ACYLATED GLUCAGON ANALOGUES

FIELD OF THE INVENTION

The present invention relates to acylated glucagon analogues and their medical use, for example in the treatment of obesity and excess weight, diabetes, and other metabolic disorders.

BACKGROUND OF THE INVENTION

Pre-proglucagon is a 158 amino acid precursor polypeptide that is differentially processed in the tissues to form a number of structurally related proglucagon-derived peptides, including glucagon (Glu), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and oxyntomodulin (OXM). These molecules are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying and intestinal growth, as well as regulation of food intake.

Glucagon is a 29-amino acid peptide that corresponds to amino acids 53 to 81 of pre-proglucagon. Oxyntomodulin (OXM) is a 37 amino acid peptide which includes the complete 29 amino acid sequence of glucagon with an octapeptide carboxyterminal extension (amino acids 82 to 89 of pre-proglucagon, and termed "intervening peptide 1" or IP-1. The major biologically active fragment of GLP-1 is produced as a 30-amino acid, C-terminally amidated peptide that corresponds to amino acids 98 to 127 of pre-proglucagon.

Glucagon helps maintain the level of glucose in the blood by binding to glucagon receptors on hepatocytes, causing the liver to release glucose – stored in the form of glycogen – through glycogenolysis. As these stores become depleted, glucagon stimulates the liver to synthesize additional glucose by gluconeogenesis. This glucose is released into the bloodstream, preventing the development of hypoglycemia.

GLP-1 decreases elevated blood glucose levels by improving glucose-stimulated insulin secretion and promotes weight loss chiefly through decreasing food intake.

OXM is released into the blood in response to food ingestion and in proportion to meal calorie content. OXM has been shown to suppress appetite and inhibit food intake in humans (Cohen et al, Journal of Endocrinology and Metabolism, 88, 4696-4701, 2003; WO 2003/022304). In addition to those anorectic effects, which are similar to those of GLP-1, OXM must also affect body weight by another mechanism, since rats treated with oxyntomodulin show less body weight gain than pair-fed rats (Bloom, Endocrinology 2004, 145, 2687). Treatment of obese rodents with OXM also improves their glucose tolerance (Parlevliet et al, Am J Physiol Endocrinol Metab, 294, E142-7, 2008) and suppresses body weight gain (WO 2003/022304).

OXM activates both the glucagon and the GLP-1 receptors with a two-fold higher potency for the glucagon receptor over the GLP-1 receptor, but is less potent than native glucagon and GLP-1 on their respective receptors. Human glucagon is also capable of activating both receptors, though with a strong preference for the glucagon receptor over the GLP-1 receptor. GLP-1 on the other hand is not capable of activating glucagon receptors. The mechanism of action of oxyntomodulin is not well understood. In particular, it is not known whether some of the extrahepatic effects of the hormone are mediated through the GLP-1 and glucagon receptors, or through one or more unidentified receptors.

Other peptides have been shown to bind and activate both the glucagon and the GLP-1 receptor (Hjort et al, Journal of Biological Chemistry, 269, 30121-30124, 1994) and to suppress body weight gain and reduce food intake (see, for example, WO 2006/134340, WO 2007/100535, WO 2008/10101, WO 2008/152403, WO 2009/155257, WO 2009/155258, WO2010/070252, WO2010/070253, WO2010/070255, WO2010/070251, WO2011/006497, WO2011/160630, WO2011/160633, WO2013/092703, WO2014/041195.

Obesity is a globally increasing health problem associated with various diseases, particularly cardiovascular disease (CVD), type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis. As a result, obesity has been found to reduce life expectancy. According to 2005 projections by the World Health Organization there are 400 million adults (age > 15) classified as obese worldwide. In the US, obesity is now believed to be the second-leading cause of preventable death after smoking.

The rise in obesity drives an increase in diabetes, and approximately 90% of people with type 2 diabetes may be classified as obese. There are 246 million people worldwide with diabetes, and by 2025 it is estimated that 380 million will have diabetes. Many have additional cardiovascular risk factors, including high/aberrant LDL and triglycerides and low HDL.

SUMMARY

In a first aspect, the present disclosure provides a compound having the formula:

R^1-P^1-P^2-R^2

wherein

R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

P^1 is a peptide having the sequence:

H-X2-X3-GTFTSDYSKYLDSΨAAHDFVEWLLSA

wherein:

X2 is selected from Aib, Ala, D-Ala, Ser, N-Me-Ser, Ac3c, Ac4c and Ac5c;

X3 is selected from Gln and His;

P^2 is absent or is a sequence of 1-20 amino acid units independently selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Cys, Glu, Lys, Arg, Dbu, Dpr and Orn;

or a pharmaceutically acceptable salt or solvate thereof;

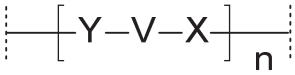
Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent having the formula $–Z^2$-Z^1;

$–Z^1$ is a fatty chain having a polar group at one end of the chain and a connection to Z^2, –X– at the end of the chain distal from the polar group,

wherein the polar group comprises a carboxylic acid or a carboxylic acid bioisostere, a phosphonic acid, or a sulfonic acid group;

and –X– is a bond, –CO–, –SO–, or $–SO_2–$;

$–Z^2–$ is a spacer of formula:



wherein:

each Y is independently –NH, –NR, –S or –O, where R is alkyl, a protecting group or forms a linkage to another part of the spacer Z^2;

each X is independently a bond, CO–, SO–, or $SO_2–$;

with the proviso that when Y is –S, X is a bond;

each V is independently a bivalent organic moiety linking Y and X;

and n is 1-10;

or a pharmaceutically acceptable salt or solvate thereof.

P^1 may have the sequence:
H-Aib-QGTFTSDYSKYLDSΨAAHDFVEWLLSA

e.g.

H-Aib-QGTFTSDYSKYLDS-K([15-carboxy-pentadecanoyl]-isoGlu)-AAHDFVEWLLSA.

The compound as disclosed herein may be:
H-H-Aib-QGTFTSDYSKYLDSΨAAHDFVEWLLSA-NH_2

e.g.

H-H-Aib-QGTFTSDYSKYLDS-K([15-carboxy-pentadecanoyl]-isoGlu)-AAHDFVEWLLSA-NH_2.

In a second aspect, the present disclosure provides a compound having the formula:
R^1-P^1-P^2-R^2
wherein
R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;
R^2 is OH or NH_2;
P^1 is a peptide having the sequence:

His-X2-X3-GTFTSDYSKYL-X15-X16-X17-X18-A-X20-DFI-X24-WLE-X28-A

wherein:
X2 is selected from Aib, Ac3c, Ac4c and Ac5c;
X3 is selected from Gln and His;
X15 is selected from Asp and Glu;
X16 is selected from Glu and Ψ;
X17 is selected from Arg and Ψ;
X18 is selected from Ala and Arg;
X20 is selected from Lys and His;
X24 is selected from Glu and Ψ;
X28 is selected from Ser and Ψ;

and P^2 is absent or is a sequence of 1-20 amino acid units independently selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Cys, Glu, Lys, Arg, Dbu, Dpr and Orn;

wherein the compound contains one and only one Ψ

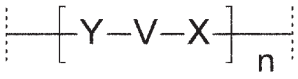
and wherein said Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent having the formula $–Z^2-Z^1$;

$–Z^1$ is a fatty chain having a polar group at one end of the chain and a connection to Z^2, –X– at the end of the chain distal from the polar group,

wherein the polar group comprises a carboxylic acid or a carboxylic acid bioisostere, a phosphonic acid, or a sulfonic acid group;

and –X– is a bond, –CO–, –SO–, or $–SO_2–$;

$–Z^2–$ is a spacer of formula:



wherein:

each Y is independently –NH, –NR, –S or –O, where R is alkyl, a protecting group or forms a linkage to another part of the spacer Z^2;

each X is independently a bond, CO–, SO–, or $SO_2–$;

with the proviso that when Y is –S, X is a bond;

each V is independently a bivalent organic moiety linking Y and X;

and n is 1-10;

or a pharmaceutically acceptable salt or solvate thereof.

In some embodiments of the second aspect:

X2 is selected from Aib and Ac4c;

X3 is Gln;

X15 is selected from Asp and Glu;

X16 is Ψ;

X17 is Arg;

X18 is Ala;

X20 is selected from Lys and His;

X24 is Glu;

X28 is Ser.

Useful combinations of residues include the following:
X2 is Ac4c and X20 is Lys;
X2 is Aib and X20 is His.

Additionally or alternatively, it may be desirable that X2 is Aib if X15 is E or that X15 is D if X2 is Ac4c.

Particularly interesting substituents Z^2Z^1 include [17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3 and [17-carboxy-heptadecanoyl]-isoGlu-GSGSGG.

P^1 may have a sequence selected from:
H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLEΨRAAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDΨRAAKDFIEWLESA and
H-Aib-QGTFTSDYSKYLEΨRAAHDFIEWLESA

e.g. from

H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA and
H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAHDFIEWLESA

The compound as disclosed herein may be selected from:
H-H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2
H-H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2
H-H-Aib-QGTFTSDYSKYLEΨRAAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2 and
H-H-Aib-QGTFTSDYSKYLEΨRAAHDFIEWLESA-NH_2

e.g. from

H-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA-NH_2

H-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2

H-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2

H-H-Ac4c-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2 and

H-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAHDFIEWLESA-NH_2

In alternative embodiments of the scond aspect:

X2 is selected from Aib and Ac4c;

X3 is selected from Gln and His;

X15 is Asp;

X16 is Glu;

X17 is selected from Arg and Ψ;

X18 is selected from Ala and Arg;

X20 is Lys;

X24 is selected from Glu and Ψ;

X28 is selected from Ser and Ψ;

In some embodiments, when X28 is Ψ, X2 is Ac4c.

In some embodiments, when X3 is His, X2 is Ac4c and X17 is Ψ.

In some embodiments, when X17 is Ψ, Z^2Z^1 is [17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3 or [17-carboxy-heptadecanoyl]-isoGlu.

In some embodiments, when X24 or X28 is Ψ, Z^2Z^1 is [17-carboxy-heptadecanoyl]-isoGlu-GSGSGG.

P^1 may have a sequence selected from:

H-Aib-QGTFTSDYSKYLDEΨAAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Ac4c-HGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨAAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Aib-QGTFTSDYSKYLDERAAKDFIΨWLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFIΨWLESA
H-Ac4c-QGTFTSDYSKYLDERRAKDFIΨWLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLEΨA and
H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLEΨA

e.g. from

H-Aib-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA
H-Ac4c-HGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-RAKDFIEWLESA
H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERRAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A and
H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2

The compound as disclosed herein may be selected from:
H-H-Aib-QGTFTSDYSKYLDEΨAAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
H-H-Ac4c-HGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDEΨAAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
H-H-Aib-QGTFTSDYSKYLDERAAKDFIΨWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERAAKDFIΨWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIΨWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLEΨA-NH_2 and
H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLEΨA-NH_2

e.g. from

H-H-Aib-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2
H-H-Ac4c-HGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-RAKDFIEWLESA-NH_2
H-H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERRAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2
H-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2 and
H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2

For the avoidance of doubt, in all aspects of the present disclosure, those positions which are not expressly stated to permit variability are fixed and thus may only include the stated residue.

In all aspects, the compound as disclosed herein comprises a residue Ψ, i.e. a residue selected from Lys, Arg, Orn and Cys in which the side chain is conjugated to a substituent –Z^2-Z^1- as decribed in more detail below.

