

AMYLIN ANALOGUES**PRIORITY**

This application claims priority from European patent application no. 16188024.0, filed 9 September 2016, the disclosure of which is hereby incorporated by reference in its
5 entirety.

FIELD OF THE INVENTION

The present invention relates to amylin analogues that are amylin receptor agonists, and to their medical use in the treatment and/or prevention of a variety of diseases, conditions or disorders, including treatment and/or prevention of excess food intake, obesity and
10 excess body weight, metabolic diseases, and other conditions and disorders described herein. In particular, the present invention relates to stable amylin analogues that have a long duration of action and are well suited for use in the form of a liquid formulation.

BACKGROUND OF THE INVENTION

Amylin is one of a family of peptide hormones that includes amylin, calcitonin, calcitonin
15 gene-related peptide, adrenomedullin and intermedin (intermedin also being known as AFP-6), and has been implicated in various metabolic diseases and disorders. Human amylin was first isolated, purified and characterized as the major component of amyloid deposits in the islets of pancreases from type 2 diabetes patients.

Native human amylin is a 37-amino acid peptide having the formula

20 H-KC()₂NTATC()₂ATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH₂

wherein H- at the N-terminus designates a hydrogen atom, corresponding to the presence of a free amino group on the N-terminal amino acid residue [i.e. the lysine (K) residue at sequence position number 1 in the sequence shown above]; wherein -NH₂ at the C-
25 terminus indicates that the C-terminal carboxyl group is in the amide form; and wherein the parentheses () associated with the two cysteine (C, Cys) residues at sequence positions 2 and 7 indicate the presence of an intramolecular disulfide bridge between the two Cys residues in question.

30 Amylin may be beneficial in treating metabolic disorders such as diabetes and/or obesity. Amylin is believed to regulate gastric emptying, and to suppress glucagon secretion and food intake, thereby regulating the rate of glucose release to the circulation. Amylin

appears to complement the actions of insulin. Compared to healthy adults, type 1 diabetes patients have no circulating amylin, and type 2 diabetes patients exhibit reduced postprandial amylin concentrations. In human trials an amylin analogue known as pramlintide, described in WO 93/10146 and having the sequence Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Pro-Ile-Leu-Pro-Pro-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr, which also possesses a disulphide bridge between the Cys residues at positions 2 and 7, has been shown to reduce body weight or reduce weight gain. An alternative amylin analogue incorporating N-methylated residues and having a reduced tendency to fibrillation, designated IAPP-GI, has been described by Yan et al. (PNAS, 103(7), 2046-2051, 2006; Angew. Chem. Int. Ed. 2013, 52, 10378-10383; WO2006/042745). IAPP-GI appears to have lower activity than native amylin, however.

WO91/07978 describes analogues of hypocalcemic peptides, including amylin, in which internal disulphide bridges are replaced with alternative cyclisations. The effect of these alternative structures on the activity of amylin analogues is not disclosed. WO99/34764 presents data showing that ^{2,7}cyclo-[2Asp,7Lys]-h-amylin has considerably lower potency than certain other amylin analogues, and human amylin itself.

Further analogues of amylin or pramlintide are described in WO2013/156594, WO2012/168430, WO2012/168431 and WO2012/168432, as well as WO2015/040182.

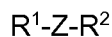
Obesity is believed to be a major causal factor in development of type 2 diabetes, which constitutes a growing and worldwide major health problem. Diseases or disorders that may develop as a consequence of untreated diabetes include cardiovascular and peripheral artery disease, micro- and macrovascular complications, stroke, and certain forms of cancer, particularly hematopoietic cancers.

There is a need in the art for further amylin analogues. For example, amylin analogues that show a reduced tendency for fibrillation and/or high chemical stability at or around pH 7 might allow for a formulation at or near physiological pH. Amylin analogues having appropriately long plasma elimination half-lives, may also enable longer intervals between dosing than is currently possible (e.g. once weekly, or even less frequently) and hence improve patient compliance. High levels of agonist activity at the amylin receptor may also be desirable.

SUMMARY OF THE INVENTION

The present invention relates to compounds which are analogues of human amylin.

In a first aspect, the invention provides an amylin analogue which is a compound having
5 the formula:



wherein

R^1 is hydrogen, C_{1-4} acyl, benzoyl or C_{1-4} alkyl, or a half-life extending moiety M, wherein M is optionally linked to Z via a linker moiety L;

10 R^2 is OH or NHR^3 , wherein R^3 is hydrogen or C_{1-3} -alkyl; and

Z is an amino acid sequence of formula I:

X1-X2-X3-X4-X5-X6-X7-Ala-Thr-X10-Arg-Leu-Ala-X14-Phe-Leu-X17-Arg-X19-X20-Phe-
Gly(Me)-Ala-Ile(Me)-X27-Ser-Ser-Thr-Glu-X32-Gly-Ser-X35-Thr-X37 (I)

wherein

15 X1 is selected from the group consisting of Arg, Lys and Glu;

X3 is selected from the group consisting of Gly, Gln and Pro;

X4 is selected from the group consisting of Thr and Glu;

X5 is selected from the group consisting of Ala and Leu;

X6 is selected from the group consisting of Thr and Ser;

20 X10 is selected from the group consisting of Glu and Gln;

X14 is selected from the group consisting of Aad, His, Asp, Asn and Arg;

X17 is selected from the group consisting of Gln, His and Thr;

X19-X20 is selected from Ser-Ser, Thr-Thr, Ala-Thr, Ala-Ala, Gly-Thr, Gly-Gly and Ala-Asn
or is absent;

25 X27 is selected from the group consisting of Leu and Pro;

X32 is selected from the group consisting of Val and Thr;

X35 is selected from the group consisting of Asn and Ser;

X37 is selected from the group consisting of Hyp and Pro; and

X2 and X7 are amino acid residues whose side chains together form a lactam bridge;

5 or a pharmaceutically acceptable salt or solvate thereof.

In some embodiments, X1 is selected from Arg and Lys.

10 In some embodiments, X3 is Gly, X4 is Thr, X5 is Ala and/or X6 is Thr, e.g. X3 is Gly, X4 is Thr, X5 is Ala and X6 is Thr.

In some embodiments, X14 is selected from His, Asp and Aad.

In some embodiments, X17 is Gln.

15

In some embodiments, X19-X20 is selected from Ser-Ser and Thr-Thr, or is absent, e.g. Ser-Ser.

In some embodiments, X32 is Val, X35 is Asn and/or X37 is Hyp.

20

Thus, Z may be an amino acid sequence of formula II:

X1-X2-Gly-Thr-Ala-Thr-X7-Ala-Thr-X10-Arg-Leu-Ala-X14-Phe-Leu-Gln-Arg-X19-X20-Phe-Gly(Me)-Ala-Ile(Me)-X27-Ser-Ser-Thr-Glu-Val-Gly-Ser-Asn-Thr-Hyp (II)

wherein

25 X1 is selected from the group consisting of Arg and Lys;

X10 is selected from the group consisting of Glu and Gln;

X14 is selected from the group consisting of Aad, Asp and His;

X19-X20 is selected from Ser-Ser and Thr-Thr or is absent;

X27 is selected from the group consisting of Leu and Pro; and

X2 and X7 are amino acid residues whose side chains together form a lactam bridge.

In some embodiments, X14 is Aad, X19-X20 is Ser-Ser and X27 is Leu.

Throughout this specification, amino acid positions of the amylin analogues are numbered
5 according to the corresponding position in native human amylin having the sequence
shown above. The sequence of Formulae I and II (and other formulae herein) contain a
two amino acid deletion corresponding to the two residues Asn21 and Asn22 of human
amylin. Thus, for ease of comparison with the amylin sequence, the Phe residue
immediately C-terminal (downstream) of position X20 is designated as position 23, since it
10 aligns with Phe23 of human amylin. Thus, the numbering of any given residue in
Formulae I and II above, and in other formulae elsewhere in this specification, reflects the
corresponding residue in human amylin when optimally aligned therewith and does not
necessarily reflect its linear position in the particular sequence.

15 (It will be apparent that any of the relevant formulae presented in this specification could
be written to include residues X21-X22 at the appropriate positions, wherein X21 and X22
are absent.)

Native amylin is known to form fibrils in aqueous solution almost instantly. Consequently,
many attempts have been made to enhance the stability of amylin analogues in liquid
20 formulations. The tendency for fibrillation can be reduced by incorporating N-methylated
residues (as mentioned above) and/or by substitution of certain amino acids at various
positions. However, despite these options the desire to further optimize the stability of
amylin analogues in aqueous solution remains. Amylin analogues with further enhanced
chemical stability in aqueous solution would facilitate development of a corresponding
25 pharmaceutical product, potentially even in the form of a ready-to-use formulation, e.g. at
or around the neutral pH range (pH 7 – 7.4).

Native amylin, and the vast majority of amylin analogues (such as pramlintide) contain a
disulphide bridge between cysteine residues at positions 2 and 7. The internal cyclisation
which this bridge provides appears to be required for full potency and activity. Although
30 compounds containing an internal disulphide bond are frequently less chemically stable
than might be desired, and the presence of the bond may contribute to dimerisation and
oligomerisation, e.g. via disulphide exchange reactions, the disulphide bond in amylin or
amylin analogues has not been reported to be a factor relevant for the low chemical
stability in aqueous formulations.

35 Few attempts to replace the disulphide bridge in amylin have been reported. As
described above, in an attempt to enhance in vivo stability and efficacy by reducing

chemical and enzymatic proteolysis, WO91/07978 proposes replacing internal disulphide bridges of hypocalcemic peptides (including e.g. calcitonin and amylin) with alternative cyclisations. However, the effect of these alternative structures on the activity of amylin analogues is not disclosed. WO99/34764 presents data showing that replacement of the
5 disulphide bridge with an intramolecular lactam bridge in the native human amylin sequence results in an amylin analogue (^{2,7}cyclo-[2Asp,7Lys]-h-amylin) having considerably lower potency than the wild type and many other amylin analogues, which may further explain why alternative cyclisation options have not been pursued further.

However, it has now been found that lactam-based cyclisations are very compatible with
10 amylin analogues having deletions at positions 21 and 22, as the replacement of the disulphide bridge by a lactam bridge leads to a substantial increase of stability in aqueous solution (see table 2) while other beneficial properties of these amylin analogues, such as low tendency for fibrillation, high activity and good solubility are retained. Although it is known that such lactam bridges reduce the activity of the peptides dramatically (see WO
15 91/07978, p 45 lines 36 to 52; WO 99/34764 p. 84, Table A), in addition it has now been found that amylin analogues of the present invention may retain high activity/show no reduction of activity at the hCT-R, hAMYR1, hAMYR2 and/or hAMYR3 receptors. Additionally or alternatively they may have excellent chemical stability and resistance to fibrillation, especially but not exclusively in the neutral pH range.

20 Thus the amylin analogue of the invention comprises a lactam bridge formed between the side chains of the residues at positions X2 and X7. For simplicity, positions 2 and 7 will be discussed by reference to the residues nominally present before lactam formation.

One of the residues at positions X2 and X7 is a residue with a side chain comprising a carboxylic acid group and the other is a residue with a side chain comprising an amine
25 group, wherein a lactam (cyclic amide) is formed between the carboxylic acid and amine groups. The amine may be a primary or secondary amine, but is typically a primary amine. Suitable amino acids whose side chains can participate in a lactam bridge include Asp, Glu and Aad (having side chains comprising carboxylic acid groups) and Dap, Dab, Orn, Lys and hLys (having side chains comprising amine groups). Any of the amino acids
30 selected from Asp, Glu and Aad may in principle form a lactam bridge with any of the amino acids selected from the group consisting of Dap, Dab, Orn, Lys and hLys.

Thus, one of the residues at position X2 and X7 may be selected from Asp, Glu and Aad, and the other may be selected from Dap, Dab, Orn, Lys and hLys.

In some embodiments the carboxylic acid component of the lactam bridge derives from the amino acid at position X2, whereas the amine component of the lactam bridge derives from the amino acid at position X7. Thus X2 may be selected from Asp, Glu and Aad, and X7 may be selected from Dap, Dab, Orn, Lys and hLys.

- 5 It may be beneficial that the side chain of the residue at position X2 is of the same length as, or shorter than, the side chain of the residue at position X7. Such residue combinations can provide benefits including higher potency as compared to other combinations.

- 10 In this context, side chain length is counted as the number of atoms in a linear chain from the first atom of the side chain (which is bonded to an atom of the peptide backbone, i.e. to the alpha carbon of the relevant residue for most amino acids) up to and including the atom which participates in the amide bond of the lactam bridge (i.e. the carbon atom of the carboxylic acid functional group or the nitrogen atom of the amine group).

