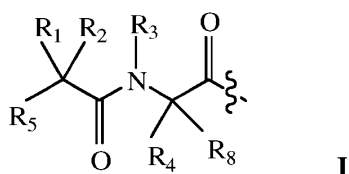
R₂ is H, or C₁-C₄ alkyl;

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

R_5 is NH_2 , 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) $_z$ -[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, optionally wherein z is 0, k and q are both 2 and p is 2.

14. The prodrug derivative of claims 12 or 13 wherein said peptide comprises a isoacyl-Thr at position 7 and a P7 dipeptide covalently linked via an amide bond to the alpha amine of said isoacyl-Thr at position 7, said P7 dipeptide comprising the structure:



wherein

R_1 comprises a side chain of (C $_1$ -C $_4$ 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 P7 spacer;

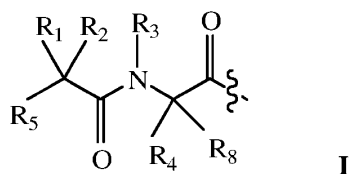
R_2 is H, or C $_1$ -C $_4$ alkyl;

R_4 and R_8 are independently H, D, C $_1$ -C $_4$ alkyl or -(CH $_2$) $_n$ CH $_2$ OH, 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, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R_5 is NH_2 , 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) $_z$ -[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.

15. The prodrug derivative of claim 14 wherein said P7 dipeptide comprises the structure:



wherein

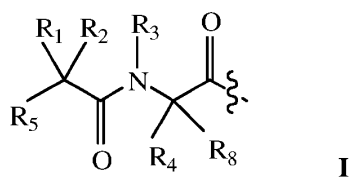
R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P7 spacer;

R₂, R₄ and R₈ are each H;

R₃ is 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 or 1.

16. The prodrug derivative of claims 14 or 15 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;

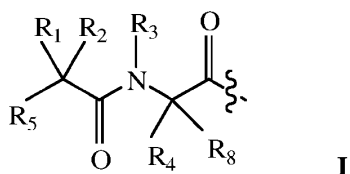
R₃ is CD₃; and

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 or 1.

17. The prodrug derivative of any one of claims 14-16 wherein said P7 spacer is

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

18. The prodrug derivative of any one of claims 12-17 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

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, wherein a C16-C30 fatty acid, a C18-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a P1 spacer;

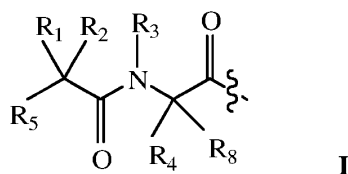
R₂ is H, or C₁-C₄ alkyl;

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 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.

19. The prodrug derivative of claim 18 wherein said P1 dipeptide comprises the structure:



wherein

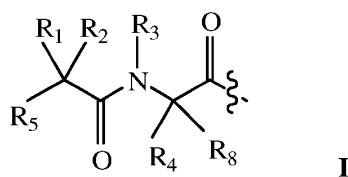
R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said P1 spacer;

R₂, R₄ and R₈ are each H;

R₃ is 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.

20. The prodrug derivative of claim 18 or 19 wherein said 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;

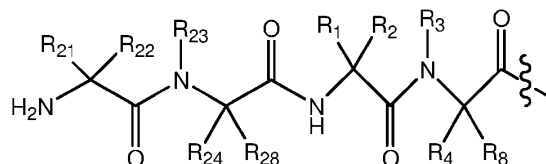
R₄ and R₈ are each D;

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.

21. The prodrug derivative of any one of claims 18-20 wherein said P1 spacer is -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid.

22. The prodrug derivative of any one of claims 12-21 wherein said dipeptide comprises a sequential dipeptide “A-B-C-D” comprising 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;

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;

R_3 is 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; and

R_{23} is CD₃, C₁-C₆ alkyl, or R_{23} and R_{24} 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 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.