The substituent is conjugated to the functional group at the distal end of the side chain from the alpha-carbon. The normal ability of the Lys, Arg, Orn or Cys side chain to participate in interactions mediated by that functional group (e.g. intra- and inter-molecular interactions) may therefore be reduced or completely eliminated by the presence of the substituent. Thus, the overall properties of the compound may be relatively insensitive to changes in the actual amino acid present as residue Ψ. Consequently, it is believed that any of the residues Lys, Arg, Orn and Cys may be present at any position where Ψ is permitted. However, in certain embodiments, it may be advantageous that the amino acid component of Ψ is Lys.

In some embodiments, –Z^1 is an acyl group of formula:

A–B–Alk–(CO)–

or a sulfonyl group of formula:

$$A–B–Alk–(SO_2)–;$$

A is –COOH or a carboxylic acid bioisostere;

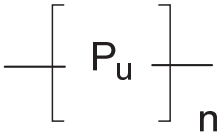
B is a bond, C_6arylene, or C_6arylene–O–;

Alk is a saturated or unsaturated fatty chain of 6 to 18 carbon atoms in length, optionally substituted with one or more substituents selected from fluoro, C_{1-4}alkyl, trifluoromethyl, hydroxymethyl, amino, hydroxyl, C_{1-4}alkoxy, oxo, and carboxyl;

$–Z^2–$ is $–S_A–$, $–S_A–S_B–$, or $–S_B–S_A–$;

$–S_A–$ is a single amino acid residue selected from γ-Glu, α-Glu, α-Asp, β-Asp, Ala, β-Ala (3-aminopropanoic acid), and Gaba (4-aminobutanoic acid);

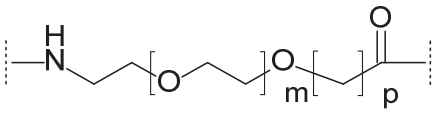
$–S_B–$ is a linker of general formula:



wherein n is 1–10 and each P_U is independently selected from P_U^{i} and P_U^{iii};

each P_U^{i} is independently a natural or unnatural amino acid residue; and

each P_U^{iii} is independently a residue of general formula:



wherein m is 0–5 and p is 1, 3, 4, or 5.

In any aspect of the present disclosure, R^1 may be selected from H and C_{1-4} alkyl (e.g. methyl).

The compounds of the invention as disclosed herein are glucagon analogue peptides. References herein to a glucagon analogue peptide should be construed as references to a compound of the invention or to a peptide P^1 or P^1-P^2 as the context requires. Reference to a compound of the invention should be taken to include any pharmaceutically acceptable salt (e.g. an acetate or chloride salt) or solvate thereof, unless otherwise stated or excluded by context.

In an aspect, the present disclosure provides a composition comprising a compound as defined herein (including pharmaceutically acceptable salts or solvates thereof, as already described) in admixture with a carrier. In preferred embodiments, the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier. The glucagon analogue peptide may be in the form of a pharmaceutically acceptable salt of the glucagon analogue.

The compounds described herein find use, *inter alia*, in preventing weight gain or promoting weight loss. By "preventing" is meant inhibiting or reducing when compared to the absence of treatment, and is not necessarily meant to imply complete cessation of weight gain. The peptides may cause a decrease in food intake and/or increased energy expenditure, resulting in the observed effect on body weight. Independently of their effect on body weight, the compounds of the invention may have a beneficial effect on glucose control and/or on circulating cholesterol levels, being capable of lowering circulating LDL levels and increasing HDL/LDL ratio. Thus the compounds of the invention can be used for direct or indirect therapy of any condition caused or characterised by excess body weight, such as the treatment and/or prevention of obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea. They may also be used for the prevention of conditions caused or characterised by inadequate glucose control or dyslipidaemia (e.g. elevated LDL levels or reduced HDL/LDL ratio), diabetes (especially Type 2 diabetes), metabolic syndrome, hypertension, atherogenic dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease. Their effects in these conditions may be as a result of or associated with their effect on body weight, or may be independent thereof.

In an aspect, the present disclosure also provides a compound as disclosed herein for use in a method of medical treatment, particularly for use in a method of treatment of a condition as described above.

The present disclosure also provides the use of a compound as disclosed herein in the preparation of a medicament for the treatment of a condition as described above. In an aspect, the present disclosure provides the use of a compound as disclosed herein in the manufacture of a medicament for preventing weight gain or promoting weight loss in an individual in need thereof. In another aspect, the present disclosure provides the use of a compound as disclosed herein in the manufacture of a medicament for lowering circulating LDL levels, and/or increasing HDL/LDL ratio in an individual in need thereof. In yet another aspect, the present disclosure provides the use of a compound as disclosed herein in the manufacture of a medicament for treatment of a condition caused or characterised by excess body weight. In a further aspect, the present disclosure provides the use of a compound as disclosed herein in the manufacture of a medicament for prevention or

recovering the expression product, and modifying the precursor peptide to yield a compound as disclosed herein.

The precursor peptide may be expressed from a nucleic acid encoding the precursor peptide in a cell or a cell-free expression system comprising such a nucleic acid.

In another aspect, there is provided a compound having the formula: R^1-P^1-R^2 wherein R^1 is hydrogen (Hy), C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl; R^2 is OH or NH_2; P^1 is a peptide having the sequence: H-X2-X3-GTFTSDYSKYLDSΨAAHDFVEWLLSA,

wherein:

X2 is selected from Aib, Ala, D-Ala, Ser, N-Me-Ser, Ac3c, Ac4c and Ac5c;

X3 is selected from Gln and His;

and Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent selected from:

(i) [17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3;

(ii) [17-Carboxy-heptadecanoyl]-isoGlu

(iii) [13-Carboxy-tridecanoyl]-isoGlu-Peg3-Peg3;

(iv) [Carboxyphenoxynonanoyl]-isoGlu-Peg3-Peg3;

(v) [13-Carboxy-tridecanoyl]-isoGlu-Peg4-Peg4;

(vi) [17-Carboxy-heptadecanoyl]-Peg3-Peg3-isoGlu;

(vii) [17-Carboxy-heptadecanoyl]-isoGlu-GSGSGG; and

(viii) [17-Carboxy-heptadecanoyl]-AA-Peg3-Peg3;

or a pharmaceutically acceptable salt or solvate thereof.

In another aspect, there is provided a compound having the formula:

R^1-P^1-R^2

wherein

R^1 is hydrogen (Hy), C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

P^1 is a peptide having the sequence:

His-X2-X3-GTFTSDYSKYL-X15-X16-X17-X18-A-X20-DFI-X24-WLE-X28-A

wherein:

X2 is selected from Aib, Ac3c, Ac4c and Ac5c;

X3 is selected from Gln and His;

X15 is selected from Asp and Glu;

X16 is selected from Glu and Ψ;

X17 is selected from Arg and Ψ;

X18 is selected from Ala and Arg;

X20 is selected from Lys and His;

X24 is selected from Glu and Ψ;

X28 is selected from Ser and Ψ;

wherein the compound contains one and only one Ψ

and wherein said Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent selected from:

(i) [17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3;

(ii) [17-Carboxy-heptadecanoyl]-isoGlu

(iii) [13-Carboxy-tridecanoyl]-isoGlu-Peg3-Peg3;

(iv) [Carboxyphenoxynonanoyl]-isoGlu-Peg3-Peg3;

(v) [13-Carboxy-tridecanoyl]-isoGlu-Peg4-Peg4;

(vi) [17-Carboxy-heptadecanoyl]-Peg3-Peg3-isoGlu;

(vii) [17-Carboxy-heptadecanoyl]-isoGlu-GSGSGG; and

(viii) [17-Carboxy-heptadecanoyl]-AA-Peg3-Peg3;

or a pharmaceutically acceptable salt or solvate thereof.

In another aspect, there is provided a compound selected from the group consisting of:

Hy-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA-NH2;

Hy-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH2;

Hy-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH2;

Hy-H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH2;

Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH2; and

Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH2.

DETAILED DESCRIPTION OF THE INVENTION

Throughout this specification, the conventional one letter and three letter codes for naturally occurring amino acids are used, as well as generally accepted abbreviations for other amino

acids, such as Aib (α-aminoisobutyric acid), Orn (ornithine), Dbu (2,4-diaminobutyric acid), Dpr (2,3-diaminopropanoic acid), Ac3c (1-amino-cyclopropanecarboxylic acid), Ac4c (1-amino-cyclobutanecarboxylic acid) and Ac5c (1-amino-cyclopentanecarboxylic acid).

Ac3c, Ac4c and Ac5c have similar structures and are to some extent interchangeable, although Ac4c may be preferred.

Glucagon is a 29-amino acid peptide that corresponds to amino acids 53 to 81 of pre-proglucagon and has the sequence His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr. Oxyntomodulin (OXM) is a 37 amino acid peptide which includes the complete 29 amino acid sequence of glucagon with an octapeptide carboxyterminal extension (amino acids 82 to 89 of pre-proglucagon, having the sequence Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala and termed "intervening peptide 1" or IP-1; the full sequence of human oxyntomodulin is thus His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala). The major biologically active fragment of GLP-1 is produced as a 30-amino acid, C-terminally amidated peptide that corresponds to amino acids 98 to 127 of pre-proglucagon.

The term "native glucagon" thus refers to native human glucagon having the sequence H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH.

Amino acids within the sequence P^1 of the compounds of the invention can be considered to be numbered consecutively from 1 to 29 in the conventional N-terminal to C-terminal direction. Reference to a "position" within P^1 should be construed accordingly, as should reference to positions within native human glucagon and other molecules.

A compound of the invention may comprise a C-terminal peptide sequence P^2 of 1-20 amino acids, for example to stabilise the conformation and/or secondary structure of the glucagon analogue peptide, and/or to render the glucagon analogue peptide more resistant to enzymatic hydrolysis, e.g. as described in WO99/46283.

When present, P^2 represents a peptide sequence of 1-20 amino acid residues, e.g. in the range of 1-15, more preferably in the range of 1-10, in particular in the range of 1-7 amino acid residues, e.g., 1, 2, 3, 4, 5, 6 or 7 amino acid residues, such as 6 amino acid residues. Each of the amino acid residues in the peptide sequence P^2 may independently be selected from Ala, Leu, Ser, Thr, Tyr, Cys, Glu, Lys, Arg, Dbu (2,4-diaminobutyric acid), Dpr (2,3-diaminopropanoic acid) and Orn (ornithine). Preferably, the amino acid residues are selected from Ser, Thr, Tyr, Glu, Lys, Arg, Dbu, Dpr and Orn, more preferably selected exclusively

from Glu, Lys, and Cys. The above-mentioned amino acids may have either D- or L-configuration, which in certain embodiments, have an L-configuration. Particularly preferred sequences P^2 are sequences of four, five, six or seven consecutive lysine residues (i.e. Lys_3, Lys_4, Lys_5, Lys_6 or Lys_7), and particularly five or six consecutive lysine residues. Other exemplary sequences of P^2 are shown in WO 01/04156. Alternatively the C-terminal residue of the sequence P^2 may be a Cys residue. This may assist in modification (e.g. PEGylation, or conjugation to albumin) of the compound. In such embodiments, the sequence P^2 may, for example, be only one amino acid in length (i.e. P^2 = Cys) or may be two, three, four, five, six or even more amino acids in length. The other amino acids therefore serve as a spacer between the peptide P^1 and the terminal Cys residue.

The peptide sequence P^2 has no more than 25% sequence identity with the corresponding sequence of the IP-1 portion of human OXM (which has the sequence Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala).

"Percent (%) amino acid sequence identity" of a given peptide or polypeptide sequence with respect to another polypeptide sequence (e.g. IP-1) is calculated as the percentage of amino acid residues in the given peptide sequence that are identical with correspondingly positioned amino acid residues in the corresponding sequence of that other polypeptide when the two are aligned with one another, introducing gaps for optimal alignment if necessary. % identity values may be determined using WU-BLAST-2 (Altschul et al., Methods in Enzymology, 266:460-480 (1996)). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11. A % amino acid sequence identity value is determined by the number of matching identical residues as determined by WU-BLAST-2, divided by the total number of residues of the reference sequence (gaps introduced by WU-BLAST-2 into the reference sequence to maximize the alignment score being ignored), multiplied by 100.