- 15 Thus common acid- and amine-containing side chains are considered to have the following side chain lengths:

Asp: 2 atoms

Glu: 3 atoms

Aad: 4 atoms

20 Dap: 2 atoms

Dab: 3 atoms

Orn: 4 atoms

Lys: 5 atoms

hLys: 6 atoms

25

In some embodiments the side chain of the residue at position X2 is shorter than the side chain of the residue at position X7.

Desirably, the length of the lactam bridge provided by the two side chains after formation of the amide bond (not including any atoms in the peptide backbone) is 4, 5, 6, 7 or 8 atoms, e.g. 5, 6, 7 or 8 atoms, or 5, 6 or 7 atoms.

- 5 Thus, suitable pairings of residues at positions X2 and X7 in which the side chain at position X2 is shorter than the side chain at position X7 include:

X2 is Asp and X7 is Lys

X2 is Asp and X7 is Orn

X2 is Asp and X7 is Dab

- 10 X2 is Asp and X7 is hLys

X2 is Dap and X7 is Aad

Examples of suitable pairings having the same side chain lengths include:

X2 is Glu and X7 is Dab

- 15 X2 is Dab and X7 is Glu

Further pairings which may nevertheless be considered include:

X2 is Asp and X7 is Dap

X2 is Aad and X7 is Dap

- 20 X2 is Dap and X7 is Asp

X2 is Dab and X7 is Asp

X2 is Orn and X7 is Asp

Pairings of particular interest are:

- 25 X2 is Asp and X7 is Lys

X2 is Asp and X7 is Orn.

In some embodiments of the formulae described above:

X1 may be Arg;

5

X10 may be Glu.

X14 may be selected from Asp and Aad.

X19-X20 may be Ser-Ser

10 X27 may be Leu

The amylin analogue may have the formula:

R^1-Z-R^2

wherein

15 R^1 is hydrogen, C_{1-4} acyl, benzoyl or C_{1-4} alkyl, or a half-life extending moiety M, wherein M is optionally linked to Z via a linker moiety L;

R^2 is OH or NHR^3 , wherein R^3 is hydrogen or C_{1-3} -alkyl; and

Z is an amino acid sequence selected from the group consisting of:

RD()GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP

RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNH-Hyp

RD()GTAT-Orn()-ATERLARFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

ED()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GEATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp

RD()GTLTk()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTASK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp
RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp
RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-hLys()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsST-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRATF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()QTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()PTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp
RD()GTATK()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATERLA-Aad-FLQRGGF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATERLA-Aad-FLQRANF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
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RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGsNT-Hyp
ED()GTATK()ATERLA-Aad-FLQRSSFGly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
KD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-Alle(Me)-LSSTEVGsNTHyp
RD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGsNT-Hyp

KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp
 R-Dap()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 R-Dab()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 R-Orn()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 R-Dap()-GTAT-Aad()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 R-Dab()-GTATE()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 R-Aad()-GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp
 RE()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp

or a pharmaceutically acceptable salt or solvate thereof.

The lactam bridge at positions 2 and 7 is indicated by parentheses () following the residues at those positions.

- 5 In some embodiments, R¹ is M or M-L-, and/or R² is NH₂.

Specific compounds of the invention include:

[19CD]-isoGlu-RD()GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 1)
[19CD]-isoGlu-RD()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 2)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 3)
[19CD]-isoGlu-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 4)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 5)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTE-VGsNT-Hyp-NH ₂	(Compound 6)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNTP-NH ₂	(Compound 7)
[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 8)

[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 9)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLARFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 10)
[19CD]-isoGlu-ED()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 11)
[19CD]-isoGlu-RD()GEATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 12)
[19CD]-isoGlu-RD()GTLTK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 13)
[19CD]-isoGlu-RD()GTASK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 14)
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 15)
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 16)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 17)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 18)
[19CD]-isoGlu-RD()GTAT-hLys()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 19)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 20)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 21)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 22)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 23)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 24)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRATF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 25)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 26)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 27)
[19CD]-isoGlu-RD()QTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 28)

[19CD]-isoGlu-RD()PTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 29)
[19CD]-isoGlu-ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE VGSNT-Hyp-NH ₂	(Compound 30)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 31)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRGGF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 32)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRANF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 33)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE VGSNT-Hyp-NH ₂	(Compound 34)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 35)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 36)
[19CD]-isoGlu-ED()GTATK()ATERLA-Aad-FLQRSSFGly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 37)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRRTTF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 38)
[19CD]-isoGlu-KD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-Alle(Me)-LSSTE VGSNTHyp-NH ₂	(Compound 39)
[19CD]-isoGlu-RD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 40)
[19CD]-isoGlu-RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 41)
[19CD]-isoGlu-RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 42)
[19CD]-isoGlu-KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 43)
[19CD]-isoGlu-KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 44)
[19CD]-isoGlu-R-Dap()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 45)
[19CD]-isoGlu-R-Dab()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 46)
[19CD]-isoGlu-R-Orn()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 47)
[19CD]-isoGlu-R-Dap()-GTAT-Aad()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE VGSNT-Hyp-NH ₂	(Compound 48)

[19CD]-isoGlu-R-Dab()-GTATE()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂ (Compound 49)

[19CD]-isoGlu-R-Aad()-GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂ (Compound 50)

[19CD]-isoGlu-RE()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂ (Compound 51)

and pharmaceutically acceptable salts and solvates thereof.

The invention further provides a composition comprising an amylin analogue as described above. The composition may be a pharmaceutical composition, and may comprise a pharmaceutically acceptable carrier, excipient or vehicle.

The invention further provides a method for the synthesis of an amylin analogue as described above. The method may comprise the steps of synthesising the peptide by solid-phase or liquid-phase methodology, and optionally isolating and/or purifying the final product. The method may further comprise the step of forming an amide bond between the side chains at positions 2 and 7.

The present invention further provides an amylin analogue of the invention for use in a method of medical treatment.

The amylin analogues are useful, inter alia, in the reduction of food intake, promotion of weight loss, and inhibition or reduction of weight gain. As a result, they may be used for treatment of a variety of conditions, diseases, or disorders in a subject, including, but not limited to, obesity and various obesity-related conditions, diseases, or disorders, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease. It will be understood that the amylin analogues may thus be administered to subjects affected by conditions characterised by inadequate control of appetite or otherwise over-feeding, such as binge-eating disorder and Prader-Willi syndrome. It will be clear that the analogues can be used for treatment of combinations of the conditions described.

Thus, the invention provides an amylin analogue of the invention for use in a method of treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess

body weight. Treatment may be achieved, for example, by control of appetite, feeding, food intake, calorie intake and/or energy expenditure.

The invention also provides an amylin analogue of the invention for use in a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

The invention also provides an amylin analogue of the invention for use in a method of prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and combinations thereof.

The invention also provides an amylin analogue of the invention for use in a method of lowering circulating LDL levels and/or increasing HDL/LDL ratio.

Effects of amylin analogues on these conditions may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

25

The invention further provides use of an amylin analogue of the invention in the manufacture of a medicament for treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight.

The invention also provides use of an amylin analogue of the invention in the manufacture of a medicament for treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health

complications of obesity or overweight such as infertility. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

- 5 The invention also provides use of an amylin analogue of the invention in the manufacture of a medicament for the prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic
- 10 dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and
- 15 combinations thereof.

The invention also provides use of an amylin analogue of the invention in the manufacture of a medicament for lowering circulating LDL levels and/or increasing HDL/LDL ratio.

- The invention further provides a method of treating, inhibiting or reducing weight gain,
- 20 promoting weight loss and/or reducing excess body weight in a subject, comprising administering a therapeutically effective amount of an amylin analogue of the invention to the subject.

- The invention also provides a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior
- 25 to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility in a subject, comprising administering a therapeutically effective amount of an amylin analogue of the invention to the subject. The subject may be affected by obesity
- 30 accompanied by at least one weight-related co-morbid condition, such as diabetes (e.g. type 2 diabetes), hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

The invention also provides a method of prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose

levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and combinations thereof, in a subject, comprising administering a therapeutically effective amount of an amylin analogue of the invention to the subject.

The invention further provides a method of lowering circulating LDL levels and/or increasing HDL/LDL ratio in a subject, comprising administering a therapeutically effective amount of an amylin analogue of the invention to the subject.

The invention further provides the use of an amylin analogue as described above in a method of cosmetic (i.e. non-therapeutic) weight loss. It will be understood that references to therapeutic uses of amylin analogues and methods comprising administration of amylin analogues may equally be taken to encompass uses and administration of such compositions.

Further aspects and embodiments of the present invention will become apparent from the disclosure below.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined herein, scientific and technical terms used herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature employed herein in connection with techniques of chemistry, molecular biology, cell and cancer biology, immunology, microbiology, pharmacology, and protein and nucleic acid chemistry, described herein, is that well known and commonly used in the art.

All publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or component, or of a stated group of integers or components, but not the exclusion of any other integer or component or group of integers or components.

The singular forms “a,” “an,” and “the” include the plurals unless the context clearly dictates otherwise.

The term “including” is used to mean “including but not limited to.” “Including” and “including but not limited to” are used interchangeably.

- 5 The terms “patient,” “subject,” and “individual” may be used interchangeably and may refer to either a human or a non-human animal. Subjects are typically mammals, including humans, non-human primates (including great apes, Old World monkeys and New World monkeys), livestock animals (e.g., bovines, porcines), companion animals (e.g., canines, felines) and rodents (e.g., mice and rats).
- 10 As used herein, the term “pharmaceutically acceptable salt” is intended to indicate a salt which is not harmful to a patient or subject to which the salt in question is administered. It may suitably be a salt chosen, e.g., among acid addition salts and basic salts. Examples of acid addition salts include chloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected among alkali metal cations, such as
- 15 sodium or potassium ions, alkaline earth metal cations, such as calcium or magnesium ions, as well as substituted ammonium ions, such as ions of the type $N(R^1)(R^2)(R^3)(R^4)^+$, where R^1 , R^2 , R^3 and R^4 independently will typically designate hydrogen, optionally substituted C_{1-6} -alkyl or optionally substituted C_{2-6} -alkenyl. Examples of relevant C_{1-6} -alkyl groups include methyl, ethyl, 1-propyl and 2-propyl groups. Examples of C_{2-6} -alkenyl
- 20 groups of possible relevance include ethenyl, 1-propenyl and 2-propenyl. Other examples of pharmaceutically acceptable salts are described in “Remington’s Pharmaceutical Sciences”, 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985 (and more recent editions thereof), in the “Encyclopaedia of Pharmaceutical Technology”, 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA
- 25 (Inc.), NY, USA, 2007, and in *J. Pharm. Sci.* 66: 2 (1977).

The term “solvate” in the context of the present invention refers to a complex of defined stoichiometry formed between a solute (*in casu*, a peptide or pharmaceutically acceptable salt thereof according to the invention) and a solvent. The solvent in this connection may, for example, be water, ethanol or another pharmaceutically acceptable - typically small-

30 molecular - organic species, such as, but not limited to, acetic acid or lactic acid. When the solvent in question is water, such a solvate is normally referred to as a hydrate.

The term “agonist” as employed in the context of the invention refers to a substance that activates the receptor type in question, typically by binding to it (i.e. as a ligand).

Each embodiment of the invention described herein may be taken alone or in combination with one or more other embodiments of the invention.

Throughout the present specification, unless naturally occurring amino acids are referred to by their full name (e.g. alanine, arginine, etc.), they are designated by their conventional three-letter or single-letter abbreviations (e.g. Ala or A for alanine, Arg or R for arginine, etc.). In the case of certain less common or non-naturally occurring amino acids (i.e. amino acids other than the 20 encoded by the standard mammalian genetic code), unless they are referred to by their full name (e.g. sarcosine, ornithine, etc.), frequently employed three- or four-character codes are employed for residues thereof, including Orn (ornithine, i.e. 2,5-diaminopentanoic acid), Aib (α -aminoisobutyric acid), Dab (2,4-diaminobutanoic acid), Dap (2,3-diaminopropanoic acid), Har (homoarginine), γ -Glu (γ -glutamic acid), Gaba (γ -aminobutanoic acid), β -Ala (i.e. 3-aminopropanoic acid), 8Ado (8-amino-3,6-dioxaoctanoic acid).

Unless otherwise indicated, reference is made to the L-isomeric forms of the amino acids in question.