23. A prodrug derivative of a GIP/GLP-1 co-agonist comprising the amino acid sequence of

YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3)

wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

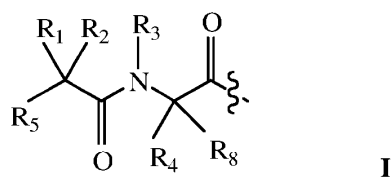
X₇ is isoacyl-Thr;

X₁₃ is Aib;

X₂₀ 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 having a molecular weight ranging from about 20k to about 40k; and

R₂₀ is COOH or CONH₂, wherein

one or more dipeptides are covalently linked via an amide bond to a primary amine of the Lys at position 16, the amine of isoacyl-Thr at position 7, and the N-terminal alpha amine, wherein the dipeptide comprises the structure:



wherein

R₁ 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;

R₂ is H, or C₁-C₄ alkyl;

R₄ 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;

R₈ is 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 pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

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

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1,

optionally wherein

I) R_1 comprises a (C1-C4 alkyl) NH_2 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 NH_2 , wherein said first and second spacers are independently

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1; or

II) R_1 comprises a (C1-C4 alkyl) NH_2 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;

R_2 is H;

R_4 and R_8 are each D;

R_3 is CD_3 ; and

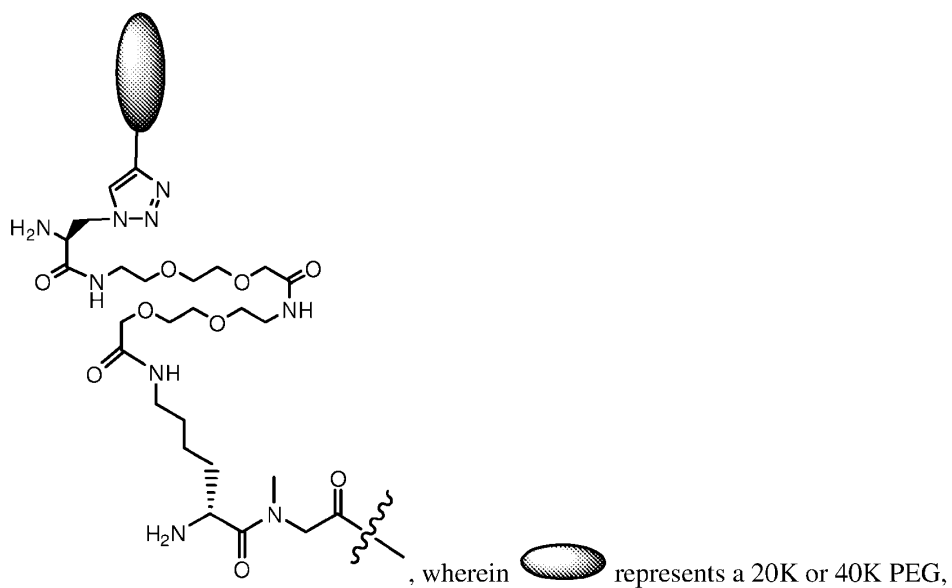
R_5 is NH_2 , wherein said first and second spacers are independently

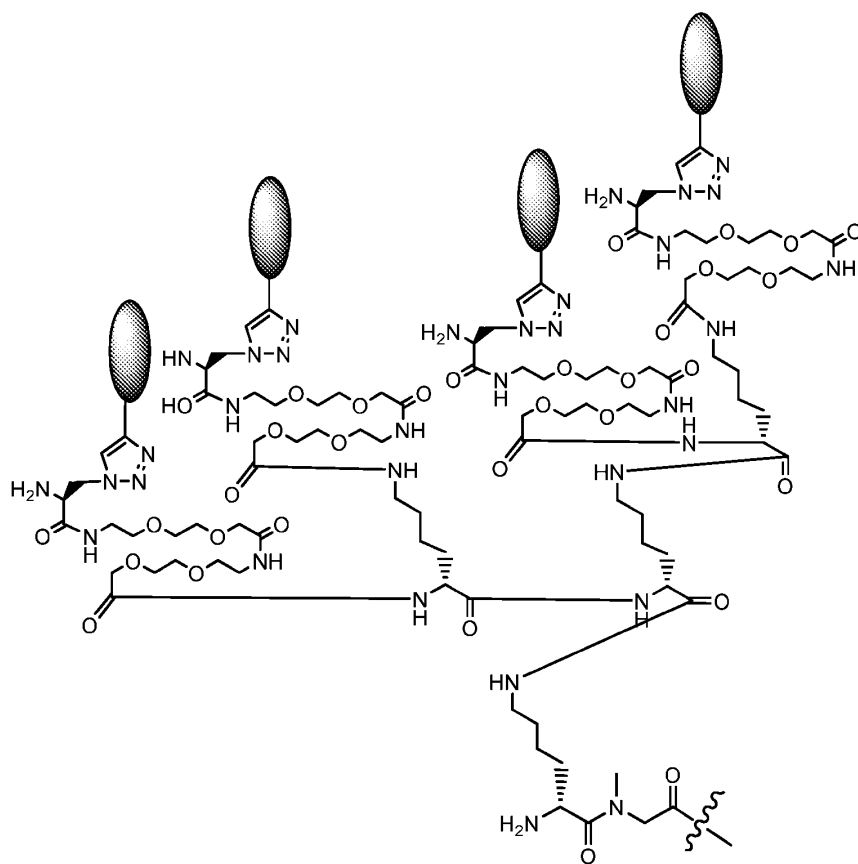
$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(alanine-triazole)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