Thus, when P^2 is aligned optimally with the 8 amino acids of IP-1, it has no more than two amino acids which are identical with the corresponding amino acids of IP-1.

In certain embodiments, P^2 is absent.

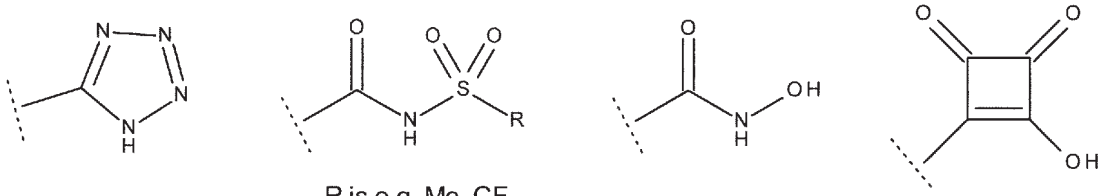
Ψ is a residue of Lys, Arg, Orn or Cys whose side chain is conjugated to a substituent Z^2-Z^1. Without wishing to be bound by any particular theory, it is thought that the substituent binds plasma proteins (e.g. albumin) in the blood stream, thus shielding the compounds of the invention from enzymatic degradation and thereby enhancing the half-life of the compounds. It may also modulate the potency of the compound, e.g. with respect to the glucagon receptor and/or the GLP-1 receptor.

The group Z^1

Z^1 is a fatty chain having a connection to Z^2, referred to herein as –X– and, at the end of the chain distal from the connection to Z^2, a polar group. –X– may be, for example, a bond, acyl (–CO–), sulfinyl (–SO–), or sulfonyl ($–SO_2–$), the connection being located at the ω-position with respect to the polar group, that is, at the end of the chain distal from the polar group.

Preferably, the polar group is an acidic or weakly acid group, for example a carboxylic acid or a carboxylic acid bioisostere, a phosphonate, or a sulfonate. The polar group may have a pK_a of between –2 and 12 in water, more preferably between 1 and 7, more preferably between 3 and 6. Certain preferred polar groups have a pK_a of between 4 and 5.

The polar group preferably comprises a carboxylic acid or carboxylic acid bioisostere. Suitable carboxylic acid bioisosteres are known in the art. Preferably the bioisostere has a proton having a pK_a similar to the corresponding carboxylic acid. Examples of suitable bioisoteres may include, not by way of limitation, tetrazole, acylsulfomides, acylhydroxylamine, and squaric acid derivatives, as shown below (--- indicates the point of attachment):



R is e.g. Me, CF_3

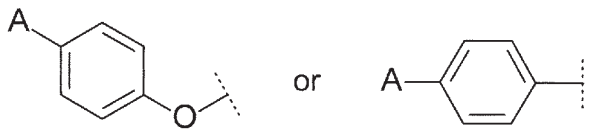
The polar group may be a group of formula A–B–, wherein A is a carboxylic acid (–COOH) or a carboxylic acid bioisostere, a phosphonic acid ($–P(O)(OH)_2$), or a sulfonic acid ($–SO_2OH$) group, and B is a bond or linker between A and the fatty chain. In some embodiments, the polar group is –COOH, that is, A is –COOH and B is a bond.

When B is a linker, it may be a cycloalkylene, heterocycloalkylene, C_6arylene, or C_{5-6}heteroarylene, or C_6arylene–O– or C_{5-6}heteroarylene–O–.

When B is phenylene it may, for example, be selected from 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, preferably 1,4-phenylene (so that A–B– is a 4-benzoic acid substituent or 4-benzoic acid bioisostere). When B is phenylene–O–, it may, for example, be selected from 1,2-phenylene–O–, 1,3-phenylene–O–, 1,4-phenylene–O–, preferably 1,4-phenylene–O. Each phenylene of B may be optionally substituted with one or more substituents selected from fluoro, methyl, trifluoromethyl, amino, hydroxyl, and C_{1-4}alkoxy, preferably methoxy. It will be appreciated that substituent identity and position may be selected to subtly alter the pK_a of the polar group. Suitable inductively or mesomerically electron-withdrawing or donating groups and their positional effects are known in the art. In some embodiments, B may be

C_{5-6}heteroarylene, for example, pyridinylene or thiofuranylene, and may be optionally substituted as described.

For example, in some embodiments, A–B– may be selected from:



Preferably, A is –COOH. In some preferred polar groups, A is a carboxylic acid and B is C_6arylene–O–.

Fatty chain as used herein refers to a moiety comprising a chain of carbon atoms, the carbon atoms being predominantly substituted with hydrogen or hydrogen-like atoms, for example, a hydrocarbon chain. Such fatty chains are often referred to as lipophilic, although it will be appreciated that substitution may alter the lipophilic properties of the overall molecule.

The fatty chain may by aliphatic. It may be entirely saturated or may include one or more double or triple bonds. Each double bond, if present, may be in the *E* or *Z* configuration. The fatty chain may also have one or more cycloalkylene or heterocycloalkylene moieties in its length, and additionally or alternatively may have one or more arylene or heteroarylene moieties in its length. For example, the fatty chain may incorporate a phenylene or piperazinylene moiety in its length as, for example, shown below (wherein --- represents the points of attachment within the chain).



The fatty chain may be derived from a fatty acid, for example, it may be derived from a medium-chain fatty acid (MCFA) with an aliphatic tail of 6–12 carbon atoms, a long-chain fatty acid (LCFA) with an aliphatic tail of 13–21 carbon atoms, or a very long-chain fatty acid (LCFA) with an aliphatic tail of 22 carbon atoms or more. Examples of linear saturated fatty acids from which suitable fatty chains may be derived include tridecylic (tridecanoic) acid, myristic (tetradecanoic) acid, pentadecylic (pentadecanoic) acid, palmitic (hexadecanoic) acid, and margaric (heptadecanoic) acid. Examples of linear unsaturated fatty acids from which suitable fatty chains may be derived include myristoleic acid, palmitoleic acid, sapienic acid and oleic acid.

The fatty chain may be connected to Z^2 by an amide linkage, a sulfinamide linkage, a sulfonamide linkage, or by an ester linkage, or by an ether, thioether or amine linkage. Accordingly, the fatty chain may have at the ω position, that is, the position distal to the polar group, a

bond to Z^2 or an acyl (–CO–), sulfinyl (–SO–), or sulfonyl ($–SO_2–$) group. Preferably, the fatty chain has an acyl (–CO–) group at the position distal to the polar group and is connected to Z^2 by an amide or ester linkage.

In some embodiments, Z^1 is a group of formula:

A–B–Alk–X–

where A–B– is the polar group defined above, X is a bond, acyl (–CO–), sulfinyl (–SO–), or sulfonyl ($–SO_2–$), and Alk is a fatty chain that may be optionally substituted with one or more substituents. The fatty chain is preferably 6 to 18 carbon atoms in length (e.g. a C_{6-18}alkylene), more preferably, 8 to 18 carbons in length (e.g. a C_{8-18}alkylene), more preferably, 12 to 16 carbons in length (e.g. C_{12-16}alkylene), and may be saturated or unsaturated. Preferably, Alk is saturated, that is, preferably Alk is alkylene.

In some embodiments, Z^1 is an acyl group of formula:

A–B–Alk–(CO)–

or a sulfonyl group of formula:

A–B–Alk–(SO_2)–.

Optional substituents on the fatty chain may be independently selected from fluoro, C_{1-4}alkyl, preferably methyl; trifluoromethyl, hydroxymethyl, amino, hydroxyl, C_{1-4}alkoxy, preferably methoxy; oxo, and carboxyl, and may be independently located at any point along the chain. In some embodiments, each optional substituent is selected from fluoro, methyl, and hydroxyl. Where more than one substituent is present, substituents may be the same or different. Preferably, the number of substituents is 0 to 3; more preferably the fatty chain is unsubstituted.

Preferably, Z^1 is an acyl group of formula:

A–B–alkylene–(CO)–

Where A and B are as defined above .

In some embodiments, Z^1 is:

4-carboxyphenoxynonanoyl HOOC–C_6H_4–O–$(CH_2)_8$–(CO)–.

Certain preferred Z^1 are derived from long-chain saturated α,ω-dicarboxylic acids of formula HOOC–$(CH_2)_{12-18}$–COOH, preferably, long-chain saturated α,ω-dicarboxylic acids having an even number of carbon atoms in the aliphatic chain. For example, and not by way of limitation, Z^1 may be:

13-carboxytridecanoyl HOOC–$(CH_2)_{12}$–(CO)–

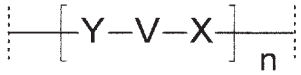
15-carboxpentadecanoyl HOOC–$(CH_2)_{14}$–(CO)–; or

17-carboxyheptadecanoyl HOOC–$(CH_2)_{16}$–(CO)–.

The carboxylic acid group may be replaced by a bioisotere as detailed herein.

The group Z^2

Z^2 is spacer that connects Z^1 to the side chain of the amino acid component of Ψ. At its most general, Z^2 is a spacer bound at one terminus by Y, which may be a nitrogen, oxygen or sufhur atom, and at the other terminus by X, which may be a bond or an acyl (–CO–), sulfinyl (–SO–), or sulfonyl ($–SO_2–$). Accordingly, Z^2 may be a spacer of formula (--- indicate points of attachment):



wherein:

Y may be –NH, –NR, –S or –O, where R may be alkyl, a protecting group or may form a linkage to another part of the spacer, with the remaining valency forming a linkage to Z^1;

X may be a bond, CO–, SO–, or $SO_2–$, with the remaining valency forming a linkage to the side chain of the amino acid component of Ψ;

V is a bivalent organic moiety linking Y and X;

and n may be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Where n is 2 or more, each Y, V, and X is independent of every other Y, V, and X.

Accordingly, Z^2 may be bound at each side by amide, sulfinamide, sulfonamide, or ester linkages or by amino, ether, or thioether linkages depending upon the nature of Y and X and the corresponding linking groups on Z^1 and the side chain. Preferably, when Y is –S, X is a bond. Where n is 2 or greater, each V may also be bound to each adjacent V by linkages as described. Preferably, linkages are amides, esters or sulfonamides, most preferably amides. Accordingly, in some embodiments, each Y is –NH or –NR and each X is CO– or $SO_2–$.

In some embodiments, Z^2 is a spacer of formula $–S_A–$, $–S_B–$, $–S_A–S_B–$ or $–S_B–S_A–$, wherein S_A and S_B are as defined below.

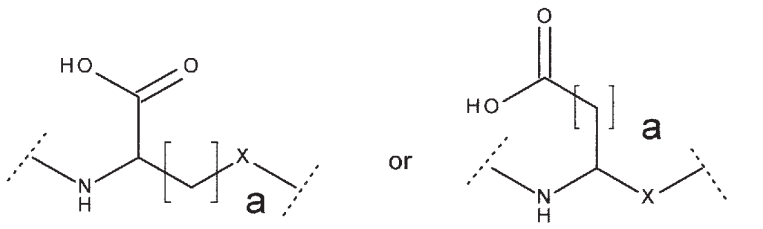
In some embodiments, Z^2 is selected from $–S_A–$ or $–S_B–S_A–$, that is, [side chain]–Z^2Z^1 is [side chain]–$S_A–Z^1$ or [side chain]–$S_B–S_A–Z^1$.