Additional abbreviations include the following:

Gly(Me):	N-methylglycine [also known as sarcosine (Sar)]
Ile(Me):	N-methylisoleucine
Aad:	2-aminoadipic acid, e.g. (2S)-2-aminoadipic acid [also (2S)-2-aminohexanedioic acid] , also known as homo-glutamic acid
Hyp:	4-hydroxyproline, e.g. (2S,4R)-4-hydroxyproline [also denoted (4R)-4-hydroxy-L-proline]
Dap:	2,3-diaminopropanoic acid, e.g. (2S)-2,3-diaminopropanoic acid
Dab:	2,4-diaminobutanoic acid, e.g. (2S)-2,4-diaminobutanoic acid
hLys:	2-amino-7-amino-heptanoic acid, also known as homo-lysine, e.g. (2S)-2-amino-7-amino-heptanoic acid

The term “therapeutically effective amount” as used herein in the context of the above-described methods of treatment or other therapeutic interventions according to the invention refers to an amount that is sufficient to cure, ameliorate, alleviate or partially

arrest the clinical manifestations of the particular disease, disorder or condition that is the object of the treatment or other therapeutic intervention in question e.g. as measured by established clinical endpoints or other biomarkers (established or experimental). A therapeutically relevant amount may be determined empirically by one skilled in the art

5 based on the indication being treated or prevented and the subject to whom the therapeutically relevant amount is being administered. For example, the skilled worker may measure one or more of the clinically relevant indicators of bioactivity described herein, e.g. plasma lipid levels, blood glucose levels or insulin release. The skilled worker may determine a clinically relevant amount through *in vitro* or *in vivo* measurements.

10 Other exemplary measures include weight gain, weight loss, and change in blood pressure.

An amount adequate to accomplish any or all of these effects is defined as a therapeutically effective amount. The administered amount and the method of administration can be tailored to achieve optimal efficacy. An amount effective for a given

15 purpose will depend, *inter alia*, on the severity of the disease, disorder or condition that is the object of the particular treatment or other therapeutic intervention, on the body weight and general condition of the subject in question, on diet, on possible concurrent medication, and on other factors well known to those skilled in the medical arts.

Determination of an appropriate dosage size and dosing regimen most appropriate for

20 administration of a peptide or pharmaceutically acceptable salt or solvate thereof according to the invention to a human may be guided by the results obtained by the present invention, and may be confirmed in properly designed clinical trials. 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

25 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 well known to the skilled person.

The terms "treatment" and grammatical variants thereof (e.g. "treated", "treating", "treat") as employed in the present context refer to an approach for obtaining beneficial or desired

30 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, stabilization (i.e. not worsening) of 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

35 survival relative to expected survival time if not receiving treatment. A subject (e.g. a human) in need of treatment may thus be a subject already afflicted with the disease or

disorder in question. The term "treatment" includes inhibition or reduction of an increase in severity of a pathological state or symptoms (e.g. weight gain or hyperglycemia) relative to the absence of treatment, and is not necessarily meant to imply complete cessation of the relevant disease, disorder or condition.

- 5 The terms "prevention" and grammatical variants thereof (e.g., "prevented", "preventing", "prevent") as employed in the present context refer to an approach for hindering or preventing the development of, or altering the pathology of, a condition, disease or disorder. Accordingly, "prevention" may refer to prophylactic or preventive measures. For the purposes of this invention, beneficial or desired clinical results include, but are not
- 10 limited to, prevention or slowing of symptoms, progression or development of a disease, whether detectable or undetectable. A subject (e.g. a human) in need of "prevention" may thus be a subject not yet afflicted with the disease or disorder in question. The term "prevention" thus includes inhibiting or slowing the onset of disease relative to the absence of treatment, and is not necessarily meant to imply permanent prevention of the
- 15 relevant disease, disorder or condition.

Synthesis of amylin analogues

- The invention further provides a method of synthesis of an amylin analogue of the invention. The amylin analogues (which may also be referred to as compounds or peptides) may suitably be manufactured by standard synthetic methods. Thus, the
- 20 peptides may be synthesized by, e.g., methods comprising synthesizing the peptide by standard solid-phase or liquid-phase methodology, either stepwise or by fragment assembly, and optionally isolating and purifying the final peptide product. In this context, reference may be made to WO 98/11125 or, *inter alia*, Fields, G.B. et al., "Principles and Practice of Solid-Phase Peptide Synthesis"; in: *Synthetic Peptides*, Gregory A. Grant
- 25 (ed.), Oxford University Press (2nd edition, 2002) and the synthesis examples herein. The method typically further comprises the step of forming an amide bond between the side chains at positions 2 and 7, e.g. as described below. In the case of solid phase synthesis, cyclisation may be performed in situ on the solid phase (e.g. resin), i.e. before removal of the peptide from the solid phase.

30

C₁₋₄ acyl groups

C₁₋₄ acyl groups that may be present as a group R¹ in the context of compounds of the present invention include formyl (i.e. methanoyl), acetyl (i.e. ethanoyl), propanoyl, 1-butanoyl and 2-methylpropanoyl groups.

35

C₁₋₄ alkyl groups

C₁₋₄ alkyl groups that may be present as a group R¹ in the context of compounds of the present invention include, but are not limited to, C₁₋₃ alkyl groups, such as methyl, ethyl, 1-propyl or 2-propyl.

5

C₁₋₃ alkyl groups

C₁₋₃ alkyl groups that may be present as a group R³ in the context of compounds of the present invention include methyl, ethyl, 1-propyl and 2-propyl.

10 Half-life extending moieties M

As described herein, the N-terminal moiety R¹ in a compound of the invention may be a half-life extending moiety M (sometimes referred to in the literature as, *inter alia*, a duration enhancing moiety or albumin binding moiety), optionally linked (covalently attached) to the peptide moiety Z via a linker moiety L. Among suitable half-life extending
15 moieties are certain types of lipophilic substituents. Without wishing to be bound by any particular theory, it is thought that such lipophilic substituents (and other classes of half-life extending moieties) bind albumin in the blood stream, thereby shielding the compound of the invention from renal filtration as well as enzymatic degradation and thus possibly enhancing the half-life of the compound *in vivo*. The lipophilic substituent may also
20 modulate the potency of the compound as an agonist to the amylin (calcitonin) receptor.

The lipophilic substituent may be attached to the N-terminal amino acid residue or to the linker L via an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulfonamide. Accordingly it will be understood that preferably the lipophilic substituent
25 includes an acyl group, a sulfonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulfonyl ester, thioester, amide, amine or sulfonamide. Preferably, an acyl group in the lipophilic substituent forms part of an amide or ester with the amino acid residue or the linker.

30 The lipophilic substituent may comprise a hydrocarbon chain having from 10 to 24 C atoms, e.g. from 14 to 22 C atoms, e.g. from 16 to 20 C atoms. Preferably it has at least 14 C atoms, and preferably has 20 C atoms or fewer. For example, the hydrocarbon chain may contain 14, 15, 16, 17, 18, 19 or 20 carbon atoms. The hydrocarbon chain may be linear or branched, and may be saturated or unsaturated. Furthermore, it can include
35 a functional group at the end of the hydrocarbon chain, e.g. a carboxylic acid group which may or may not be protected during synthesis. From the discussion above it will also be understood that the hydrocarbon chain is preferably substituted with a moiety which forms

part of the attachment to the N-terminal amino acid residue of the peptide moiety Z or to the linker L, for example an acyl group, a sulfonyl group, an N atom, an O atom or an S atom.

- 5 Most preferably, the hydrocarbon chain is substituted with an acyl group, and accordingly the hydrocarbon chain may be part of an alkanoyl group, for example a dodecanoyl, 2-butyloctanoyl, tetradecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl or eicosanoyl group. Examples of functionalized hydrocarbon chains are 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl and 19-carboxy-nonadecanoyl.

10

- As mentioned above, a lipophilic substituent M may be linked to the N-terminal amino acid residue of Z via a linker L. In embodiments, the linker moiety L may itself comprise one, two, three or more linked sub-moieties L^1 , L^2 , L^3 , ..etc. When the linker L comprises only one such moiety, it is attached to the lipophilic substituent and to the N-terminal amino acid residue of Z. The linker may then be attached to the lipophilic substituent and to the N-terminal amino acid residue of Z independently by means of an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulfonamide bond. Accordingly, it may include two moieties independently selected from acyl, sulfonyl, an N atom, an O atom and an S atom. The linker may consist of a linear or branched C_{1-10} hydrocarbon chain or more preferably a linear C_{1-5} hydrocarbon chain. Furthermore the linker can be substituted with one or more substituents selected from C_{1-6} alkyl, amino C_{1-6} alkyl, hydroxy C_{1-6} alkyl and carboxy C_{1-6} alkyl.
- 15
- 20

- In some embodiments the linker may comprise one or more (e.g. one, two or three) linked amino acid residues, which may each independently be a residue of any naturally occurring or non-naturally occurring amino acid. For example, the linker may comprise one, two or three linked amino acid residues, each of which may independently be a residue of Gly, Pro, Ala, Val, Leu, Ile, Met, Cys, Phe, Tyr, Trp, His, Lys, Arg, Gln, Asn, α -Glu, γ -Glu, ϵ -Lys, Asp, β -Asp, Ser, Thr, Gaba, Aib, β -Ala (i.e. 3-aminopropanoyl), 4-aminobutanoyl, 5-aminopentanoyl, 6-aminohexanoyl, 7-aminoheptanoyl, 8-aminooctanoyl, 9-aminononanoyl, 10-aminodecanoyl or 8Ado (i.e. 8-amino-3,6-dioxaoctanoyl).
- 25
- 30

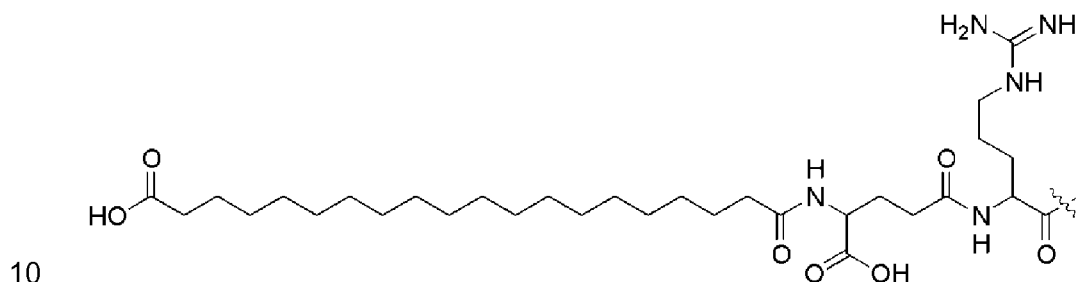
- References to γ -Glu, ϵ -Lys, and β -Asp indicate residues of amino acids which participate in bonds via their side chain carboxyl or amine functional groups. Thus γ -Glu, and β -Asp participate in bonds via their alpha amino and side chain carboxyl groups, while ϵ -Lys participates via its carboxyl and side chain amino groups. In the context of the present invention, γ -Glu and isoGlu are used interchangeably.
- 35

In certain embodiments, the linker comprises or consists of one, two or three independently selected residues of Glu, γ -Glu, ϵ -Lys, β -Ala, 4-aminobutanoyl, 8-aminooctanoyl or 8Ado.

5

Linkers consisting of isoGlu and isoGlu-isoGlu may be particularly preferred.

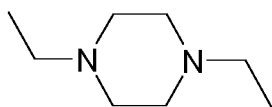
An example of a lipophilic substituent comprising a lipophilic moiety M and linker L is shown in the formula below:



Here, the backbone nitrogen of an Arg residue (present at position X1 of the amylin analogue's peptide sequence Z) is covalently attached to the side chain carboxyl group of a Glu moiety via an amide linkage. A 19-carboxy-nonadecanoyl group is covalently attached to the alpha amino group of the Glu linker via an amide linkage. Thus the Glu linker is in an iso-Glu (or γ -Glu) configuration. This combination of lipophilic moiety and linker, attached to an Arg residue, may be referred to by the shorthand notation [19CD]-isoGlu-R, e.g. when shown in formulae of specific compounds.

20 The skilled person will be well aware of suitable techniques for preparing the compounds employed in the context of the invention. For examples of suitable chemistry, see, e.g., WO98/08871, WO00/55184, WO00/55119, Madsen et al (*J. Med. Chem.* 2007, 50, 6126-32), and Knudsen et al. 2000 (*J. Med Chem.* 43, 1664-1669).