24. The prodrug derivative of claim 23 wherein the dipeptide linked to position 7, and optionally at position 1 and/or 16, comprises a branched polyethylene chain having four branches of 5K each, or four branches of 10K each.

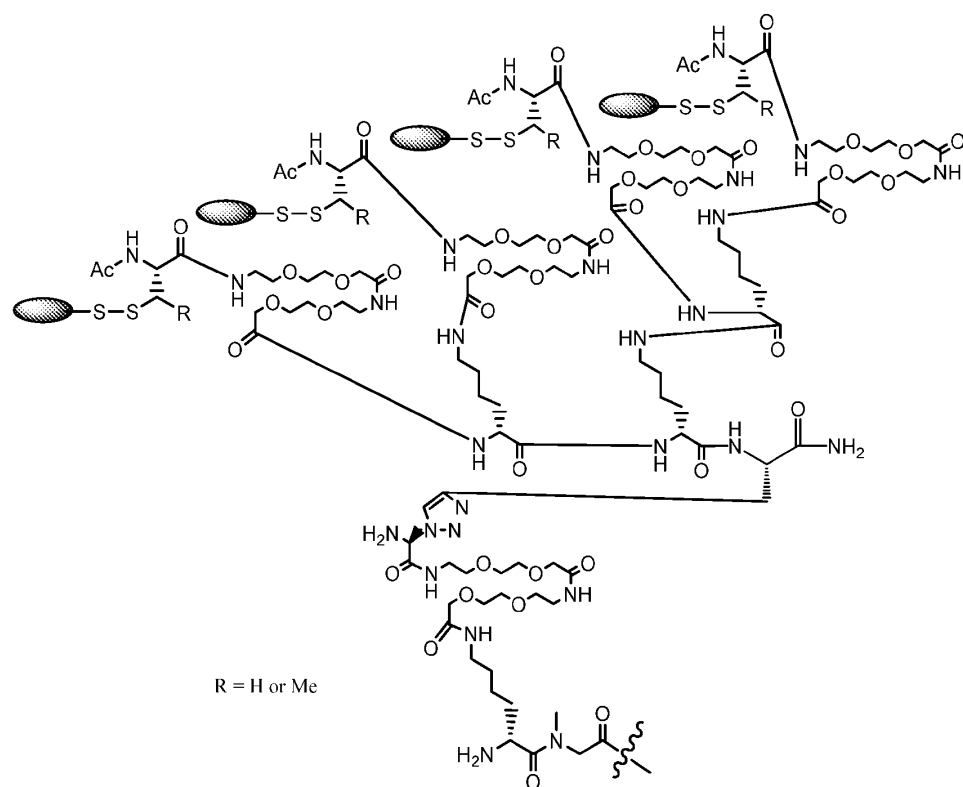
25. The prodrug derivative of claim 24 wherein each of the branches of the branched polyethylene chains are covalently linked to the dipeptide of the GIP/GLP-1 co-agonist peptide via a disulfide linkage.