The group S_A

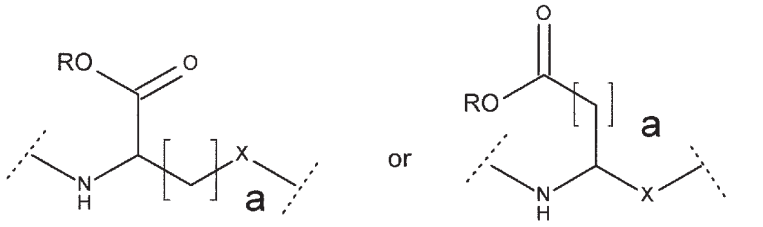
S_A may be a single amino acid residue or a residue of an amino acid derivative, especially an amino acid derivative residue having a sulfinyl or sulfonyl in place of the carboxy moiety at the C terminus. Additionally or alternatively, the single amino acid residue may have an oxygen or sulfur atom in place of the nitrogen atom at the N terminus. Preferably, S_A is a single amino acid residue.

In some embodiments, the amino acid may be selected from γ-Glu, α-Glu, α-Asp, β-Asp, Ala, β-Ala (3-aminopropanoic acid), and Gaba (4-aminobutanoic acid). It will be understood that amino acids may be D or L, or a racemic or enantioenriched mixture. In some embodiments, the amino acid is an L-amino acid. In some embodiments, the amino acid is a D-amino acid.

In some preferred embodiments, S_A has a carboxylic acid substituent, with γ-Glu, α-Glu, α-Asp, and β-Asp, and sulfinyl and sulfonyl derivatives thereof, being preferred. Accordingly, in some embodiments, the amino acid residue is:



where –X– is –CO–, –SO–, $–SO_2–$, preferably –CO–, and a is 1 or 2, preferably 2. In some embodiments, the carboxylic acid is an ester, and the amino acid residue is:

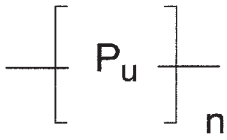


where –X– is –CO–, –SO–, $–SO_2–$, preferably –CO–, and a is 1 or 2, preferably 2, and R is C_{1-4}alkyl or C_6aryl. Preferably R is C_{1-4}alkyl, preferably methyl or ethyl, more preferably ethyl.

Preferably, S_A is γ-Glu.

The group S_B

S_B may be a linker of general formula:



wherein P_U is a polymeric unit and n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. One terminus of the linker S_B is an –NH, –NR, –S or –O, wherein R may be alkyl, a protecting group or may form a linkage to another part of the polymeric unit; while the other is a bond or CO–, SO– or SO_2– . Accordingly, each polymeric unit P_U may be bound at each side by amide, sulfinamide, sulfonamide, or ester linkages or by amino, ether, or thioether linkages depending upon the nature of Y and X and the corresponding linking groups on Z^1, S_A, and Lys.

In some embodiments, each P_U may be independently a unit of formula:



wherein:

Y may be –NH, –NR, –S or –O, wherein R may be alkyl, a protecting group or may form a linkage to another part of the spacer, with the remaining valency forming a linkage to Z^1;

X may be a bond, CO–, SO–, or SO_2–, with the remaining valency forming a linkage to Lys;

and V is a bivalent organic moiety linking Y and X.

In some embodiments, V is the α-carbon of a natural or unnatural amino acid, that is V is –CHR^{AA}–, wherein R^{AA} is an amino acid side chain; or V is an optionally substituted $C_{1\text{-}6}$alkylene, or V is a chain comprising one or more units of ethylene glycol in series, also known as PEG chain, for example, $–CH_2CH_2–(OCH_2CH_2)_m–O–(CH_2)_p–$, where m is 0, 1, 2, 3, 4, or 5, and p is 1, 2, 3, 4, or 5; when X is CO–, p is preferably 1, 3, 4, or 5. Optional alkylene substituents include fluoro, methyl, hydroxy, hydroxymethy, and amino.

Preferred P_U units include:

(i). Single amino acid residues: $P_U{}^i$;

(ii). Dipeptide residues: $P_U{}^{ii}$; and

(iii). Amino-$(PEG)_m$-carboxylic acid residues: $P_U{}^{iii}$,

and may be present in any combination or order. For example, S_B may comprise one or more of each of $P_U{}^i$, $P_U{}^{ii}$, and $P_U{}^{iii}$ in any order, or may comprise one or more units of $P_U{}^i$, $P_U{}^{ii}$, and $P_U{}^{iii}$ only, or one of more units selected from $P_U{}^i$ and $P_U{}^{ii}$, $P_U{}^i$ and $P_U{}^{iii}$, or $P_U{}^{ii}$ and $P_U{}^{iii}$.

(i). P_U^i *single amino acid residues*

Each P_U^i may be independently selected from any natural or unnatural amino acid residue and, for example, may be selected from Gly, Pro, Ala, Val, Leu, Ile, Met, Cys, Phe, Tyr, Trp, His, Lys, Arg, Gln, Asn, α-Glu, γ-Glu, Asp, Ser Thr, Gaba, Aib, β-Ala, 5-aminopentanoyl, 6-aminohexanoyl, 7-aminoheptanoyl, 8-aminooctanoyl, 9-aminononanoyl, and 10-aminodecanoyl. Preferably, P_U^i amino acid residues are selected from Gly, Ser, Ala, Thr, and Cys, more preferably from Gly and Ser.

In some embodiments, S_B is $-(P_U^i)_n-$, wherein n is 1 to 8, more preferably 5 to 7, most preferably 6. In some preferred embodiments, S_B is $-(P_U^i)_n-$, n is 6 and each P_U^i is independently selected from Gly or Ser, with a preferred sequence being -Gly-Ser-Gly-Ser-Gly-Gly-.

(ii). P_U^{ii} *dipeptide residues*

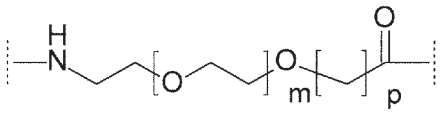
Each P_U^{ii} may be independently selected from any dipeptide residue comprising two natural or unnatural amino acid residues bound by an amide linkage. Preferred P_U^{ii} dipeptide residues include Gly-Gly, Gly-Ser, Ser-Gly, Gly-Ala, Ala-Gly, and Ala-Ala, more preferably Gly-Ser and Gly-Gly.

In some embodiments, S_B is $-(P_U^{ii})_n-$, wherein n is 2 to 4, more preferably 3, and each P_U^{ii} is independently selected from Gly-Ser and Gly-Gly. In some preferred embodiments S_B is $-(P_U^{ii})_n-$, n is 3 and each P_U^{ii} is independently selected from Gly-Ser and Gly-Gly, with a preferred sequence being -(Gly-Ser)-(Gly-Ser)-(Gly-Gly).

Amino acids having stereogenic centres within P_U^i and P_U^{ii} may be racemic, enantioenriched, or enantiopure. In some embodiments, the or each amino acid is independently an L-amino acid. In some embodiments, the or each amino acid is independently a D-amino acid.

(iii). P_U^{iii} *amino-(PEG)$_m$-carboxylic acid residues*

Each P_U^{iii} may be independently a residue of general formula:



wherein m is 0, 1, 2, 3, 4, or 5, preferably 1 or 2, and p is 1, 3, 4, or 5, preferably 1.

In some embodiments, m is 1 and p is 1, that is, P_U^{iii} is a residue of 8-amino-3,6-dioxaoctanoic acid (also known as {2-[2-aminoethoxy]ethoxy}acetic acid and H_2N-PEG_3-COOH). This residue is referred to herein as $-PEG_3-$.

In some embodiments, m is 2 and p is 1, that is, P_U^{iii} is a residue of 11-amino-3,6,9-trioxaundecanoic acid (also known as H_2N-PEG_4-COOH). This residue is referred to herein as $-PEG_4-$

In some embodiments, S_B is $-(P_U^{iii})_n-$, wherein n is 1 to 3, more preferably 2.

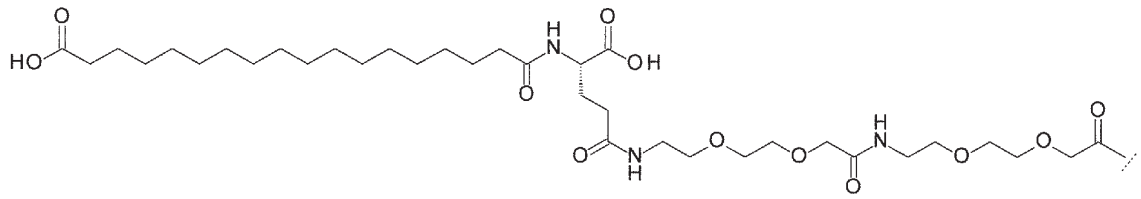
In some preferred embodiments, S_B is selected from $-PEG_3-PEG_3-$ and $-PEG_4-PEG_4-$.

Preferred $-Z^2-Z^1$

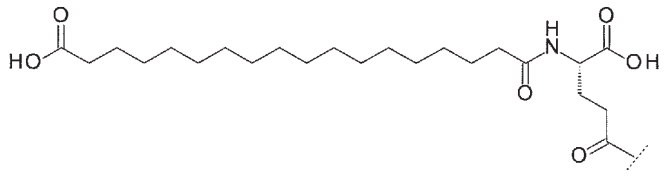
It will be understood that the above preferences may be independently combined to give preferred $-Z^2$-Z^1 combinations.

Some preferred $-Z^2$-Z^1 combinations are shown below (in each case, --- indicates the point of attachment to the side chain of the amino acid component of Ψ:

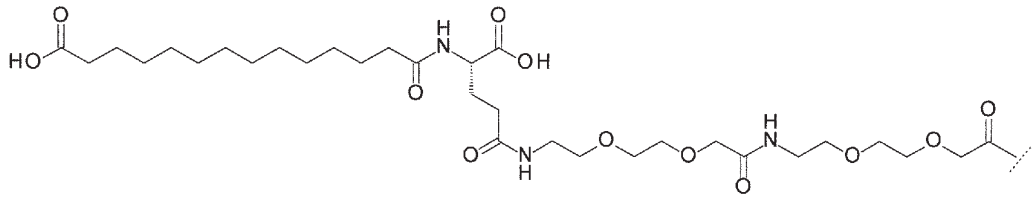
(i) [17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3



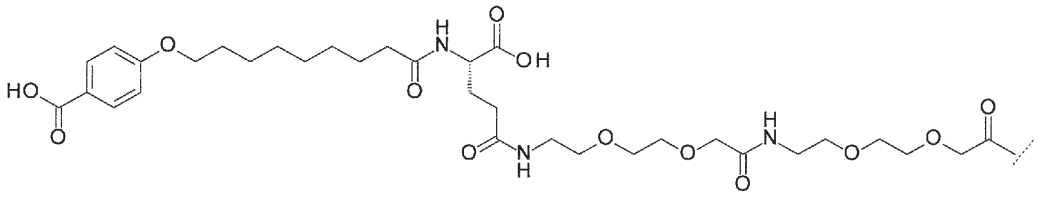
(ii) [17-Carboxy-heptadecanoyl]-isoGlu



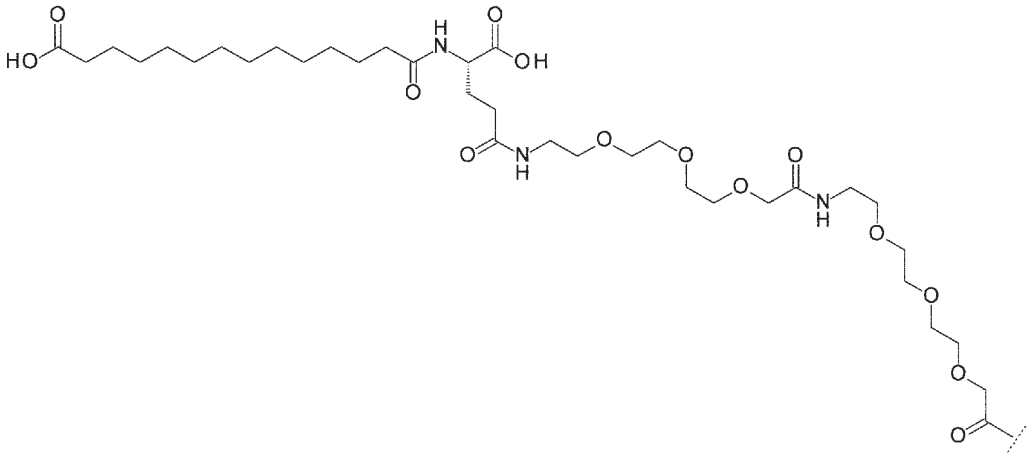
(iii) [13-Carboxy-tridecanoyl]-isoGlu-Peg3-Peg3