25 The hydrocarbon chain in a lipophilic substituent may be further substituted. For example, it may be further substituted with up to three substituents selected from NH_2 , OH and COOH. If the hydrocarbon chain is further substituted, it is preferably further substituted with only one substituent. Alternatively or additionally, the hydrocarbon chain may include a cycloalkane or heterocycloalkane moiety, for example as shown below:

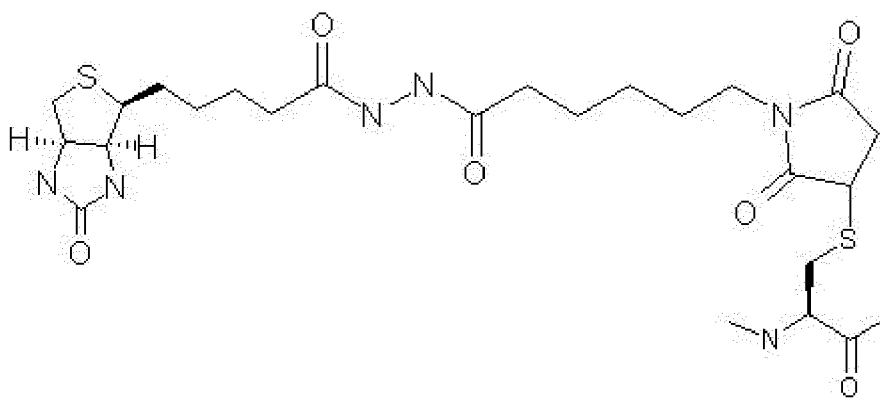


In some embodiments, the cycloalkane or heterocycloalkane moiety is a six-membered ring, e.g. a piperidine ring.

In alternative embodiments of the present invention, the N-terminal amino acid of Z in a compound of the invention may be linked (covalently attached) to a biotinylc substituent, optionally via a linker moiety L. Without wishing to be bound by any particular theory, it is likewise believed that such biotinylc substituents bind to albumin in the blood stream, thereby shielding the compound of the invention from enzymatic degradation and thus possibly enhancing the half-life of the compound *in vivo*. A linker, when present, may provide spacing between the peptide moiety Z and the biotinylc substituent.

The biotinylc substituent may be attached to the N-terminal amino acid residue or to the linker via an maleimide ester bond, a sulfonyl ester bond, a thioester bond, an amide bond, an amine bond or a sulfonamide bond. Accordingly it will be understood that the biotinylc substituent preferably comprises an maleimido group, an acyl group, a sulfonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulfonyl ester, thioester, amide, amine or sulfonamide bond in question.

Examples of biotinylc substituents may include



Biotin is known as Vitamin H or Coenzyme R, and is a water-soluble B-complex vitamin (vitamin B7). It has been shown to increase oral uptake of certain drugs.

Efficacy of compounds

The compounds of the invention are amylin receptor agonists, i.e. they are capable of binding to, and inducing signaling by, one or more receptors or receptor complexes regarded as physiological receptors for human amylin. These include the human calcitonin receptor hCT-R, as well as complexes comprising the human calcitonin receptor
5 hCT-R and at least one of the human receptor activity modifying proteins designated hRAMP1, hRAMP2 and hRAMP3. Complexes between hCT-R and hRAMP1, hRAMP2 and hRAMP3 are designated hAMYR1, hAMYR2 and hAMYR3 (i.e. human amylin receptors 1, 2 and 3) respectively.

Without wishing to be bound by theory, a compound may be considered an amylin
10 receptor agonist if it has agonist activity at one or more of hAMYR1, hAMYR2 and hAMYR3, e.g. against hAMYR1 and/or hAMYR3, e.g. at hAMYR3.

Typically an amylin receptor agonist will also have agonist activity at hCT-R when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3. Typically, the agonist will have activity at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and
15 hRAMP3) which is less than 10-fold higher than its activity at any one of hAMYR1, hAMYR2 and hAMYR3 (i.e. its activity at all of these receptors) in a comparable assay. Agonist activity at hCT-R may be less than 5-fold higher than agonist activity at hAMYR1, hAMYR2 and hAMYR3, substantially equal to (e.g. +/- 10%) agonist activity at hAMYR1, hAMYR2 and hAMYR3, or less than agonist activity at hAMYR1, hAMYR2 and hAMYR3.
20 In this regard, it may be sufficient just to compare activity between hCT-R and hAMYR3.

The ability to induce cAMP formation (i.e. to induce adenylate cyclase activity) as a result of binding to the relevant receptor or receptor complex is typically regarded as indicative of agonist activity. Other intracellular signaling pathways or events may also be used as read-outs for amylin receptor agonist activity. These may include calcium release, β -
25 arrestin recruitment, receptor internalization, kinase activation or inactivation, lipase activation, inositol phosphate release, diacylglycerol release or nuclear transcription factor translocation.

A suitable comparable assay format would utilize cells which express hCT-R and which differ only in their expression of hRAMP1, 2 and 3. For example, a "base" cell line which
30 does not express any of hCT-R, hRAMP1, hRAMP2 and hRAMP3 may be engineered to generate cells which express (i) hCT-R, and (ii) one of hAMYR1, hAMYR2 and hAMYR3 (i.e. hCT-R plus one of hRAMP1, hRAMP2 and hRAMP3), e.g. hAMYR3. The base cells will typically be mammalian cells and may be primate cells. They may be non-human primate cells. Preferably the base cell does not express any of CT-R, RAMP1, RAMP2 or
35 RAMP3 (whether human, or native to the base cell if the base cell is non-human). The

base cells may be fibroblast cells. Suitable non-human fibroblast base cells include COS7 cells, from African green monkey, which do not express native CT-R or RAMPs.

Comparative activity may be measured by any suitable means, such as via determination of EC₅₀ values as described below. It will be apparent that the same biological read-out
5 must be for both receptor types.

Compounds of the present invention may exhibit a number of advantageous properties in relation to human amylin and existing analogues thereof, such as pramlintide, IAPP-GI, and analogues described in WO2012/168430, WO2012/168431 and WO2012/168432. As compared to human amylin or any of those analogues, compounds of the invention
10 may, for example, exhibit improved efficacy (e.g., in the form of improved *in vitro* activity or potency at one or more of the receptors hCT-R, hAMYR1, hAMYR2 or hAMYR3. Additionally or alternatively, compounds of the invention may exhibit improved solubility in aqueous media, especially at pH values in the range from 4 to 7.5, or at a range of pH values across that range. Moreover, compounds of the present invention may additionally
15 or alternatively exhibit reduced tendency to undergo fibrillation in pharmaceutically relevant aqueous media, especially at pH values in the range from 4 to 7, or at a range of pH values across that range. Furthermore, compounds of the present invention may additionally or alternatively exhibit improved chemical stability (i.e. reduced tendency to undergo chemical degradation) in aqueous media, especially at pH values in the range
20 from 4 to 9, or at a range of pH values across that range.

Compounds of the invention may thus be well suited for formulation in acidic media (e.g. pH 4) and in neutral or near-neutral media (e.g. pH 7 or 7.4). In contrast to pramlintide, for example, which generally exhibits poor chemical stability and rapid fibrillation in pharmaceutically relevant aqueous media at neutral pH, compounds of the invention may
25 be thus well suited for co-formulation with, for example, insulin, various insulin analogues and/or other therapeutic (e.g. anti-diabetic or anti-obesity) agents that require a neutral or near-neutral formulation pH.

In general it is preferred to use a biological assay which measures intracellular signalling caused by binding of the compound to the relevant receptor, as discussed above.
30 Activation of the calcitonin/amylin receptor by compounds of the invention (which behave as agonists of the receptor) induces cAMP formation and activation of other intracellular signaling pathways and events. Thus, production of cAMP or any other suitable parameter in suitable cells expressing the receptor can be used to monitor agonist activity towards the receptor.

The skilled person will be aware of suitable assay formats, and examples are provided below. For example, the assays may make use of the human calcitonin receptor (hCT-R, .e.g. isoform 2 of the hCT-R) or the hAMYR3 receptor (see the examples below). Where sequences of precursor proteins are referred to, it should be understood that assays may
5 make use of the mature protein, lacking the signal sequence.

EC₅₀ values may be used as a numerical measure of agonist potency at a given receptor. An EC₅₀ 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 EC₅₀ [hCT-R] lower than the EC₅₀ [hCT-R] of native human amylin, or lower than
10 that of pramlintide, in a particular assay may be considered to have higher potency or activity at the receptor than native human amylin, or higher than that of pramlintide, respectively.

In some embodiments of compounds of the present invention, the EC₅₀ towards hCT-R is below 1.5 nM (e.g. 0.001 to 1.5 nM).

15 In some embodiments of compounds of the present invention, the EC₅₀ towards hCT-R is below 0.9 nM (e.g. 0.001 to 0.9 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hCT-R is below 0.5 nM (e.g. 0.001 to 0.5 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hCT-R is
20 below 0.3 nM (e.g. 0.001 to 0.3 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hCT-R is below 0.2 nM (e.g. 0.001 to 0.2 nM).

The EC₅₀ at hCT-R may be an indication of the effect of a compound on food intake, weight gain and/or weight loss. Compounds with lower EC₅₀ values at hCT-R may have a
25 greater effect on these parameters.

In some embodiments of compounds of the present invention, the EC₅₀ towards hAMYR3 is below 1.0 nM (e.g. 0.001 to 1.0 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hAMYR3
30 is below 0.5 nM (e.g. 0.001 to 0.5 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hAMYR3 is below 0.4 nM (e.g. 0.001 to 0.4 nM).

In some embodiments of compounds of the present invention, the EC₅₀ towards hAMYR3 is below 0.3 nM (e.g. 0.001 to 0.3 nM).

- 5 In some embodiments of compounds of the present invention, the EC₅₀ towards hAMYR3 is below 0.2 nM (e.g. 0.001 to 0.2 nM).

The EC₅₀ at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3) may be less than the EC₅₀ at any or all of hAMYR1, hAMYR2 and hAMYR3, e.g. at hAMYR3.

- 10 For example, the EC₅₀ at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3) may be less than 10-fold lower than the EC₅₀ at any or all of hAMYR1, hAMYR2 and hAMYR3, e.g. at hAMYR3.

The EC₅₀ at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3) may be less than 5-fold lower than the EC₅₀ at any or all of hAMYR1, hAMYR2 and

- 15 hAMYR3, e.g. at hAMYR3.

The EC₅₀ at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3) may be substantially equal to (e.g. +/- 50%) the EC₅₀ at any or all of hAMYR1, hAMYR2 and hAMYR3, e.g. at hAMYR3.

- 20 The EC₅₀ at hCT-R (when expressed in the absence of hRAMP1, hRAMP2 and hRAMP3) may be higher than the EC₅₀ at any or all of hAMYR1, hAMYR2 and hAMYR3, e.g. at hAMYR3.

Such assays may be performed under the conditions described in Example 2 below.

- 25 Additionally or alternatively, compounds of the invention may show excellent resistance to fibrillation. For example, they may show no detectable fibrillation after 96 hours at pH 4.0 and/or pH 7.0, e.g. at 40°C, e.g. under the conditions described in Example 4.

- 30 Additionally or alternatively, compounds of the invention may show excellent chemical stability, i.e. resistance to degradation in solution. For example, they may retain at least 70% purity, at least 75% purity, at least 80% purity, at least 85% purity, at least 90% purity, or at least 95% purity after incubation at pH 4, pH 6, and/or pH 7 at 40°C for 72 hours, or for 14 days, e.g. under the conditions described in Example 5.

Therapeutic uses

The compounds of the invention are useful, *inter alia*, in the reduction of food intake, promotion of weight loss, and inhibition or reduction of weight gain. They may therefore provide an attractive treatment option for, *inter alia*, obesity and metabolic diseases

5 caused, characterised by, or associated with, excess body weight.

Thus, the compounds may be used in a method of treating, inhibiting or reducing weight gain, promoting weight loss, reducing food intake, and/or reducing excess body weight. Treatment may be achieved, for example, by control of appetite, feeding, food intake, calorie intake and/or energy expenditure.

- 10 The compounds may be used in a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as
- 15 infertility.

- The compounds may also be used in in a method of prevention or treatment of Alzheimer's disease, diabetes, type 1 diabetes, type 2 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome,
- 20 hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure, arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure as a diabetic complication) coronary heart disease,
- 25 peripheral artery disease or stroke.

The compounds may also be useful in lowering circulating LDL levels and/or increasing HDL/LDL ratio.

The effects of the compounds described above may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

- 30 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).

5 Individuals with 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 factor for this syndrome appears to be abdominal obesity.

Pharmaceutical compositions

10 The invention also extends to compositions, such as pharmaceutical compositions, comprising amylin analogues. As with all aspects of the invention, it is to be understood that reference to an amylin analogue encompasses reference to pharmaceutically acceptable salts and solvates.

15 The amylin analogues of the present invention may be formulated as pharmaceutical compositions which are suited for administration with or without storage, and which typically comprise a therapeutically effective amount of at least one peptide of the invention, together with a pharmaceutically acceptable carrier, excipient or vehicle.