26. The prodrug derivative of any one of claims 23-25 wherein the first amino acid of the dipeptide comprises a structure selected from the group consisting of:



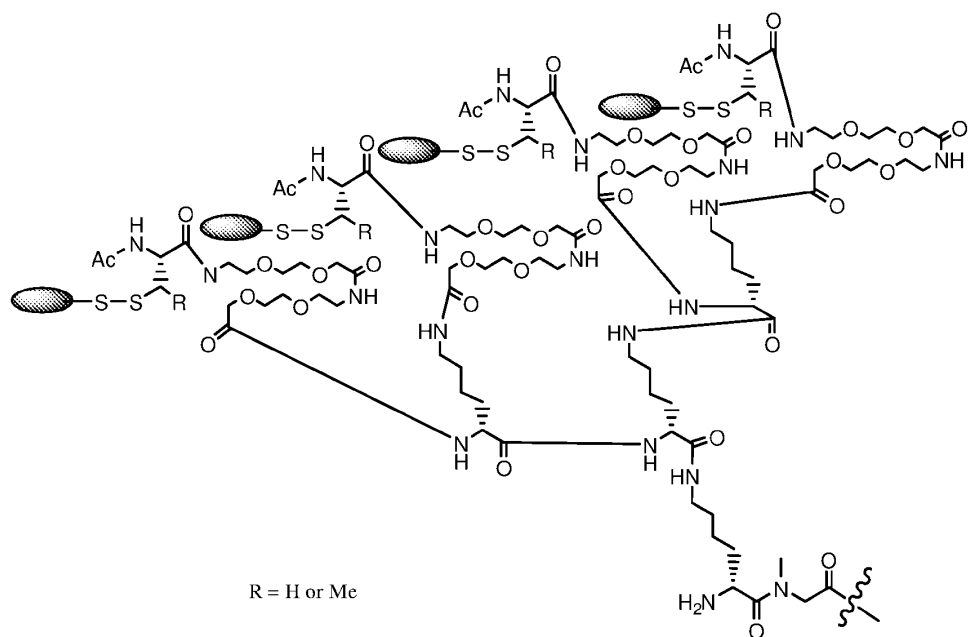


wherein  represents a 5K or 10K PEG,



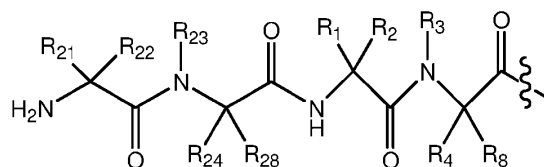
represents a 5K or 10K PEG and Ac = acyl group, and

, wherein



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

27. A GIP/GLP-1 co-agonist peptide comprising SEQ ID NO: 3, wherein
- X₂ is Aib;
 - X₆ is alpha methylated Phe;
 - X₇ is isoacyl-Thr;
 - X₁₃ is Aib;
 - X₂₀ 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; and
 - R₂₀ is COOH or CONH₂, wherein
 - a sequential dipeptide "A-B-C-D" comprising the structure of



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;

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

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 prodrug derivative of a peptide having GIP/GLP-1 co-agonist activity, said peptide comprising the sequence of

YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 2)
or YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3)

wherein

X₂ is Aib;

X₆ is alpha methylated Phe;

X₇ is isoacyl-Thr;

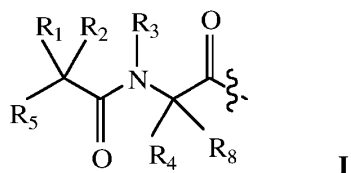
X₁₃ is Aib;

X₂₀ 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 P20 spacer comprising the structure:

-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-

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

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 via a P1 spacer;

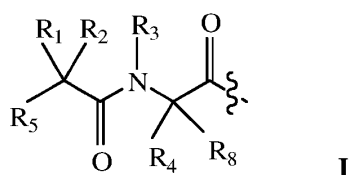
R₂ is H;

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

R₅ is NH₂, wherein said P1 spacer and P20 spacer 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.