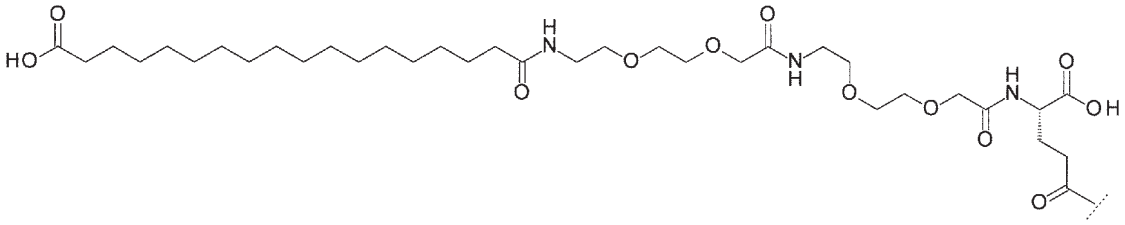
(iv) [Carboxyphenoxynonanoyl]-isoGlu-Peg3-Peg3



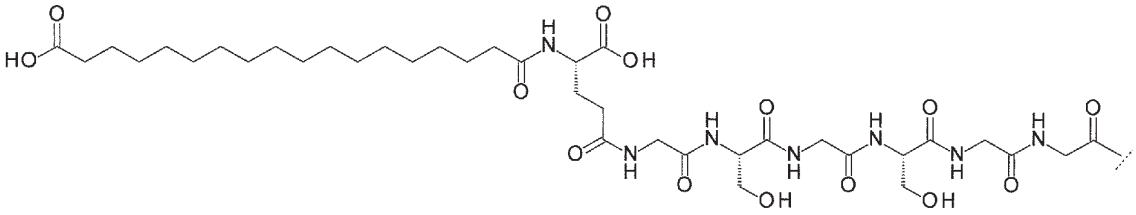
(v) [13-Carboxy-tridecanoyl]-isoGlu-Peg4-Peg4



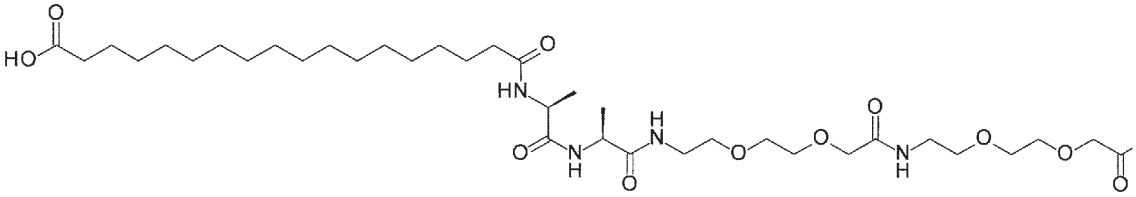
(vi) [17-Carboxy-heptadecanoyl]-Peg3-Peg3-isoGlu



(vii) [17-Carboxy-heptadecanoyl]-isoGlu-GSGSGG



(viii) [17-Carboxy-heptadecanoyl]-AA-Peg3-Peg3



The presence of the polar group at the end of Z^1 is believed to enhance the pharmacokinetic properties of the compound, for example, by increasing half life and/or mean residence time, and reducing clearance. The linker may also contribute to these pharmacokinetic properties. Linkers comprising more than one amino acid unit (or moieties of similar size) may improve pharmacokinetic properties compared to those consisting of just one amino acid unit or the like. These properties may enable the compound to be administered less frequently than an equivalent compound with the same peptide backbone but no modification or a different modification (e.g. a substituent with an aliphatic fatty chain lacking a polar group and/or having a shorter linker moiety).

Without wishing to be bound by any particular theory, the inventors have found that, especially when longer linkers were included, the polar or charged group at the end of Z^1 may be capable of participating in an undesirable intra-molecular nteraction with the free N-terminus of the molecule which might compromise the beneficial effects of the polar group on pharmacokinetics. The peptide backbones of the compounds described herein are belived to adopt relatively well-defined helical secondary structure, so the capacity of the polar group to engage in such interactions may depend on its location within the molecule. When located towards the C-terminus, interaction with the N-terminus may be relatively unlikely. However, the inventors were surprised to find that the substituent could be located at residues 16 and 17 of the molecule without necessarily compromising the pharmacokinetic benefits obtained.

The term "conjugated" is used here to describe the physical attachment of one identifiable chemical moiety to another, and the structural relationship between such moieties. It should not be taken to imply any particular method of synthesis.

The skilled reader will be well aware of suitable techniques that can be used to perform the coupling reactions using general synthetic methodologies listed e.g. in "Comprehensive Organic Transformations, A Guide to Functional Group Preparations", 2nd edition, Larock, R. C.; Wiley-VCH: New York, 1999. Such transformations may take place at any suitable stage during the synthesis process.

Peptide synthesis

The compounds of the present invention may be manufactured either by standard synthetic methods, recombinant expression systems, or any other state of the art method. Thus the glucagon analogues may be synthesized in a number of ways, including, for example, a method which comprises:

(a) synthesizing the peptide by means of solid-phase or liquid-phase methodology, either stepwise or by fragment assembly, and isolation and purifying of the final peptide product; or

(b) expressing a precursor peptide sequence from a nucleic acid construct that encodes the precursor peptide, recovering the expression product, and modifying the precursor peptide to yield a compound of the invention.

Expression is typically performed from a nucleic acid encoding the precursor peptide, which may be perfomed in a cell or a cell-free expression system comprising such a nucleic acid.

It is preferred to synthesize the analogues of the invention by means of solid-phase or liquid-phase peptide synthesis. In this context, reference is made to WO 98/11125 and, among many others, Fields, GB et al., 2002, "Principles and practice of solid-phase peptide synthesis". In: Synthetic Peptides (2nd Edition), and the Examples herein.

For recombinant expression, the nucleic acid fragments encoding the precursor peptide will normally be inserted in suitable vectors to form cloning or expression vectors. The vectors can, depending on purpose and type of application, be in the form of plasmids, phages, cosmids, mini-chromosomes, or virus, but also naked DNA which is only expressed transiently in certain cells is an important vector. Preferred cloning and expression vectors (plasmid vectors) are capable of autonomous replication, thereby enabling high copy-numbers for the purposes of high-level expression or high-level replication for subsequent cloning.

In general outline, an expression vector comprises the following features in the 5'→3' direction and in operable linkage: a promoter for driving expression of the nucleic acid fragment, optionally a nucleic acid sequence encoding a leader peptide enabling secretion (to the extracellular phase or, where applicable, into the periplasma), the nucleic acid fragment encoding the precursor peptide, and optionally a nucleic acid sequence encoding a terminator. They may comprise additional features such as selectable markers and origins of replication. When operating with expression vectors in producer strains or cell lines it may be preferred that the vector is capable of integrating into the host cell genome. The skilled person is very familiar with suitable vectors and is able to design one according to their specific requirements.

The vectors of the invention are used to transform host cells to produce the precursor peptide. Such transformed cells can be cultured cells or cell lines used for propagation of the nucleic acid fragments and vectors, and/or used for recombinant production of the precursor peptides.

Preferred transformed cells are micro-organisms such as bacteria [such as the species Escherichia (e.g. E. coli), Bacillus (e.g. Bacillus subtilis), Salmonella, or Mycobacterium (preferably non-pathogenic, e.g. M. bovis BCG), yeasts (e.g., Saccharomyces cerevisiae and Pichia pastoris), and protozoans. Alternatively, the transformed cells may be derived from a multicellular organism, i.e. it may be fungal cell, an insect cell, an algal cell, a plant cell, or an animal cell such as a mammalian cell. For the purposes of cloning and/or optimised expression it is preferred that the transformed cell is capable of replicating the nucleic acid fragment of the invention. Cells expressing the nucleic fragment can be used for small-scale or large-scale preparation of the peptides of the invention.

When producing the precursor peptide by means of transformed cells, it is convenient, although far from essential, that the expression product is secreted into the culture medium.

Efficacy

Binding of the relevant compounds to GLP-1 or glucagon (Glu) receptors may be used as an indication of agonist activity, but in general it is preferred to use a biological assay which measures intracellular signalling caused by binding of the compound to the relevant receptor. For example, activation of the glucagon receptor by a glucagon agonist will stimulate cellular cyclic AMP (cAMP) formation. Similarly, activation of the GLP-1 receptor by a GLP-1 agonist will stimulate cellular cAMP formation. Thus, production of cAMP in suitable cells expressing one of these two receptors can be used to monitor the relevant receptor activity. Use of a suitable pair of cell types, each expressing one receptor but not the other, can hence be used to determine agonist activity towards both types of receptor.

The skilled person will be aware of suitable assay formats, and examples are provided below. The GLP-1 receptor and/or the glucagon receptor may have the sequence of the receptors as described in the examples. For example, the assays may employ the human glucagon receptor (Glucagon-R) having primary accession number GI:4503947 and/or the human glucagon-like peptide 1 receptor (GLP-1R) having primary accession number GI:166795283. (in that where sequences of precursor proteins are referred to, it should of course be understood that assays may make use of the mature protein, lacking the signal sequence).

EC_{50} values may be used as a numerical measure of agonist potency at a given receptor. An EC_{50} value is a measure of the concentration of a compound required to achieve half of that compound's maximal activity in a particular assay. Thus, for example, a compound having EC_{50}[GLP-1] lower than the EC_{50}[GLP-1] of glucagon in a particular assay may be considered to have higher GLP-1 receptor agonist potency than glucagon.

The compounds described in this specification are typically GluGLP-1 dual agonists, as determined by the observation that they are capable of stimulating cAMP formation at both

the glucagon receptor and the GLP-1 receptor. The stimulation of each receptor can be measured in independent assays and afterwards compared to each other.

By comparing the EC_{50} value for the GLP-1 receptor (EC_{50} [GLP-1-R]) with the EC_{50} value for the Glucagon receptor, (EC_{50} [GlucagonR]) for a given compound. the relative GLP-1R selectivity can be calculated as follows:

Relative GLP-1R selectivity [compound] = (EC_{50} [GLP-1R]) / (EC_{50} [Glucagon-R])

The term "EC_{50}" stands for the half maximal Effective Concentration, typically at a particular receptor, or on the level of a particular marker for receptor function, and can refer to an inhibitory or an antagonistic activity, depending on the specific biochemical context.

Without wishing to be bound by any particular theory, a compound's relative selectivity may allow its effect on the GLP-1 or glucagon receptor to be compared directly to its effect on the other receptor. For example, the higher a compound's relative GLP-1 selectivity is, the more effective that compound may be on the GLP-1 receptor as compared to the glucagon receptor. Typically the results are compared for glucagon and GLP-1 receptors from the same species, e.g. human glucagon and GLP-1 receptors, or murine glucagon and GLP-1 receptors.