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
20 Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985. For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. Suitable pH-buffering agents may, e.g., be phosphate, citrate, acetate, tris(hydroxymethyl)aminomethane (TRIS), N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), ammonium bicarbonate,
25 diethanolamine, histidine, arginine, lysine or acetate (e.g. as sodium acetate), or mixtures thereof. The term further encompasses any carrier agents listed in the US Pharmacopeia for use in animals, including humans.

A pharmaceutical composition of the invention may be in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active
30 component or components. The unit dosage form may be presented as a packaged preparation, the package containing discrete quantities of the preparation, for example, packaged tablets, capsules or powders in vials or ampoules. The unit dosage form may also be, e.g., a capsule, cachet or tablet in itself, or it may be an appropriate number of any of these packaged forms. A unit dosage form may also be provided in single-dose

injectable form, for example in the form of a pen device containing a liquid-phase (typically aqueous) composition. Compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for e.g. oral, intravitreal, rectal, vaginal, nasal, topical, enteral or
5 parenteral (including subcutaneous (sc), intramuscular (im), intravenous (iv), intradermal and transdermal) administration or administration by inhalation. 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 pharmaceutical formulation.

Subcutaneous or transdermal modes of administration may in some cases be suitable for
10 peptides of the invention.

Further embodiments relate to devices, dosage forms and packages used to deliver the pharmaceutical formulations of the present invention. Thus, at least one peptide in a stable or preserved formulation or solution described herein can be administered to a patient in accordance with the present invention via a variety of delivery methods,
15 including by sc or im injection, or by transdermal, pulmonary or transmucosal administration, or by implant, or by use of an osmotic pump, cartridge, micro-pump or other means recognized by a person of skill in the art.

Still further embodiments relate to oral formulations and oral administration. Formulations for oral administration may rely on the co-administration of adjuvants (e.g. resorcinols
20 and/or nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to artificially increase the permeability of the intestinal walls, and/or the co-administration of enzymatic inhibitors (e.g. pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) or trasylol) to inhibit enzymatic degradation. The active constituent compound of a solid-type dosage form for oral administration can be mixed
25 with at least one additive, such as sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, alginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, or glyceride. These dosage forms can also contain other type(s) of additives, e.g. an inactive diluting agent, a lubricant (such as magnesium stearate), a paraben, a
30 preserving agent (such as sorbic acid, ascorbic acid or alpha-tocopherol), an antioxidant (such as cysteine), a disintegrant, binder, thickener, buffering agent, pH-adjusting agent, sweetening agent, flavoring agent or perfuming agent.

Dosages

A typical dosage of an amylin analogue as employed in the context of the present invention may be in the range from about 0.0001 to about 100 mg/kg body weight per day, such as from about 0.0005 to about 50 mg/kg body weight per day, such as from about 0.001 to about 10 mg/kg body weight per day, e.g. from about 0.01 to about 1 mg/kg body weight per day, administered in one or more doses, such as from one to three doses. The exact dosage employed will depend, *inter alia*, on: the nature and severity of the disease or disorder to be treated, on the sex, age, body weight and general condition of the subject to be treated, on possible other, concomitant, disease or disorder that is undergoing or is to undergo treatment, as well as on other factors that will be known to a medical practitioner of skill in the art.

An amylin analogue of the invention may be administered continuously (e.g. by intravenous administration or another continuous drug administration method), or may be administered to a subject at intervals, typically at regular time intervals, depending on the desired dosage and the pharmaceutical composition selected by the skilled practitioner for the particular subject. Regular administration dosing intervals include, e.g., once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, and the like. Such regular peptide administration regimens may, in certain circumstances such as, e.g., during chronic long-term administration, be advantageously interrupted for a period of time so that the medicated subject reduces the level of, or stops taking, the medication, often referred to as taking a "drug holiday." Drug holidays are useful for, e.g., maintaining or regaining sensitivity to a drug especially during long-term chronic treatment, or to reduce unwanted side-effects of long-term chronic treatment of the subject with the drug. The timing of a drug holiday depends on the timing of the regular dosing regimen and the purpose for taking the drug holiday (e.g., to regain drug sensitivity and/or to reduce unwanted side effects of continuous, long-term administration). In some embodiments, the drug holiday may be a reduction in the dosage of the drug (e.g. to below the therapeutically effective amount for a certain interval of time). In other embodiments, administration of the drug is stopped for a certain interval of time before administration is started again using the same or a different dosing regimen (e.g. at a lower or higher dose and/or frequency of administration). A drug holiday of the invention may thus be selected from a wide range of time-periods and dosage regimens. An exemplary drug holiday is two or more days, one or more weeks, or one or more months, up to about 24 months of drug holiday. So, for example, a regular daily dosing regimen with a peptide of the invention may, for example, be interrupted by a drug holiday of a week, or two weeks, or four weeks, after which time the preceding, regular dosage regimen (e.g. a daily or a weekly dosing regimen) is resumed. A variety of other drug holiday regimens are envisioned to be useful for administering peptides of the invention.

Thus, the peptide may be delivered via an administration regime which comprises two or more administration phases separated by respective drug holiday phases.

During each administration phase, the peptide is administered to the recipient subject in a therapeutically effective amount according to a pre-determined administration pattern. The administration pattern may comprise continuous administration of the drug to the recipient subject over the duration of the administration phase. Alternatively, the administration pattern may comprise administration of a plurality of doses of the peptide to the recipient subject, wherein said doses are spaced by dosing intervals.

10

A dosing pattern may comprise at least two doses per administration phase, at least five doses per administration phase, at least 10 doses per administration phase, at least 20 doses per administration phase, at least 30 doses per administration phase, or more.

Said dosing intervals may be regular dosing intervals, which may be as set out above, including once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, or a regular and even less frequent dosing interval, depending on the particular dosage formulation, bioavailability, and pharmacokinetic profile of the peptide.

20

An administration phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2 months, at least 3 months, at least 6 months, or more.

Where an administration pattern comprises a plurality of doses, the duration of a possible following drug holiday phase is longer than the dosing interval used in that administration pattern. Where the dosing interval is irregular, the duration of a drug holiday phase may be greater than the mean interval between doses over the course of the administration phase. Alternatively the duration of the drug holiday may be longer than the longest interval between consecutive doses during the administration phase.

30

The duration of a possible drug holiday phase may be at least twice that of the relevant dosing interval (or mean thereof), at least 3 times, at least 4 times, at least 5 times, at least 10 times, or at least 20 times that of the relevant dosing interval or mean thereof.

35

Within these constraints, a drug holiday phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2 months, at

least 3 months, at least 6 months, or more, depending on the administration pattern during the previous administration phase.

- An administration regime entailing the use of drug holiday comprises at least 2 administration phases. Consecutive administration phases are separated by respective drug holiday phases. Thus the administration regime may comprise at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, or at least 30 administration phases, or more, each separated by respective drug holiday phases.
- Consecutive administration phases may utilise the same administration pattern, although this may not always be desirable or necessary. However, if other drugs or active agents are administered in combination with a peptide of the invention, then typically the same combination of drugs or active agents is given in consecutive administration phases. In certain embodiments, the recipient subject is a human.

15

Combination therapy

- An amylin analogue of the invention may be administered as part of a combination therapy together with another active agent for the treatment of the disease or disorder in question, e.g. an anti-diabetic agent, an anti-obesity agent, an agent for treatment of metabolic syndrome, an anti-dyslipidemia agent, an anti-hypertensive agent, a proton pump inhibitor, or an anti-inflammatory agent. In such cases, the two active agents may be given together or separately, e.g. as constituents in the same pharmaceutical composition or formulation, or as separate formulations.

- Thus a peptide of the invention may have some benefit if administered in combination with an anti-diabetic agent of known type, including, but not limited to, metformin, a sulfonylurea, a glinide, a DPP-IV inhibitor, a glitazone, a GLP-1 receptor agonist (including GLP-1 or a GLP-1 analogue, an exendin-4 or an exendin-4 analogue, any other GLP-1 receptor agonist including liraglutide (Saxenda™, Victoza™), Dulaglutide or Albiglutide or a glucagon-GLP-1 dual agonist, e.g. as described in WO2008/101017, WO2008/152403, WO2010/070252, WO2010/070253, WO2010/070255, WO2010/070251, WO2011/006497, WO2011/160630, WO2011/160633, WO2013/092703, WO2014/041195, WO2015/055801, WO2015/055802), an SGLT2 inhibitor (i.e. an inhibitor of sodium-glucose transport, e.g. a gliflozin such as empagliflozin, canagliflozin, dapagliflozin or ipragliflozin), a GPR40 agonist (FFAR1/FFA1 agonist, e.g. fasiglifam), or an insulin or an insulin analogue. Examples of appropriate insulin analogues include, but are not limited to, Lantus™, Novorapid™, Humalog™,

Novomix™, Actraphane™ HM, Levemir™ Degludec™ and Apidra™. Other relevant anti-diabetic agents in this connection include GLP-1 receptor agonists, such as exenatide (Byetta™ and Bydureon™ exendin-4) and Byetta LAR™, lixisenatide (Lyxumia™) and liraglutide (Victoza™).

- 5 Moreover, a peptide of the invention may be used in combination with an anti-obesity agent of known type, including, but not limited to, peptide YY or an analogue thereof, neuropeptide Y (NPY) or an analogue thereof, a cannabinoid receptor 1 antagonist, a lipase inhibitor, Human proinsulin Peptide (HIP), a melanocortin receptor 4 agonist, a GLP-1 receptor agonist (including GLP-1 or a GLP-1 analogue, an exendin-4 or an exendin-4
- 10 analogue, any other GLP-1 receptor agonist including liraglutide (Saxenda™, Victoza™), Dulaglutide or Albiglutide or a glucagon-GLP-1 dual agonist, e.g. as described in WO2008/101017, WO2008/152403, WO2010/070252, WO2010/070253, WO2010/070255, WO2010/070251, WO2011/006497, WO2011/160630, WO2011/160633, WO2013/092703, WO2014/041195, WO2015/055801,
- 15 WO2015/055802), Orlistat™, Sibutramine™, phentermine, a melanin concentrating hormone receptor 1 antagonist, CCK, amylin, pramlintide and leptin, as well as analogues thereof.

A peptide of the invention may further be used in combination with an anti-hypertension agent of a known type, including, but not limited to, an angiotensin-converting enzyme

20 inhibitor, an angiotensin II receptor blocker, a diuretic, a beta-blocker or a calcium channel blocker.

A peptide of the invention may still further be used in combination with an anti-dyslipidemia agent of known type, including, but not limited to, a statin, a fibrate, a niacin, a PCSK9 (Proprotein convertase subtilisin/kexin type 9) inhibitor, or a cholesterol

25 absorption inhibitor.

A peptide of the invention may also be used in combination with a proton pump inhibitor (i.e. a pharmaceutical agent possessing pharmacological activity as an inhibitor of H⁺/K⁺-ATPase) of known type, including, but not limited to, an agent of the benzimidazole derivative type or of the imidazopyridine derivative type, such as Omeprazole™,

30 Lansoprazole™, Dexlansoprazole™, Esomeprazole™, Pantoprazole™, Rabeprazole™, Zolpidem™, Alpidem™, Saripidem™ or Necopidem™.

In addition, with regard to anti-inflammatory treatment, a peptide of the invention may be beneficial if administered in combination with an anti-inflammatory agent of known type, including, but not limited to:

steroids and corticosteroids, such as beclomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and hydrocortisone;

non-steroidal anti-inflammatory agents (NSAIDs), such as propionic acid derivatives (e.g. alminoprofen, benoxaprofen, bucloxic acid, carprofen, fenbufen, fenoprofen, fluprofen, 5 flurbiprofen, ibuprofen, indoprofen, ketoprofen, miroprofen, naproxen, oxaprozin, piroprofen, pranoprofen, suprofen, tiaprofenic acid and tioxaprofen); acetic acid derivatives (e.g. indomethacin, acetaminophen, alclofenac, clidanac, diclofenac, fenclofenac, fenclozic acid, fentiazac, furofenac, ibufenac, isoxepac, oxpinac, sulindac, tiopinac, tolmetin, zidometacin and zomepirac); fenamic acid derivatives (e.g. flufenamic acid, meclofenamic 10 acid, mefenamic acid, niflumic acid and tolfenamic acid); biphenylcarboxylic acid derivatives (e.g. diflunisal and flufenisal); oxicams (e.g. isoxicam, piroxicam, sudoxicam and tenoxicam); salicylates (e.g. acetylsalicylic acid and sulfasalazine); and pyrazolones (e.g. apazone, bezpiperylon, feprazone, mofebutazone, oxyphenbutazone and phenylbutazone);

15 COX II inhibitors, such as rofecoxib and celecoxib; preparations of interferon beta (e.g. interferon beta-1a or interferon beta-1b);

and certain other compounds, such as 5-aminosalicylic acid and prodrugs and 20 pharmaceutically acceptable salts thereof.