29. The prodrug derivative of claim 28 wherein X₁₆ is Lys, modified by the covalent linkage of a P16 dipeptide (A-B) to the side chain amine via an amide bond, wherein said P16 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 P16 spacer;

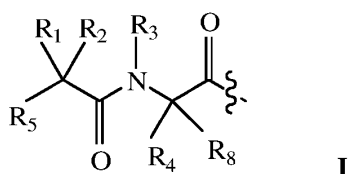
R₂ is H, or C₁-C₄ alkyl;

R_4 and R_8 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;

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

R_5 is NH₂, 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-[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. The prodrug derivative of claim 29 wherein said P16 dipeptide comprises the
structure:



wherein

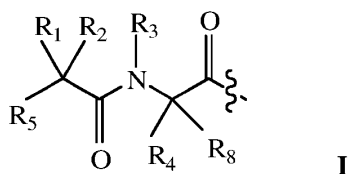
R_1 comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-
C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally
via said P16 spacer;

R_2 , R_4 and R_8 are each H;

R_3 is C₁-C₆ alkyl; and

R_5 is NH₂, wherein said P16 spacer comprises -[COCH₂(OCH₂CH₂)_k-NH]_q-
(gamma glutamic acid)_p, k is 2, q is 1 or 2 and p is 0 or 1.

31. The prodrug derivative of claim 29 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 P16 spacer;

R₂ is H;

R₄ and R₈ are each D;

R₃ is CD₃; and

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

32. The prodrug derivative of claim 30 or 31 wherein said P16 spacer is -[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid), and the acyl group is a C18-C20 diacid.

33. The prodrug derivative of any one of claims 12-32 wherein the first amino acid of said P1, P7, or P16 dipeptide (A-B) is an amino acid in the D-configuration.

34. The prodrug derivative of any one of claims 12-33 wherein R₄ is (C₁-C₄ alkyl)phenyl, or R₃ and R₄ together with the atoms to which they are attached form a piperidine ring.

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

YX₂EGTX₆TSDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 2) or YX₂EGTX₆X₇SDYSIX₁₃LDKIAQX₂₀AFVQWLIAGGPSSGAPPPS-R₂₀ (SEQ ID NO: 3)

wherein

X₂ is Aib;

X₆ is alpha methylated Phc;

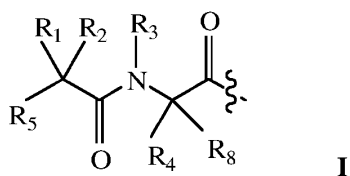
X₇ is isoacyl-Thr;

X₁₃ is Aib;

X₂₀ is an amino acid comprising a (C1-C8 alkyl)NH₂ side chain that has been acylated with a C16-C30 acyl group, a C16-C30 phosphonic fatty acid, or a C16-C30 alkyl group, optionally via a P20 spacer; and

R₂₀ is CONH₂, 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

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 via a P1 spacer;

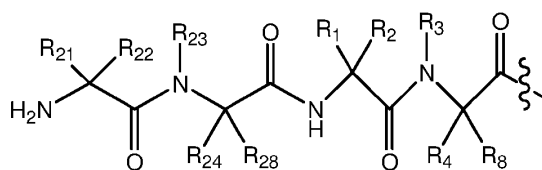
R₂ each H;

R₄ and R₈ are independently H or D;

R₃ is CD₃, CH₃, or R₃ and R₄ together with the atoms to which they are attached form a piperidine ring; and

R₅ is NH₂, wherein said P1 spacer and P20 spacer 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.

36. The prodrug derivative of any one of claims 12-35 wherein any one or more of the P1, P7 or P16 dipeptides comprise a sequential dipeptide structure of



covalently linked via an amide bond to said peptide,

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 DD1 spacer;

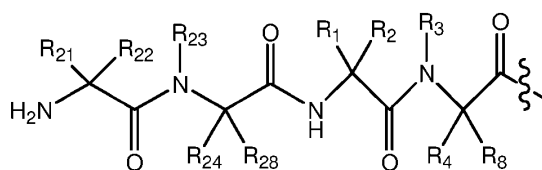
R_{21} is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

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 is C₁-C₆ alkyl, CD₃, 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.

37. The prodrug derivative of claim 35 wherein said sequential dipeptide comprises the structure:



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 DD1 spacer;

R_{21} is selected from an acylated amino acid having a side chain comprising $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

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

R_3 and R_{23} are independently C_1-C_6 alkyl; 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.