The compounds of the invention may have a higher relative GLP-1R selectivity than human glucagon in that for a particular level of glucagon-R agonist activity, the compound may display a higher level of GLP-1R agonist activity (i.e. greater potency at the GLP-1 receptor) than glucagon. It will be understood that the absolute potency of a particular compound at the glucagon and GLP-1 receptors may be higher, lower or approximately equal to that of native human glucagon, as long as the appropriate relative GLP-1R selectivity is achieved.

Nevertheless, the compounds of this invention may have a lower EC_{50} [GLP-1R] than human glucagon. The compounds may have a lower EC_{50}[GLP-1-R] than glucagon while maintaining an EC_{50} [Glucagon-R] that is less than 10-fold higher than that of human glucagon, less than 5-fold higher than that of human glucagon, or less than 2-fold higher than that of human glucagon.

The compounds of the invention may have an EC_{50} [Glucagon-R] that is less than two-fold that of human glucagon. The compounds may have an EC_{50} [Glucagon-R] that is less than two-fold that of human glucagon and have an EC_{50} [GLP-1R] that is less than half that of human glucagon, less than a fifth of that of human glucagon, or less than a tenth of that of human glucagon.

The relative GLP-1R selectivity of the compounds may be between 0.05 and 20. For example, the compounds may have a relative selectivity of 0.05-0.20, 0.1-0.30, 0.2-0.5, 0.3-0.7, or 0.5-1.0; 1.0-2.0, 1.5-3.0, 2.0-4.0 or 2.5-5.0; or 0.05-20, 0.075-15, 0.1-10, 0.15-5, 0.75-2.5 or 0.9-1.1.

In certain embodiments, it may be desirable that EC_{50} of any given compound for both the Glucagon-R and GLP-1R, e.g. for the human glucagon and GLP-1 receptors, should be less than 1 nM.

Therapeutic uses

The compounds of the invention may provide attractive treatment and/or prevention options for, *inter alia*, obesity and metabolic diseases including diabetes, as discussed below.

Diabetes comprises a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Acute signs of diabetes include excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, notably the eyes, kidneys, nerves, heart and blood vessels. Diabetes is classified into type 1 diabetes, type 2 diabetes and gestational diabetes on the basis on pathogenetic characteristics.

Type 1 diabetes accounts for 5-10% of all diabetes cases and is caused by auto-immune destruction of insulin-secreting pancreatic β-cells.

Type 2 diabetes accounts for 90-95% of diabetes cases and is a result of a complex set of metabolic disorders. Type 2 diabetes is the consequence of endogenous insulin production becoming insufficient to maintain plasma glucose levels below the diagnostic thresholds.

Gestational diabetes refers to any degree of glucose intolerance identified during pregnancy.

Pre-diabetes includes impaired fasting glucose and impaired glucose tolerance and refers to those states that occur when blood glucose levels are elevated but below the levels that are established for the clinical diagnosis for diabetes.

A large proportion of people with type 2 diabetes and pre-diabetes are at increased risk of morbidity and mortality due to the high prevalence of additional metabolic risk factors including abdominal obesity (excessive fat tissue around the abdominal internal organs), atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and/or high LDL cholesterol, which foster plaque buildup in artery walls), elevated blood

pressure (hypertension) a prothrombotic state (e.g. high fibrinogen or plasminogen activator inhibitor–1 in the blood), and proinflammatory state (e.g., elevated C-reactive protein in the blood).

Conversely, obesity confers an increased risk of developing pre-diabetes, type 2 diabetes as well as e.g. certain types of cancer, obstructive sleep apnea and gall-blader disease.

Dyslipidaemia is associated with increased risk of cardiovascular diasese. High Density Lipoprotein (HDL) is of clinical importance since an inverse correlation exists between plasma HDL concentrations and risk of atherosclerotic disease. The majority of cholesterol stored in atherosclerotic plaques originates from LDL and hence elevated concentrations Low Density Lipoproteins (LDL) is closely associated with atherosclerosis. The HDL/LDL ratio is a clinical risk indictor for atherosclerosis and coronary atherosclerosis in particular.

Metabolic syndrome is characterized by a group of metabolic risk factors in one person. They include abdominal obesity (excessive fat tissue around the abdominal internal organs), atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and/or high LDL cholesterol, which foster plaque buildup in artery walls), elevated blood pressure (hypertension), insulin resistance and glucose intolerance, prothrombotic state (e.g. high fibrinogen or plasminogen activator inhibitor–1 in the blood), and proinflammatory state (e.g., elevated C-reactive protein in the blood).

Individuals with the metabolic syndrome are at increased risk of coronary heart disease and other diseases related to other manifestations of arteriosclerosis (e.g., stroke and peripheral vascular disease). The dominant underlying risk factors for this syndrome appear to be abdominal obesity.

Without wishing to be bound by any particular theory, it is believed that the compounds of the invention act as dual agonists both on the human glucagon-receptor and the human GLP1-receptor, abbreviated here as dual GluGLP-1 agonists. The dual agonist may combine the effect of glucagon, e.g. on fat metabolism, with the effect of GLP-1, e.g. on blood glucose levels and food intake. They may therefore act to accelerate elimination of excessive adipose tissue, induce sustainable weight loss, and improve glycaemic control. Dual GluGLP-1 agonists may also act to reduce cardiovascular risk factors such as high cholesterol, high LDL-cholesterol or low HDL/LDL cholesterol ratios.

The compounds of the present invention can therefore be used in a subject in need thereof as pharmaceutical agents for preventing weight gain, promoting weight loss, reducing excess body weight or treating obesity (e.g. by control of appetite, feeding, food intake, calorie intake, and/or energy expenditure), including morbid obesity, as well as associated diseases and

health conditions including but not limited to obesity linked inflammation, obesity linked gallbladder disease and obesity induced sleep apnea. The compounds of the invention may also be used for treatment of conditions caused by or associated with impaired glucose control, including metabolic syndrome, insulin resistance, glucose intolerance, pre-diabetes, increased fasting glucose, type 2 diabetes, hypertension, atherosclerois, arteriosclerosis, coronary heart disease, peripheral artery disease and stroke, in a subject in need thereof. Some of these conditions can be associated with obesity. However, the effects of the compounds of the invention on these conditions may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

The synergistic effect of dual GluGLP-1 agonists may also result in reduction of cardiovascular risk factors such as high cholesterol and LDL, which may be entirely independent of their effect on body weight.

Thus the invention provides the use of a compound of the invention in the treatment of a condition as described above, in an individual in need thereof.

The invention also provides a compound of the invention for use in a method of medical treatment, particularly for use in a method of treatment of a condition as described above.

In a preferred aspect, the compounds described may be used in treating diabetes, esp. type 2 diabetes.

In a specific embodiment, the present invention comprises use of a compound for treating diabetes, esp. type 2 diabetes in an individual in need thereof.

In a not less preferred aspect, the compounds described may be used in preventing weight gain or promoting weight loss.

In a specific embodiment, the present invention comprises use of a compound for preventing weight gain or promoting weight loss in an individual in need thereof.

In a specific embodiment, the present invention comprises use of a compound in a method of treatment of a condition caused or characterised by excess body weight, e.g. the treatment and/or prevention of obesity, morbid obesity, morbid obesity prior to surgery, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, prediabetes, diabetes, esp. type 2 diabetes, hypertension, atherogenic dyslipidimia, atherosclerois, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease in an individual in need thereof.

In another aspect, the compounds described may be used in a method of lowering circulating LDL levels, and/or increasing HDL/LDL ratio.

In a specific embodiment, the present invention comprises use of a compound in a method of lowering circulating LDL levels, and/or increasing HDL/LDL ratio in an individual in need thereof.

In another aspect, the compounds described may be used in a method of lowering circulating triglyceride levels.

Pharmaceutical compositions

The compounds of the present invention may be formulated as pharmaceutical compositions prepared for storage or administration. Such a composition typically comprises a therapeutically effective amount of a compound of the invention, in the appropriate form, in a pharmaceutically acceptable carrier.

The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy, and may depend on such factors as weight, diet, concurrent medication and other factors, well known to those skilled in the medical arts. The dosage sizes and dosing regimen most appropriate for human use may be guided by the results obtained by the present invention, and may be confirmed in properly designed clinical trials. The compounds of the present invention may be particularly useful for treatment of humans.

An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Such considerations are known to the skilled person.

The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. pH buffering agents may be phosphate, citrate, acetate, tris/hydroxymethyl)aminomethane (TRIS), N-Tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid (TAPS), ammonium bicarbonate,

diethanolamine, histidine, which is a preferred buffer, arginine, lysine, or acetate or mixtures thereof. The term further encompasses any agents listed in the US Pharmacopeia for use in animals, including humans.

The term "pharmaceutically acceptable salt" refers to a salt of any one of the compounds of the invention. Salts include pharmaceutically acceptable salts such as acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions $^{+}N(R^3)_3(R^4)$, where R^3 and R^4 independently designates optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences" ,17th edition. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in the Encyclopaedia of Pharmaceutical Technology.

"Treatment" is an approach for obtaining beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures in certain embodiments. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. By treatment is meant inhibiting or reducing an increase in pathology or symptoms (e.g. weight gain, hyperglycemia) when compared to the absence of treatment, and is not necessarily meant to imply complete cessation of the relevant condition.

The pharmaceutical compositions can be in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparations, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms. It may be provided in single dose injectable form, for example in the form of a pen. In certain embodiments, packaged forms include a label or insert with instructions for use. Compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents

include those used in formulations suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

Subcutaneous or transdermal modes of administration may be particularly suitable for the compounds described herein.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions there of, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Combination therapy

A compound or composition of the invention may be administered as part of a combination therapy with an agent for treatment of obesity, hypertension, dyslipidemia or diabetes.

In such cases, the two active agents may be given together or separately, and as part of the same pharmaceutical formulation or as separate formulations.

Thus a compound or composition of the invention can further be used in combination with an anti-obesity agent, including but not limited to a glucagon-like peptide receptor 1 agonist, peptide YY or analogue thereof, cannabinoid receptor 1 antagonist, lipase inhibitor, melanocortin receptor 4 agonist, melanin concentrating hormone receptor 1 antagonist, phentermine (alone or in combination with topiramate), a combination of norepinephrine/dopamine reuptake inhibitor and opioid receptor antagonist (e.g. a combination of bupropion and naltrexone), or a serotonergic agent (e.g. lorcaserin).

A compound or composition of the invention can be used in combination with an anti-hypertension agent, including but not limited to an angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, diuretics, beta-blocker, or calcium channel blocker.

A compound or composition of the invention can be used in combination with a dyslipidaemia agent, including but not limited to a statin, a fibrate, a niacin and/or a cholesterol absorbtion inhibitor.

Further, a compound or composition of the invention can be used in combination with an anti-diabetic agent, including but not limited to a biguanide (e.g. metformin), a sulfonylurea, a meglitinide or glinide (e.g. nateglinide), a DPP-IV inhibitor, an SGLT2 inhibitor, a glitazone, a different GLP-1 agonist, an insulin or an insulin analogue. In a preferred embodiment, the compound or salt thereof is used in combination with insulin or an insulin analogue, DPP-IV inhibitor, sulfonylurea or metformin, particularly sulfonylurea or metformin, for achieving adequate glycemic control. Examples of insulin analogues include but are not limited to Lantus, Novorapid, Humalog, Novomix, and Actraphane HM, Levemir and Apidra.