Metformin has also been demonstrated to have anti-inflammatory properties (see, e.g., Haffner et al., *Diabetes* 54: 1566-1572 (2005)) and as such may also be useful in combination with compounds (peptides) of the invention.

25

Devices and kits

In some embodiments, the invention relates to a device comprising an amylin analogue or pharmaceutical composition of the invention, for delivery of the analogue to a subject. Via such devices, amylin analogues can be administered to a patient via a variety of delivery 30 methods, including: intravenous, subcutaneous, intramuscular or intraperitoneal injection; oral administration; transdermal administration; pulmonary or transmucosal administration; administration by implant, osmotic pump, cartridge or micro pump; or by other means recognized by a person of skill in the art.

In some embodiments, the invention relates to a kit comprising an amylin analogue of the invention or a pharmaceutical composition of the invention. In certain embodiments, the kit further comprises packaging and/or instructions for use.

5 The device or kit may be useful for combination therapy as described above. Thus the device or kit may further comprise a further active agent, e.g. an anti-diabetic agent, an anti-obesity agent, an agent for treatment of metabolic syndrome, an anti-dyslipidemia agent, an anti-hypertensive agent, a proton pump inhibitor, or an anti-inflammatory agent as described above, or a pharmaceutical composition comprising such an active agent.

10 **EXAMPLES**

The following examples demonstrate certain specific embodiments of the present invention. The following examples were carried out using standard techniques that are well known and routine to those of skill in the art, except where otherwise described in detail. It is to be understood that these examples are for illustrative purposes only and do
15 not purport to be wholly definitive as to conditions or scope of the invention. As such, they should not be construed as limiting the scope of the present invention in any way.

Abbreviations employed in the examples include:

	Acm:	acetaminomethyl
	Alloc:	allyloxycarbonyl
20	Boc:	tert-butoxycarbonyl
	BSA:	bovine serum albumin
	cAMP:	cyclic adenosine monophosphate
	COMU TM :	(1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate
25	DCM:	dichloromethane
	DIPEA:	diisopropylethylamine
	DMEM:	Dulbecco's Modified Eagle Medium
	DMF:	N,N-dimethylformamide
	DODT:	3,6-dioxa-1,8-octanedithiol

	ESI-MS:	electron spray ionization mass spectrometry
	EtOH:	ethanol
	Et ₂ O:	diethyl ether
	FCS:	fetal calf serum
5	Fmoc:	9-fluorenylmethoxycarbonyl
	HATU:	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
	HEPES:	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
	HPLC:	high performance liquid chromatography
10	IBMX:	3-isobutyl-1-methylxanthine
	MeCN:	acetonitrile
	MS:	mass spectrometry
	NEP:	N-ethylpyrrolidone
	NMP	N-methylpyrrolidone
15	OAlI:	allylester
	PBS:	phosphate-buffered saline
	p-ERK:	phosphorylated extracellular regulated kinase
	RP-HPLC:	reverse phase high performance liquid chromatography
	TFA:	trifluoroacetic acid
20	TIS:	triisopropylsilane
	Trt:	trityl (i.e. triphenylmethyl)
	v/v:	volume/volume
	w/v:	weight/volume

The following examples are provided to illustrate certain embodiments of the invention and are not intended to limit the scope of the invention.

Measurement of physiological parameters

- Unless otherwise specified, whole-blood glucose levels were determined on tail-vein blood samples by the Biosen (EKF Diagnostic, Germany) enzyme-based electrode method. Blood samples were analyzed for glycated hemoglobin (HbA1c) using a Cobas c111 analyzer (Roche Diagnostics, Mannheim, Germany).

Example 1: Synthesis of compounds

The following compounds were synthesised:

[19CD]-isoGlu-RD()GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 1)
[19CD]-isoGlu-RD()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 2)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 3)
[19CD]-isoGlu-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 4)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 5)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 6)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 7)
[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 8)
[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 9)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLARFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 10)
[19CD]-isoGlu-ED()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 11)
[19CD]-isoGlu-RD()GEATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 12)
[19CD]-isoGlu-RD()GTLTK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 13)

[19CD]-isoGlu-RD()GTASK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 14)
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 15)
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 16)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 17)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 18)
[19CD]-isoGlu-RD()GTAT-hLys()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 19)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 20)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 21)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 22)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 23)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 24)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRATF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 25)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 26)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 27)
[19CD]-isoGlu-RD()GTAT-Orn()ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 28)
[19CD]-isoGlu-RD()PTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 29)
[19CD]-isoGlu-ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTE-VGSNT-Hyp-NH ₂	(Compound 30)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 31)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRGGF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 32)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRANF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 33)

[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 34)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 35)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 36)
[19CD]-isoGlu-ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 37)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 38)
[19CD]-isoGlu-KD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNTHyp-NH ₂	(Compound 39)
[19CD]-isoGlu-RD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 40)
[19CD]-isoGlu-RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 41)
[19CD]-isoGlu-RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 42)
[19CD]-isoGlu-KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTE-VGSNT-Hyp-NH ₂	(Compound 43)
[19CD]-isoGlu-KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp-NH ₂	(Compound 44)
[19CD]-isoGlu-R-Dap()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 45)
[19CD]-isoGlu-R-Dab()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 46)
[19CD]-isoGlu-R-Orn()-GTATD()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 47)
[19CD]-isoGlu-R-Dap()-GTAT-Aad()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 48)
[19CD]-isoGlu-R-Dab()-GTATE()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 49)
[19CD]-isoGlu-R-Aad()-GTAT-Dap()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 50)
[19CD]-isoGlu-RE()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 51)

For comparison purposes, three compounds having disulphide bridges (instead of lactam bridges) and two uncyclised compounds were synthesised:

[19CD]-isoGlu-RDGTAT-Orn-ATERLAHFLQRSSF-Sar-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	Ref. 1
[19CD]-isoGlu-RC() ₂ GTATC() ₂ ATERLAHFLQRSSF-Sar-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	Ref. 2
[19CD]-isoGlu-RDGTAT-Orn-ATERLA-Aad-FLQRSSF-Sar-A-Ile(Me)-LSSTE-VGsNT-Hyp-NH ₂	Ref. 3
[19CD]-isoGlu-RC() ₂ GTATC() ₂ ATERLA-Aad-FLQRSSF-Sar-A-Ile(Me)-LSSTE-VGsNT-Hyp-NH ₂	Ref. 4
[19CD]-isoGlu-RC() ₂ NTATC() ₂ ATQRLADFLQRSSF-Sar-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	Ref. 5

A further reference compound, designated NN96, is {N- α -[(S)-4-carboxy-4-(19-carboxynonadecanoylamino)butyryl]-[Arg1,Glu14,His17,Pro37]-pramlintide; disclosed as Example 96 in WO 2012/168430 A2, and having the amino acid sequence

5 RC()₂NTATC()₂ATQRLAEFLHHSSNFGPILPPTNVGSNTP.

Parentheses “()” indicate intramolecular lactam bridges (or disulphide bridges, where appropriate) formed between the side chains of the residues at positions 2 and 7 of the relevant amino acid sequences.

10 Unless otherwise specified, reagents and solvents employed in the following were available commercially in standard laboratory reagent or analytical grade, and were used without further purification.

General procedures for solid-phase synthesis of peptides

15 A CEM Liberty Peptide Synthesizer or a CEM Liberty Blue Peptide Synthesizer was employed, using standard Fmoc chemistry. TentaGel™ S Ram resin (1 g; 0.25 mmol/g) was swelled in DMF (10 ml) prior to use and transferred between tube and reaction vessel using DCM and DMF. Pseudoprolines [i.e. dipeptides employed to minimize aggregation during peptide synthesis, such as Fmoc-Phe-Thr(ψ -Me,Me-Pro)-OH and Fmoc-Asp-Ser(ψ -Me,Me-Pro)-OH and Fmoc-Ser-Ser(ψ -Me,Me-Pro)-OH] were used where
20 appropriate, and non-naturally occurring amino acids and other suitable building blocks were employed without any changes to the general procedure.

The following optical isomers of particular amino acids (including non-naturally occurring amino acids) were employed in the synthesis of the compounds:

Hyp: (2S,4R)-4-hydroxyproline [also denoted (4R)-4-hydroxy-L-proline].

Aad: (2S)-2-aminoadipic acid

Dab: (2S)-2,4-diaminobutanoic acid

Dap: (2S)-2,3-diaminopropanoic acid

5 hLys: (2S)-2-amino-7-amino-heptanoic acid, also known as homo-lysine

Gly(Me): N-methylglycine, also known as sarcosine

Ile(Me): N-methylisoleucine

Coupling:

10 CEM Liberty Peptide Synthesizer: an Fmoc-amino acid in DMF/DCM (2:1; 0.2 M; 5 mL) was added to the resin in a CEM Discover microwave unit together with COMU/DMF (0.5 M; 2 mL) and DIPEA/NMP (2.0 M; 1 mL). The coupling mixture was heated to 75 °C for 5 min while nitrogen was bubbled through the mixture. The resin was then washed with DMF (4 x 10 mL). Alternatively the coupling was done without heating and the reaction
15 time extended to 60 min in this case.

CEM Liberty Blue Peptide Synthesizer: an Fmoc-amino acid in DMF (0.2 M; 5 mL) was added to the resin in a CEM Discover microwave unit together with DIC/DMF (0.5 M; 2 mL) and Oxyma/DMF (2.0 M; 1 mL). The coupling mixture was heated to 90 °C for 2 min while nitrogen was bubbled through the mixture. The resin was then washed with DMF (4
20 x 10 mL). Alternatively the coupling was done without heating and the reaction time extended to 60 min in this case.

Independent of CEM Synthesizer type, in the case of difficult couplings (e.g. coupling of a residue immediately after an N-methylated amino acid residue or other sterically hindered amino acid residue as recognized by a person of skill in the art) the coupling was repeated
25 one or more times.

Deprotection:

Piperidine/DMF (1:4, i.e. 1 part piperidine to 4 parts DMF by volume; 10 mL) was added to the resin for initial deprotection, and the mixture was microwave-heated (40 °C; 30 sec).

The reaction vessel was drained and a second portion of piperidine/DMF (1:4; 10 mL) was added and heated (75 °C; 3 min) again. The resin was then washed with DMF (6 x 10 mL).

Oxidative cyclisation

5 Intramolecular ring formation (disulfide bridge formation) between Cys residues in positions 2 and 7 (initially coupled in the form of Ac₂S-protected cysteines) was performed with the peptide still attached to the resin, using 163 mg thallium(III) trifluoroacetate [Tl(TFA)₃] in 5 mL NMP in a simultaneous Ac₂S-deprotection and disulfide-formation step. (alternative method: addition of 10eq of iodine to a 50 mM solution of resin bound peptide in acetic acid and stirring for 18-24 h)

10 *Lactam cyclisation*

The following procedure for the coupling of Asp and Lys is representative for all lactam formations where the amino acid side chain containing the carboxyl-function is protected with OAll and the amino acid side chain containing the amino group is Alloc-protected. After assembly of the full peptide sequence, deprotection of Asp(OAll) in position 2 and 15 Lys(Alloc) in position 7 was performed using 29 mg tetrakis(triphenylphosphine)palladium(0) and phenylsilane 125 µL in 20 mL DCM. Subsequently, the lactam bridge was formed between the Asp residue (2) and Lys residue (7) using 414 mg HCTU and 346 µL DIPEA in 20 mL DMF. Both steps were performed with the peptide still attached to the resin.

20 *Cleavage:*

The resin was washed with EtOH (3 x 10 mL) and Et₂O (3 x 10 mL) and dried to constant weight at room temperature (r.t.). The crude peptide was cleaved from the resin by treatment with TFA/TIS/H₂O (90:5:5; 40 mL; 2 h; r.t.) or alternatively with TFA/DODT (95:5; 40 mL; 2 h; r.t.). Most of the TFA was removed under reduced pressure, and the 25 crude peptide was precipitated and washed three times with Et₂O and dried to constant weight at room temperature.