38. The prodrug derivative of claim 36 or 37 wherein said DD1 spacer and DD2 spacer are each $-[COCH_2(OCH_2CH_2)_2-NH]-(\text{gamma glutamic acid})$ -, and the acyl group is a C18-C20 diacid.

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

$YX_2EGTX_6X_7SDYSIX_{13}LDKIAQX_{20}AFVQWLIAGGPSSGAPPPS-R_{20}$ (SEQ ID NO: 3)

wherein

X_2 is Aib;

X_6 is alpha methylated Phe;

X_7 is isoacyl-Thr;

X_{13} is Aib;

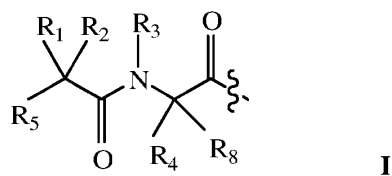
X_{20} is an amino acid comprising a $(C_1-C_4 \text{ alkyl})NH_2$ side chain that has been pegylated, optionally via a first spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k; and

R_{20} is $COOH$ or $CONH_2$, wherein

i) a dipeptide is covalently linked via an amide bond to the amine of 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:



wherein

R₁ 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;

R₂ is H, or C₁-C₄ alkyl;

R₄ 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;

R₈ is 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 pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

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

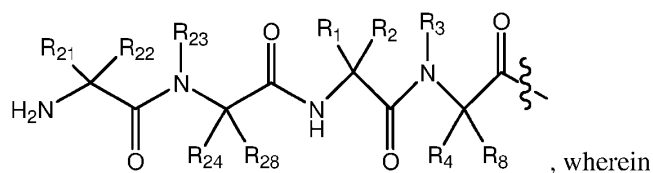
-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1.

40. The prodrug derivative of claim 39 wherein said dipeptide is linked to the amine of isoacyl-Thr at position 7 of the peptide, and at the N-terminal alpha amine of the peptide.

41. The prodrug derivative of claim 40 wherein the dipeptide linked to the amine of isoacyl-Thr at position 7 of the peptide, is the same as the dipeptide linked to the N-terminal alpha amine of the peptide.

42. The prodrug derivative of any of claims 39-41 wherein one or more of the

- i) the dipeptide linked to the amine of isoacyl-Thr at position 7 of the peptide, or
- ii) the dipeptide linked to the N-terminal alpha amine, or
- iii) both i) and ii) is a sequential dipeptide "A-B-C-D" comprising the structure of



R_1 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;

R_{21} 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;

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 is 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; and

R_{23} is CD₃, C₁-C₆ alkyl, or R_{23} and R_{24} 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

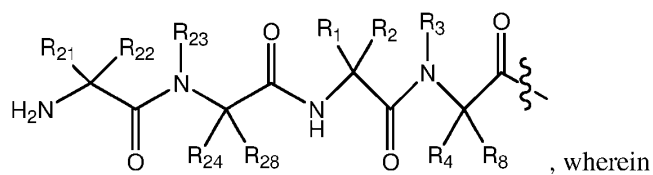
-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

43. A prodrug derivative of the peptide of claim 11, wherein

X_{20} 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; and

a sequential dipeptide is linked to the N-terminal alpha amine, wherein said

a sequential dipeptide "A-B-C-D" comprises the structure of



R_1 and R_{21} independently comprises $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}CO_2H$ or $-(CH_2)_4NH(COCH_2(OCH_2CH_2)_2NH)_2-\gamma E-COC_{18}H_{36}PO_3H_2$;

R_2 , and R_{22} are each H;

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

R_4 , R_8 , R_{22} , R_{24} and R_{28} are independently H or D.

44. A pharmaceutical composition comprising the peptide of any one of claims 1 to 43 and a pharmaceutically acceptable carrier.

45. A kit comprising a pharmaceutical composition of claim 44 and a device for administering said pharmaceutical composition to the patient.

46. The kit of claim 45, wherein the device comprises a syringe comprising the pharmaceutical composition.

47. A method of reducing weight gain or inducing weight loss, comprising administering to a patient in need thereof a pharmaceutical composition of claim 43 in an amount effective to reduce weight gain or induce weight loss.

48. A method of treating diabetes, comprising administering to a patient in need thereof a pharmaceutical composition of claim 44 in an amount effective to lower blood glucose levels.