EXAMPLES

Example 1: General synthesis of glucagon analogues

Solid phase peptide synthesis (SPPS) was performed on a microwave assisted synthesizer using standard Fmoc strategy in NMP on a polystyrene resin (TentaGel S Ram). HATU was used as coupling reagent together with DIPEA as base. Piperidine (20% in NMP) was used for deprotection. Pseudoprolines: Fmoc-Phe-Thr(psiMe,Mepro)-OH and Fmoc-Asp-Ser(psiMe,Mepro)-OH (purchased from NovaBiochem) were used where applicable.

Abbreviations employed are as follows:

Boc:	tert-butyloxycarbonyl
ivDde:	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)3-methyl-butyl
Dde:	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl
DCM:	dichloromethane
DMF:	*N,N*-dimethylformamide
DIPEA:	diisopropylethylamine
EDT:	1,2-ethanedithiol
EtOH:	ethanol
Et_2O:	diethyl ether
HATU:	*N*-[(dimethylamino)-1*H*-1,2,3-triazol[4,5-*b*]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
MeCN:	acetonitrile
NMP:	*N*-methylpyrrolidone
TFA:	trifluoroacetic acid

TIS: triisopropylsilane

Cleavage:

The crude peptide was cleaved from the resin by treatment with 95/2.5/2.5 % (v/v) TFA/TIS/ water at room temperature (r.t.) for 2 hours. Most of the TFA was removed at reduced pressure and the crude peptide was precipitated and washed with diethylether and allowed to dry to constant weight at ambient temperature.

The following compounds were synthesised:

1 H-H-Aib-QGTFTSDYSKYLDS-K([15-carboxy-pentadecanoyl]-isoGlu)-AAHDFVEWLLSA-NH_2.

2 H-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA-NH_2

3 H-H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

4 H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2

5 H-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2

6 H-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2

7 H-H-Ac4c-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2

8 H-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAHDFIEWLESA-NH2

9 H-H-Ac4c-HGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2

10 H-H-Aib-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2

11 H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2

12 H-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-RAKDFIEWLESA-NH_2

13 H-H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

14 H-H-Ac4c-QGTFTSDYSKYLDERRAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

15 H-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2

16 H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2

The acylated GLP-1 analogue semaglutide was also synthesised, and has the structure: H-H-[2-methyl-Ala]-EGTFTSDVSSYLEGQAA-K([17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-EFIAWLVRGRG-OH.

Example 2: Glucagon receptor and GLP-1-receptor efficacy assays

The cDNA encoding either the human glucagon receptor (Glucagon-R) (primary accession number P47871) or the human glucagon-like peptide 1 receptor (GLP-1R) (primary accession number P43220) were synthesized and cloned into a mammalian expression vector containing a Zeocin resistance marker.

The mammalian expression vectors encoding the Glucagon-R or the GLP-1-R were transfected into Chinese hamster ovary (CHO) cells by the Attractene method method. Stably expressing clones were obtained by Zeocin selection (250µg/mL) upon limited dilution of cells resistant to the selection pressure. Glucagon-R and GLP-1-R cell clones expressing were picked, propagated and tested in the Glucagon-R and GLP-1-R efficacy assays as described below. One Glucagon-R expressing clone and one GLP-1-R expressing clone were chosen for compound profiling.

CHO cells expressing the human Glucagon-R, or human GLP-1-R were seeded 24 hours prior to the assay at 30,000 cells per well in 96-well microtiter plates in culture in 100 µl growth medium. On the day of analysis, growth medium was removed and the cells were washed once with 200 µl of assay buffer (Krebs-Ringer- buffer – KRBH). The buffer was removed and the cells were incubated for 15 min at room temperature in 10µl KRBH (KRBH + 10 mM HEPES, 5 mM NaHCO3, 0.1 % (V/V) BSA) with 0.1 mM IBMX in deionized water containing increasing concentrations of test peptides.. The reaction was stopped by the addition of lysis buffer (0.1 % w/v BSA, 5 mM HEPES, 0.3 % v/v Tween-20). After cell lysis for 10min at room temperature, lysates were transferred to 384-well plates and 10µl of acceptor/donorbead mixture as contained in the AlphaScreen™ cAMP Functional Assay Kit was added. After one hour of incubation at room temperature in the dark, the cAMP content was determined applying the AlphaScreen™ cAMP Functional Assay Kit from Perkin-Elmer according to manufacturer instructions. EC_{50} and relative efficacies compared to reference compounds (glucagon and GLP-1) were calculated applying computer aided curve fitting.. The GLP-1/glucagon ratio is calculated as defined earlier. See Table 1.

Compound	EC50 hGCGR CHO-K1 [nM]	EC50 hGLP-1R CHO-K1 [nM]	Ratio GLP-1/ Glucagon
1	0.21 nM	0.38 nM	1.81
2	0.13 nM	1.76 nM	13.54
3	1.48 nM	0.70 nM	0.47
4	0.45 nM	0.70 nM	1.56
5	0.18 nM	0.83 nM	4.61
6	0.44 nM	1.43 nM	3.25
7	0.11 nM	0.97 nM	8.82
8	0.31 nM	0.80 nM	2.58
9	0.07 nM	0.97 nM	13.86
10	1.08 nM	0.41 nM	0.38
11	0.28 nM	0.56 nM	2.00
12	0.07 nM	0.48 nM	6.86
13	0.52 nM	0.33 nM	0.63
14	0.18 nM	0.60 nM	3.33
15	0.92 nM	0.61 nM	0.65
16	0.16 nM	0.53 nM	3.31

Table 1

Example 3: Agonistic activity on endogenous GLP-1 receptor

Agonistic activity of the test compounds on endogenous GLP-1 receptors was determined using a murine insulinoma cell line. Intracellular cAMP was used an indicator of receptor activation.

Cells were cultured for 24h at a density of 10,000 cells/well in a 384-well plate. Medium was removed and 10 µL KRBH buffer (NaCl 130 mM, KCl 3.6 mM, NaH_2PO_4 0.5 mM, $MgSO_4$ 0.5 mM, $CaCl_2$ 1.5 mM) containing test compound or GLP-1 (at increasing concentrations from 0.1 pM to 100 nM) or solvent control (0.1% (v/v) DMSO) was added to the wells for 15 minutes at a temperature of 26°C.

The cellular cAMP content is measured using the AlphaScreen cAMP Functional Assay Kit (Perkin Elmer). Measurement was performed using the Envision (PerkinElmer) according to manufacturer's recommendations.

Results were converted into cAMP concentrations using a cAMP standard curve prepared in KRBH buffer containing 0.1% (v/v) DMSO. The resulting cAMP curves were plotted as absolute cAMP concentrations (nM) over log (test compound concentration) and analyzed using the curve fitting program XLfit.

Parameters calculated to describe both the potency as well as the agonistic activity of each test compound on the endogenous GLP-1 receptors were:
pEC50 (negative logarithmic value of EC50, a concentration resulting in a half-maximal elevation of cAMP levels, reflecting the potency of the test compound);
Percent control (%CTL)(% cAMP elevation for each test compound concentration normalized based on the GLP-1-induced maximum cAMP response (100 %CTL)). See Table 2.

Compound	EC50 [nM]
1	0.60 nM
2	0.69 nM
3	0.15 nM
4	0.40 nM
5	0.65 nM
6	0.54 nM
7	0.47 nM
8	0.36 nM
9	0.84 nM
10	0.60 nM
11	0.72 nM
12	0.81 nM
13	0.37 nM
14	0.38 nM
15	0.25 nM
16	0.34 nM

Table 2.

Example 4: Agonistic activity on endogenous glucagon receptor

Agonistic activity of the test compounds on endogenous glucagon receptor was deterimed by measuring their effect on rate of glycogen synthesis in primary rat hepatocytes. Upon activation of the glucagon receptor, an inhibition of the glycogen synthesis rate is expected. Rate of glycogen synthesis was determined by counting the amount of radioactively labeled glucose incorporated into the cellular glycogen stores in a defined period of time.

Primary rat hepatocytes were cultured at a density of 40,000 cells/well in a 24-well plate for 24 hours at 37°C and 5% CO_2.

Medium was discarded and the cells washed with PBS. 180 µL of KRBH-based buffer containing 0.1% BSA and glucose at a concentration of 22.5 mM was then added to the wells, followed by test compound and 40 µCi/ml D-[U14C] glucose (20µL each). Incubation was continued for 3 hours.

At the end of the incubation period, the incubation buffer was aspirated and cells washed once with ice-cold PBS before lysis by incubation for 30 min at room temperature with 100 µL 1 mol/l NaOH.

Cell lysates were transferred to 96-well filter plates and glycogen precipitated by incubating the filter-plates for 120 min at 4°C followed by washing the filter plates 4 times with ice-cold ethanol (70%). The resulting precipitates were filtered to dryness and the amount of incorporated ^{14}C-glucose determined by using a Topcount scintillation counter according to manufacturer's recommendations.

Wells with vehicle controls (0.1% (v/v) DMSO in KRBH buffer) were included as reference for non-inhibited glycogen synthesis (100 %CTL). Wells without added D-[U^{14}C] glucose were included as controls for non-specific background signal (subtracted from all values). Endogenous glucagon peptide was used as a positive control.

All treatments were performed at least in duplicates.

Parameters calculated to describe both the potency as well as the agonistic activity of each test compound on the endogenous glucagon receptor are pEC50 and %CTL.

%CTL is determined by calculating the percentage of CPM/well in the presence of the test compound compared to the CPM/well of the vehicle control after subtracting the background CPM/well:

[CPM/well(basal) - CPM/well(sample)]* 100 / [CPM/well(basal) - CPM/well(control)]

An activator of the glucagon receptor will result in an inhibition of the glycogen synthesis rate and will give %CTL values between 0%CTL (complete inhibition) and 100%CTL (no observable inhibition).

The resulting activity curves were plotted as absolute counts (unit: cpm/sample) over log (test compound concentration) and analyzed using the curve fitting program XLfit.

pEC50 (negative logarithmic value of EC50) reflects the potency of the test compound.

Compound	EC50 [nM]
1	0.85 nM
2	0.11 nM
3	0.94 nM
4	1.79 nM
5	0.21 nM
6	0.80 nM
7	0.34 nM
8	0.29 nM
9	0.11 nM
10	1.53 nM
11	0.95 nM
12	0.45 nM
13	0.43 nM
14	0.19 nM
15	3.63 nM
16	0.19 nM

Table 3.

The terms EC_{50} and pEC_{50} quoted in relation to GLP-1R activation could equally be regarded as IC_{50} and pIC_{50} in relation to glycogen synthesis.

Example 5: Estimate of pharmacokinetic parameters

Pharmacokinetic parameters of the test compounds were determined after intravenous administration to Han/Wistar rats. The acylated GLP-1 analogue semaglutide was also tested for comparison purposes.

Male Wistar rats were obtained from Charles River (Germany) weighing approximately 180 to 210 g at time of arrival at the test facility. Rats were caged in European standard rat cages type IV with light cycle of 12-hour dark and 12-hour light. During the study rats were housed in standard rat cages type III. Both diet Altromin 1324 (Altromin, Germany) and water was administered ad libitum during the whole experimental period. The animals were housed in the test facility for at least 4 days in order to assure proper acclimatization.

The compounds were first dissolved in 0.1% aqueous ammonia to a nominal concentration of 2 mg/ml, and then diluted to the desired dosing strength (10 µM) in sterile PBS containing 25 mM phosphate buffer, pH 7.4. Intravenous injections corresponding to 20 nmol/kg were given via a lateral tail vein.