Purification and characterisation:

The crude peptide was purified by preparative reverse phase HPLC using a gradient of solvent A (0.1% aqueous TFA) and solvent B (0.1% TFA, 90% MeCN in water) on either a 30 PerSeptive Biosystems VISION Workstation or a Gilson system (Pumps: "Pump 305", "331 Pump", "332 Pump", "402 Syringe Pump"; column changer "Valvemate® II" UV detector "UV/Vis-155"; and the fraction collector "GX 281" equipped with a suitable column

and a fraction collector) or a Waters Autopurification HPLC/MS System (2525 pump System, Waters 2996 DAD, sample manager Waters 2767, MS ZQ single quadrupole. Columns: XSelect CSH 130 Prep C18 5 mm ODB 30x150 mm or Kinetex 5 mm C8 100A 150x21,2 mm). Fractions were analysed by analytical HPLC and MS, and relevant
5 fractions were pooled and lyophilised. The final product was characterized by HPLC and MS.

One of skill in the art will appreciate that standard methods of peptide synthesis may be used to generate the compounds of the invention.

10 Example 2

hCT-R and hAMYR3 assays

For the assessment of the in vitro activity of test peptides, cell lines expressing recombinant human calcitonin receptor (hCT-R) or recombinant human amylin 3 receptor (hAMYR3) in the background of the human astrocytoma cell line 1321N1 were purchased
15 from DiscoverX Corporation (Cat.No. 95-0161C6 and 95-0166C6). The hAMYR3 is a hetero-oligomer of the calcitonin receptor (Isoform 2; Gene ID 799) and RAMP3 (Gene ID 10268) that forms when both genes are expressed in the same cell. The hCT-R cell line expresses only the recombinant human calcitonin receptor gene (Isoform 2; Gene ID 799). Upon activation of hCT-R or hAMYR3 by the test peptides the formation of cAMP is
20 induced and was measured using the AlphaScreen® cAMP Assay kit from Perkin-Elmer (Cat.No. 6760635R).

Briefly, 1321N1 cells expressing hCT-R or hAMYR3 were seeded in 384-well microtiter plates (Falcon Optilux White, Cat.-No. 10448642) at 5,000 cells in 50 µL growth medium per well (AssayComplete 1321N1 Cell Culture Kit, DiscoverX Corp.), and incubated for 24
25 h at 37 °C, 5 % CO₂. On the day of analysis growth medium was removed and the cells were stimulated by adding 10 µL of stimulation buffer (10 nM Hepes pH 7.4, 140 nM NaCl, 3.6 nM KCl, 0.5 nM NaH₂PO₄, 0.5nM MgSO₄, 1.5 nM CaCl₂, 5 nM NaHCO₃, 0.5 nM IBMX, 0.1% BSA) containing increasing concentrations of test peptides, and incubation for 45 min at room temperature. The stimulation was stopped by adding 5.6 µL/well donor bead
30 detection mix (5 mM Hepes pH7.4, 0.5% TWEEN 20, 0.1% BSA, 0.05 mg/mL donor beads, 62.5 nM biotinylated cAMP) and 4.5 µL/well acceptor bead solution (5 mM Hepes pH7.4, 0.5% TWEEN 20, 0.1% BSA, 0.05 mg/mL acceptor beads). After thorough mixing the plates were incubated in the dark for 1 h at room temperature and the cAMP content of the resulting cell lysates was estimated according to the AlphaScreen® cAMP Assay

manufacturer's instructions. EC50 values were estimated by computer-aided curve fitting of results of at least 7 different compound concentrations.

The *in vitro* activity results (expressed as EC₅₀ values) are summarized in Table 1, below.

Example 3

5 Solubility determination

Test peptide is weighed into a suitable vial and the respective buffer (acetate buffer pH 4.0, phosphate buffer pH 6, histidine buffer pH 6 and 7; all at 40 nM concentration) added to a total volume of 0.5 mL.

- 10 Vials are shaken at room temperature for 2 h and filtered through a 0.45 µm filter. The resulting solutions are analyzed by RP-HPLC on a C18 column with gradient elution using a formic acid/acetonitrile/water eluent system. The area of the main peak is determined by UV spectroscopy at 230 nm at each sampling time point. The dissolved concentration is calculated by an external standard method.

Example 4

15 Assessment of physical stability

- Aggregation in the form of fibril formation was detected using the amyloid-specific dye Thioflavin T (ThT), which is frequently employed to demonstrate the presence of fibrils in solution (see, e.g., Groenning, M., J. Chem. Biol. **3**(1) (2010), pp. 1-18; Groenning et al., J. Struct. Biol. **158** (2007) pp. 358-369; and Levine, H., III, Protein Sci. **2** (1993) pp. 404-410)
- 20 Test peptides (2 mg/mL) were dissolved in demineralized water adjusted to pH 2.5 with HCl, at ambient temperature (typically 25°C). Solutions containing (i) 1 mg/mL of test peptide, 40 µM ThT and 50 mM phosphate (Ph) buffer (pH 7.0), (ii) 1 mg/mL of test peptide, 40 µM ThT and 50 mM histidine (His) buffer (pH 7.0), and (iii) 1 mg/mL of test peptide, 40 µM ThT and 50 mM acetate (Ac) buffer (pH 4.0), were loaded in a 96-well
- 25 black fluorescence plate (clear bottom) in triplicate. Data were collected at fixed intervals of 10 min, each preceded by 300 s of automixing (agitation), over a period of 96 hours at 40 °C. Physical stability is determined by measuring the fluorescence intensity over time. A significant increase in intensity is rated as fibrillation detected (FD). Data are summarized in Table 1 below.

Example 5Assessment of chemical stability

Samples of each test peptide were dissolved in acetate buffer pH 4 and phosphate buffer pH 6 and 7 (all buffer 40 mM). The final peptide concentration was 1 mg/mL. Samples
5 were placed in glass vials and incubated at 40°C. The samples were analyzed by RP-HPLC on a C8 column with gradient elution using a trifluoroacetic acid/acetonitrile/water eluent system. The area-percentage (area-%) of the main peak was determined by UV spectroscopy at 220 nm at each sampling time point.

The % degradation was calculated by subtracting the main peak area percentage at start
10 ($t = 0$) from the main peak area percentage at each sampling time point.

The results of the chemical stability assessment after 3 days of incubation time are summarized as % degradation in Table 1 (below).

The results of the chemical stability assessment after 14 days of incubation time are summarized as % degradation in Table 2 (below).

15 In an alternative analysis, peptides were dissolved in MilliQ water and pH adjusted to 4.0, 7.5 or 9.0. Samples were placed in glass vials and incubated at 40°C. The samples were analyzed by RP-HPLC on a C8 column with gradient elution using a trifluoroacetic acid/acetonitrile/water eluent system. The area-percentage (area-%) of the main peak was determined by UV spectroscopy at 220 nm at each sampling time point.

20 The % degradation was calculated by subtracting the main peak area percentage at start ($t = 0$) from the main peak area percentage at each sampling time point.

The results of the chemical stability assessment after 8 days of incubation time are summarized as % degradation in Table 3 (below).

Table 1. EC₅₀, chemical stability and fibrillation data

Compound no.	hCT-R	hAMYR 3	Fibrillation* (in buffer)			% degradation after 72h at 40 °C		
	EC50 [nM]	EC50 [nM]	pH4 acetate	pH7 histidine	pH7 phosphate	pH4 acetate	pH6 phosphate	pH7 phosphate
1	13.6	9.3						
2	0.105	0.032						
3	0.081	0.035	FND	FND	FD	< 1	3.2	1.2
4	0.160	0.092	FND	FND	FND	< 1	< 1	< 1
5	0.114	0.032						
6	0.327	0.011						
7	0.289	0.032						
8	0.129	0.059	FND	FND	FND	< 1	< 1	< 1
9	0.027	0.013						
10	0.345	0.068	FD	FND	FND	< 1	< 1	< 1
11	0.068	0.024	FND	FND	FND	< 1	< 1	< 1
12	0.893	0.999						
13	1.411	0.456						
14	0.386	0.108						
15	0.056	0.017	FND	FD	FD	< 1	< 1	< 1
16	0.043	0.035						
17	0.058	0.025	FND	FND	FND	1.2	< 1	< 1
18	0.085	0.031	FND	FND	FND	< 1	< 1	< 1
19	0.028	0.006						
20	0.009	0.003	FD	FND	FND	1.0	1.3	2.0
21	0.037	0.010	FND	FND	FND	< 1	< 1	< 1
22	0.474	0.051						
23	1.931	0.706						
24	0.038	0.013						
25	0.844	0.336	FND	FND	FND	-	-	< 1
26	0.769	0.120						

27	0.521	0.325	FND	FND	FND	< 1	< 1	1.7
28	1.229	0.268						
29	1.070	0.312						
30	0.232	0.122	FD	FND	FND			
31	0.227	0.066						
32	0.006	0.005	FD	FND	FND	1.3	<1	2.2
33	0.019	0.009	FND	FND	FND	< 1	1	< 1
34	0.206	0.063	FND	FND	FND	< 1	< 1	2.8
35	0.066	0.028	FND	FND	FND	< 1	< 1	1.4
36	0.026	0.007	FD	FND	FND	2.5	2.1	1.0
37	0.116	0.031	FD	FND	FND	-	< 1	3.0
38	0.114	0.060	FD	FND	FND	< 1	< 1	< 1
39	0.094	0.024	FD	FND	FND	< 1	< 1	< 1
40	0.028	0.008	FD	FND	FND	1.1	< 1	< 1
41	0.095	0.024	FND	FND	FND	< 1	< 1	< 1
42	0.006	0.003						
43	0.021	0.007	FD	FD	FD			
44	0.020	0.009	FD	FND	FND			
45	299	279						
46	7.5	5.5						
47	10.8	7.7						
48	0.107	0.020						
49	1.038	0.442						
50	3.3	1.5						
51	0.861	0.306						
Ref. 1	20.3	17.1						
Ref. 2	0.019	0.003						
Ref. 3	18.2	13.2						
Ref. 4	0.075	0.031	FND	FND	FND			
Ref. 5	0.063	0.028	FD	FND	FND			

*FND = fibrillation not detected; FD = fibrillation detected

Table 2. Chemical stability

Compound no.	% degradation after 14 days at 40 °C		
	pH4 acetate	pH6 phosphate	pH7 phosphate
32	<1	<1	<1
41	1.1	<1	<1
10	1.9	1.8	9.1
14	2.2	<1	2.6
18	<1	1.0	1.9
37	ND	<1	<1
36	ND	1.6	<1
4	1.6	<1	6.4
38	ND	ND	<1
20	ND	<1	3.7
16	ND	<1	ND
3	ND	1.0	ND
35	2	<1	<1
Ref. 2	<1	4.6	ND
Ref. 4	3.2	12.6	20.3
Ref. 5	3.0	5.1	12.7
NN96	3.0	10.1	33.2

ND – not determined

Table 3. Chemical stability

Compound no.	% degradation after 8 days at 40°C		
	pH4	pH7.5	pH9
51	3.1	4.6	4.6
49	1.7	2.1	3.0
48	1.8	2.3	2.5
19	3.9	6.2	4.2

Example 6Pharmacokinetic (PK) profiling in rats

- 5 Sprague Dawley male rats were given a single subcutaneous (sc) bolus of each peptide to be tested, as specified below.

30 nmol/kg doses of compound were administered. Blood samples were drawn from the tail vein prior to dosing and at 24 and 96 hours after dosing. The rats were euthanized immediately after the last blood sampling by concussion and cervical dislocation.

- 10 The dosing vehicle used for each test peptide was a mannitol-containing histidine buffer (pH 7.0). Plasma samples were analyzed after precipitation with ethanol by liquid chromatography mass spectrometry (LC-MS/MS). Mean plasma concentrations were used for calculation of the pharmacokinetic parameters.

- 15 Plasma terminal elimination half-life ($t_{1/2}$) was determined as $\ln(2)/\lambda_z$, where λ_z is the magnitude of the slope of the log linear regression of the log concentration versus time profile during the terminal phase.

Results

The plasma terminal elimination half-lives ($t_{1/2}$) for all tested peptides were determined to be in the range of 21.3 h to 36.1 h.

Table 4: in vivo half life

Compound No.	t _{1/2} [h]
3	21.3
4	31.9
18	30.1
20	31.1
35	36.1
38	31.8

Example 7Effect on acute food intake and body weight in normal Sprague Dawley rats

- 5 Sprague Dawley (SD) rats were obtained from Janvier Labs, France. The animals arrived at least 14 days before the study start to allow acclimatization to experimental conditions. From arrival and throughout the study, the rats were housed in groups of 2 to 4 (n = 2-4) in a light-, temperature- and humidity-controlled room. Animals had access ad libitum to food (KLIBA 3430, Provimi Kliba AG, Switzerland) and water (domestic quality tap water)
- 10 during the entire study. Per group 6-8 rats were included. A vehicle group and positive control group were included in each set of tests. Rats were dosed subcutaneously (sc) once in the morning 1 hour before turning off the lights, using a body weight-corrected dose (30 nmol/kg) of test peptide. Dosing volume was 2 ml/kg. Food intake was recorded online using an automated food intake system (HM02, MBRose, Denmark) for 4 days or
- 15 manually at 0 h for a predose and then 24, 48, 72 and 96 h post dosing. Body weight was measured daily.