Blood samples (200 µl) were collected from the periorbital plexus at time points 0.08, 0.25, 0.5, 1, 2, 4, 8, 24, 32 and 48 h post dosing into K_3EDTA tubes and centrifuged for 5 minutes at 4°C within 20 minutes of sampling. Plasma samples (>100 µl) were transferred to 96-well PCR plates, immediately frozen and kept at -20°C until analysed for plasma concentration for the respective GLP-1-glucagon compound using LC-MS/MS. Individual plasma concentration-time profiles were analysed by a non-compartmental approach using ToxKin™ version 3.2 (Unilog IT Services), and the resulting pharmacokinetic parameters were determined. See Table 4.

Compound	Clearance (ml/min/kg)	Terminal half life (h)	Mean Residence Time (h)
2	0.11	9.1	13.6
3	0.056	23.4	28.7
4	0.11	13.7	17.6
Semaglutide	0.10	9.0	11.4

Table 4.

CLAIMS

1. A compound having the formula:

R^1-P^1-R^2

wherein

R^1 is hydrogen (Hy), C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

P^1 is a peptide having the sequence:

H-X2-X3-GTFTSDYSKYLDSΨAAHDFVEWLLSA

wherein:

X2 is selected from Aib, Ala, D-Ala, Ser, N-Me-Ser, Ac3c, Ac4c and Ac5c;

X3 is selected from Gln and His;

and Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent selected from:

(i) [17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3;

(ii) [17-Carboxy-heptadecanoyl]-isoGlu

(iii) [13-Carboxy-tridecanoyl]-isoGlu-Peg3-Peg3;

(iv) [Carboxyphenoxynonanoyl]-isoGlu-Peg3-Peg3;

(v) [13-Carboxy-tridecanoyl]-isoGlu-Peg4-Peg4;

(vi) [17-Carboxy-heptadecanoyl]-Peg3-Peg3-isoGlu;

(vii) [17-Carboxy-heptadecanoyl]-isoGlu-GSGSGG; and

(viii) [17-Carboxy-heptadecanoyl]-AA-Peg3-Peg3;

or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to claim 1 wherein P^1 has the sequence:
H-Aib-QGTFTSDYSKYLDSΨAAHDFVEWLLSA

3. A compound according to claim 2 which is:
H-H-Aib-QGTFTSDYSKYLDSΨAAHDFVEWLLSA-NH_2

4. A compound having the formula:
R^1-P^1-R^2
wherein
R^1 is hydrogen (Hy), C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;
R^2 is OH or NH_2;
P^1 is a peptide having the sequence:

His-X2-X3-GTFTSDYSKYL-X15-X16-X17-X18-A-X20-DFI-X24-WLE-X28-A

wherein:
X2 is selected from Aib, Ac3c, Ac4c and Ac5c;
X3 is selected from Gln and His;
X15 is selected from Asp and Glu;
X16 is selected from Glu and Ψ;
X17 is selected from Arg and Ψ;
X18 is selected from Ala and Arg;
X20 is selected from Lys and His;
X24 is selected from Glu and Ψ;
X28 is selected from Ser and Ψ;

wherein the compound contains one and only one Ψ

and wherein said Ψ is a residue of Lys, Arg, Orn or Cys in which the side chain is conjugated to a substituent selected from:

(i) [17-Carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3;

(ii) [17-Carboxy-heptadecanoyl]-isoGlu

(iii) [13-Carboxy-tridecanoyl]-isoGlu-Peg3-Peg3;

(iv) [Carboxyphenoxynonanoyl]-isoGlu-Peg3-Peg3;

(v) [13-Carboxy-tridecanoyl]-isoGlu-Peg4-Peg4;

(vi) [17-Carboxy-heptadecanoyl]-Peg3-Peg3-isoGlu;

(vii) [17-Carboxy-heptadecanoyl]-isoGlu-GSGSGG; and

(viii) [17-Carboxy-heptadecanoyl]-AA-Peg3-Peg3;

or a pharmaceutically acceptable salt or solvate thereof.

5. A compound according to claim 4 wherein:

X2 is selected from Aib and Ac4c;

X3 is Gln;

X15 is selected from Asp and Glu;

X16 is Ψ;

X17 is Arg;

X18 is Ala;

X20 is selected from Lys and His;

X24 is Glu;

X28 is Ser.

6. A compound according to claim 4 or claim 5 wherein:

X2 is Ac4c and X20 is Lys;

X2 is Aib and X20 is His.

7. A compound according to any one of claims 4 to 6 wherein

X2 is Aib if X15 is E; or

X15 is D if X2 is Ac4c.

8. A compound according to any one of claims 4 to 7 wherein P^1 has a sequence selected from:

H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA

H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA

H-Aib-QGTFTSDYSKYLEΨRAAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDΨRAAKDFIEWLESA and

H-Aib-QGTFTSDYSKYLEΨRAAHDFIEWLESA

9. A compound according to claim 8 which is selected from:

Hy-H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2

Hy-H-Aib-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2

Hy-H-Aib-QGTFTSDYSKYLEΨRAAKDFIEWLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDΨRAAKDFIEWLESA-NH_2 and

Hy-H-Aib-QGTFTSDYSKYLEΨRAAHDFIEWLESA-NH_2

10. A compound according to claim 4 wherein:

X2 is selected from Aib and Ac4c;

X3 is selected from Gln and His;

X15 is Asp;

X16 is Glu;

X17 is selected from Arg and Ψ;

X18 is selected from Ala and Arg;

X20 is Lys;

X24 is selected from Glu and Ψ;

X28 is selected from Ser and Ψ;

11. A compound according to claim 10 wherein, when X28 is Ψ, X2 is Ac4c.

12. A compound according to claim 10 wherein, when X3 is His, X2 is Ac4c and X17 is Ψ.

13. A compound according to claim 10 wherein P^1 has a sequence selected from:

H-Aib-QGTFTSDYSKYLDEΨAAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Ac4c-HGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨAAKDFIEWLESA

H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA

H-Aib-QGTFTSDYSKYLDERAAKDFIΨWLESA

H-Ac4c-QGTFTSDYSKYLDERAAKDFIΨWLESA

H-Ac4c-QGTFTSDYSKYLDERRAKDFIΨWLESA

H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLEΨA and
H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLEΨA

14. A compound according to claim 13 which is selected from:
Hy-H-Aib-QGTFTSDYSKYLDEΨAAKDFIEWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
Hy-H-Ac4c-HGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDEΨAAKDFIEWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDEΨRAKDFIEWLESA-NH_2
Hy-H-Aib-QGTFTSDYSKYLDERAAKDFIΨWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFIΨWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDERRAKDFIΨWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLEΨA-NH_2 and
Hy-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLEΨA-NH_2

15. A compound according to claim 1 wherein P1 has the sequence: H-Aib-QGTFTSDYSKYLDS-K([15-carboxy-pentadecanoyl]-isoGlu)-AAHDFVEWLLSA.

16. A compound according to claim 15 which is: H-H-Aib-QGTFTSDYSKYLDS-K([15-carboxy-pentadecanoyl]-isoGlu)-AAHDFVEWLLSA-NH_2.

17. A compound according to any one of claims 4 to 7 wherein the substituent is [17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3 or [17-carboxy-heptadecanoyl]-isoGlu-GSGSGG.

18. A compound according to claim 10 or claim 12 wherein, when X17 is Ψ, the substituent is [17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3 or [17-Carboxy-heptadecanoyl]-isoGlu.

19. A compound according to claim 10 or claim 11 wherein, when X24 or X28 is Ψ, the substituent is [17-carboxy-heptadecanoyl]-isoGlu-GSGSGG.

20. A compound according to claim 8 wherein P^1 has a sequence selected from:
H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA
H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA and
H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAHDFIEWLESA

21. A compound according to claim 20 which is selected from:
Hy-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA-NH_2
Hy-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2
Hy-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2
Hy-H-Ac4c-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2 and
Hy-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAHDFIEWLESA-NH2

22. A compound according to claim 13 wherein P^1 has a sequence selected from:
H-Aib-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA
H-Ac4c-HGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA
H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-RAKDFIEWLESA H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERRAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA
H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A and

H-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2

23. A compound according to claim 22 which is selected from:

Hy-H-Aib-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2

Hy-H-Ac4c-HGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-AAKDFIEWLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu)-RAKDFIEWLESA-NH_2

Hy-H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDERRAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2

Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2 and

Hy-H-Ac4c-QGTFTSDYSKYLDERRAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2.

24. A compound which is:

Hy-H-Aib-QGTFTSDYSKYLD-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAAKDFIEWLESA-NH_2.

25. A compound which is:

Hy-H-Aib-QGTFTSDYSKYLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-RAAKDFIEWLESA-NH_2.

26. A compound which is:

Hy-H-Ac4c-QGTFTSDYSKYLDE-K([17-carboxy-heptadecanoyl]-isoGlu-Peg3-Peg3)-RAKDFIEWLESA-NH_2.

27. A compound which is:

Hy-H-Aib-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2.

28. A compound which is:
Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFI-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-WLESA-NH_2.

29. A compound which is:
Hy-H-Ac4c-QGTFTSDYSKYLDERAAKDFIEWLE-K([17-carboxy-heptadecanoyl]-isoGlu-GSGSGG)-A-NH_2.

30. A composition comprising a compound according to any one of the preceding claims in admixture with a carrier.

31. A composition according to claim 30 wherein the composition is a pharmaceutical composition, and the carrier is a pharmaceutically acceptable carrier.

32. A compound according to any one of claims 1 to 29 for use in a method of medical treatment.

33. Use of a compound according to any one of claims 1 to 29 in the manufacture of a medicament for use in a therapeutic method of preventing weight gain or promoting weight loss in an individual in need thereof.

34. Use of a compound according to any one of claims 1 to 29 in the manufacture of a medicament for use in a therapeutic method of lowering circulating LDL levels, and/or increasing HDL/LDL ratio in an individual in need thereof.

35. Use of a compound according to any one of claims 1 to 29 in the manufacture of a medicament for prevention or treatment of obesity, morbid obesity, morbid obesity prior to surgery, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, diabetes, metabolic syndrome, hypertension, atherogenic dyslipidimia, atherosclerois, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease.

36. The use according to any one of claims 33 to 35 wherein the compound is to be administered as part of a combination therapy together with an agent for treatment of diabetes, obesity, dyslipidemia or hypertension.

37. The use according to claim 36 wherein the agent for treatment of diabetes is a biguanide (e.g. metformin), a sulfonylurea, a meglitinide or glinide (e.g. nateglinide), a DPP-IV inhibitor, an SGLT2 inhibitor, a glitazone, a different GLP-1 agonist, an insulin or an insulin analogue.

38. The use according to claim 36, wherein the agent for treatment of obesity is a glucagon-like peptide receptor 1 agonist, peptide YY receptor agonist or analogue thereof, cannabinoid receptor 1 antagonist, lipase inhibitor, melanocortin receptor 4 agonist, melanin concentrating hormone receptor 1 antagonist, phentermine, a combination of norepinephrine/dopamine reuptake inhibitor and opioid receptor antagonist (e.g. a combination of phentermine and topiramate), a combination of bupropion and naltrexone, or a serotonergic agent.

39. The use according to claim 36 wherein the agent for treatment of hypertension is an angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, diuretic, beta-blocker, or calcium channel blocker.

40. The use according to claim 36 wherein the agent for treatment of dyslipidaemia is a statin, a fibrate, a niacin and/or a cholesterol absorbtion inhibitor.

41. A therapeutic kit comprising a compound according to any of claims 1 to 29 or a composition according to claim 30 or 31.

42. A method of synthesis of a compound according to any one of claims 1 to 29.

43. A method of producing a compound according to any one of claims 1 to 29, the method comprising expressing a precursor peptide sequence from a nucleic acid construct that encodes the precursor peptide, recovering the expression product, and modifying the precursor peptide to yield a compound according to any one of claims 1 to 29.

44. A method according to claim 43 comprising modifying the precursor peptide to introduce the substituent at residue Ψ.