Statistical analyses were performed using GraphPad™ Prism version 7. The measured parameters were compared using two-way ANOVA followed by Dunnett's multiple comparison tests. Differences were considered statistically significant at p < 0.05.

20 Results

- 48 h after dosing, each of the tested compounds (except for compounds 30 and 37) had given rise to a clear, statistically significant inhibition of food intake (vehicle-corrected, in %). This reduction in food intake was reflected by a decrease in body weight (vehicle-
- 25 corrected, in %). Normal feeding behavior was subsequently resumed.

Table 5. Effect on food intake and body weight

Compound No.	Food intake reduction in % at 48h Dose: 30 nmol/kg	Body weight reduction in % at 48h Dose: 30 nmol/kg
41	75	11
35	72	12
18	58	10
39	76	9.2
40	83	7.3
37	24	4.3
30	< 20	0
36	44 (at dose of 10 nmol/kg)	5.2 (at dose of 10 nmol/kg)
4	34	4.6
38	43	8.2
20	49	7.8
15	71	7.7
16	66	7.4
3	34	5.6

5 Example 8

Acute fed basal glucose changes in diabetic ZDF rats

Male Zucker Diabetic Fatty rat (ZDF-Lepr^{fa}/Crl) are obtained from Charles River, US. Animals are acclimatised to experimental conditions for at least 14 days before the start of the study. From arrival and throughout the study, the rats are housed in groups of 2 (n = 2) in a light-, temperature- and humidity-controlled room. Animals have access ad libitum to food (KLIBA 2437, Provimi Kliba AG, Switzerland) and water (domestic quality tap water) during the entire study.

Rats are randomized based on blood glucose, HbA1c and body weight into a designated test and vehicle group. The total number of animals per group is 10 (n = 10). Rats are dosed subcutaneously (sc) once in the morning using a body weight-corrected dose (10 nmol/kg) of test peptide. Dosing volume is 2 ml/kg.

Blood glucose levels in tail blood samples taken 24, 72, 96 and 168 hours post dosing and blood glucose levels are determined using a glucometer (GlucoSmart Swing™; MSP Bodmann GmbH, Germany).

- 5 Statistical analyses are performed using GraphPad™ Prism version 7. The measured parameters are compared using two-way ANOVA followed by Sidak multiple comparison tests. Differences are considered statistically significant at $p < 0.05$.

CLAIMS

1. An amylin analogue which is a compound having the formula:
R¹-Z-R²
- 5 wherein
- R¹ is hydrogen, C₁₋₄ acyl, benzoyl or C₁₋₄ alkyl, or a half-life extending moiety M,
wherein M is optionally linked to Z via a linker moiety L;
R² is OH or NHR³, wherein R³ is hydrogen or C₁₋₃-alkyl; and
Z is an amino acid sequence of formula I:
- 10 X1-X2-X3-X4-X5-X6-X7-Ala-Thr-X10-Arg-Leu-Ala-X14-Phe-Leu-X17-Arg-X19-X20-
Phe-Gly(Me)-Ala-Ile(Me)-X27-Ser-Ser-Thr-Glu-X32-Gly-Ser-X35-Thr-X37 (I)
wherein
- X1 is selected from the group consisting of Arg, Lys and Glu;
X3 is selected from the group consisting of Gly, Gln and Pro;
- 15 X4 is selected from the group consisting of Thr and Glu;
X5 is selected from the group consisting of Ala and Leu;
X6 is selected from the group consisting of Thr and Ser;
X10 is selected from the group consisting of Glu and Gln;
X14 is selected from the group consisting of Aad, His, Asp, Asn and Arg;
- 20 X17 is selected from the group consisting of Gln, His and Thr;
X19-X20 is selected from Ser-Ser, Thr-Thr, Ala-Thr, Ala-Ala, Gly-Thr, Gly-Gly and
Ala-Asn or is absent;
X27 is selected from the group consisting of Leu and Pro;
X32 is selected from the group consisting of Val and Thr;
- 25 X35 is selected from the group consisting of Asn and Ser;
X37 is selected from the group consisting of Hyp and Pro; and
X2 and X7 are amino acid residues whose side chains together form a lactam bridge,
wherein:
- X2 is Asp and X7 is Lys;
- 30 X2 is Asp and X7 is Orn;
X2 is Asp and X7 is Dab;
X2 is Asp and X7 is hLys;
X2 is Dap and X7 is Aad;
X2 is Glu and X7 is Dab; or
- 35 X2 is Dab and X7 is Glu;
or a pharmaceutically acceptable salt or solvate thereof.

2. An amylin analogue or pharmaceutically acceptable salt according to claim 1 wherein:
- (i) X1 is selected from Arg and Lys;
 - (ii) X3 is Gly, X4 is Thr, X5 is Ala and/or X6 is Thr;
 - 5 (iii) X3 is Gly, X4 is Thr, X5 is Ala and X6 is Thr;
 - (iv) X14 is selected from His, Asp and Aad;
 - (v) X17 is Gln;
 - (vi) X19-X20 is selected from Ser-Ser and Thr-Thr, or is absent; and/or
 - (vii) X32 is Val, X35 is Asn and/or X37 is Hyp.
- 10
3. An amylin analogue or pharmaceutically acceptable salt according to claim 1 wherein Z is an amino acid sequence of formula II:
- X1-X2-Gly-Thr-Ala-Thr-X7-Ala-Thr-X10-Arg-Leu-Ala-X14-Phe-Leu-Gln-Arg-X19-X20-Phe-Gly(Me)-Ala-Ile(Me)-X27-Ser-Ser-Thr-Glu-Val-Gly-Ser-Asn-Thr-Hyp (II)
- 15 wherein
- X1 is selected from Arg and Lys;
 - X10 is selected from the group consisting of Glu and Gln;
 - X14 is selected from the group consisting of Aad, Asp and His;
 - X19-X20 is selected from Ser-Ser and Thr-Thr or is absent;
 - 20 X27 is selected from the group consisting of Leu and Pro; and
 - X2 and X7 are amino acid residues whose side chains together form a lactam bridge, wherein:
 - X2 is Asp and X7 is Lys;
 - X2 is Asp and X7 is Orn;
 - 25 X2 is Asp and X7 is Dab;
 - X2 is Asp and X7 is hLys;
 - X2 is Dap and X7 is Aad;
 - X2 is Glu and X7 is Dab; or
 - X2 is Dab and X7 is Glu.
- 30
4. An amylin analogue or pharmaceutically acceptable salt according to claim 3 wherein X14 is Aad, X19-X20 is Ser-Ser and X27 is Leu.
5. An amylin analogue or pharmaceutically acceptable salt according to claim 1
- 35 wherein Z is an amino acid sequence selected from the group consisting of:
- RD()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;

RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLARFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 ED()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GEATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTLTK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTASK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-hLys()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRATF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()QTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()PTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;
 RD()GTATK()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTATK()ATERLA-Aad-FLQRGGF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTATK()ATERLA-Aad-FLQRANF-Gly(Me)-A-Ile(Me)-LSSTEVGSNHyp;
 RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGSNHyp;

RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp;
ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
RD()GTATK()ATERLA-Aad-FLQRSTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
KD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
RD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp;
KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
KD()GTATK()ATQRLANFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGSNT-Hyp;
R-Dap()-GTAT-Aad()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
R-Dab()-GTATE()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp; and
RE()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp;
wherein parentheses "()" indicate an intramolecular lactam bridge formed between
the side chains of the residues at positions 2 and 7.

6. An amylin analogue or pharmaceutically acceptable salt according to any one of the preceding claims wherein R¹ is M or M-L-.

5 7. An amylin analogue or pharmaceutically acceptable salt according to claim 6
wherein M is a lipophilic substituent comprising a hydrocarbon chain having from 10
to 24 C atoms, e.g. from 14 to 22 C atoms, e.g. from 16 to 20 C atoms, wherein the
lipophilic substituent optionally comprises a carboxylic acid group at the end of the
hydrocarbon chain, and further optionally wherein the lipophilic substituent is a 15-
10 carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl or 19-carboxy-nonadecanoyl
moiety.

8. An amylin analogue or pharmaceutically acceptable salt according to claim 6
or claim 7 wherein the linker L comprises a residue of Gly, Pro, Ala, Val, Leu, Ile,
15 Met, Cys, Phe, Tyr, Trp, His, Lys, Arg, Gln, Asn, α -Glu, γ -Glu, ϵ -Lys, Asp, β -Asp, Ser,
Thr, Gaba, Aib, β -Ala (i.e. 3-aminopropanoyl), 4-aminobutanoyl, 5-aminopentanoyl,
6-aminohexanoyl, 7-aminoheptanoyl, 8-aminooctanoyl, 9-aminononanoyl, 10-
aminodecanoyl or 8Ado (i.e. 8-amino-3,6-dioxaoctanoyl), e.g. wherein L is a residue
of Glu, γ -Glu, ϵ -Lys, β -Ala, 4-aminobutanoyl, 8-aminooctanoyl or 8Ado.

9. An amylin analogue or pharmaceutically acceptable salt according to claim 8 wherein R¹ is a 19-carboxy-nonadecanoyl group covalently attached to the alpha amino group of an iso-glutamic acid linker ([19CD]-isoGlu).

5

10. An amylin analogue or pharmaceutically acceptable salt according to any one of the preceding claims wherein R² is NH₂.

11. An amylin analogue according to claim 1 which is:

10

[19CD]-isoGlu-RD()GTAT-Dab()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 2)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 3)
[19CD]-isoGlu-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 4)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGST-Hyp-NH ₂	(Compound 5)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 6)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTP-NH ₂	(Compound 7)
[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 8)
[19CD]-isoGlu-RD()GTAT-Orn()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGST-Hyp-NH ₂	(Compound 9)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLARFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 10)
[19CD]-isoGlu-ED()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 11)
[19CD]-isoGlu-RD()GEATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 12)
[19CD]-isoGlu-RD()GTLTK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 13)
[19CD]-isoGlu-RD()GTASK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH ₂	(Compound 14)

[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 15)
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 16)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 17)
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 18)
[19CD]-isoGlu-RD()GTAT-hLys()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 19)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 20)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 21)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 22)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 23)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 24)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRATF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 25)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 26)
[19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 27)
[19CD]-isoGlu-RD()QTAT-Orn()-ATERLA-Aad-FLQRGTF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 28)
[19CD]-isoGlu-RD()PTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 29)
[19CD]-isoGlu-ED()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 30)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRAAF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 31)
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRGGF-Gly(Me)-A-Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 32)

[19CD]-isoGlu-RD() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 33)
[19CD]-isoGlu-RD() Ile(Me)-PSSTEVGsNT-Hyp-NH ₂	(Compound 34)
[19CD]-isoGlu-RD() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 35)
[19CD]-isoGlu-RD() Ile(Me)-LSSTETGsNT-Hyp-NH ₂	(Compound 36)
[19CD]-isoGlu-ED() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 37)
[19CD]-isoGlu-RD() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 38)
[19CD]-isoGlu-KD() AlIe(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 39)
[19CD]-isoGlu-RD() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 40)
[19CD]-isoGlu-RD() LSSTEVGsNT-Hyp-NH ₂	(Compound 41)
[19CD]-isoGlu-RD() LSSTETGsNT-Hyp-NH ₂	(Compound 42)
[19CD]-isoGlu-KD() LSSTEVGsNT-Hyp-NH ₂	(Compound 43)
[19CD]-isoGlu-KD() LSSTETGsNT-Hyp-NH ₂	(Compound 44)
[19CD]-isoGlu-R-Dap() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 48)
[19CD]-isoGlu-R-Dab() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 49)
[19CD]-isoGlu-RE() Ile(Me)-LSSTEVGsNT-Hyp-NH ₂	(Compound 51)

or a pharmaceutically acceptable salt or solvate thereof; wherein parentheses "()" indicate an intramolecular lactam bridge formed between the side chains of the residues at positions 2 and 7.

- 5 12. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()
PSSTEVGsNT-Hyp-NH₂.

13. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
- 5
14. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
- 10
15. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTAT-hLys()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
16. An amylin analogue according to claim 1 which is:
15 [19CD]-isoGlu-RD()GTAT-Orn()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
17. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
- 20
18. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTETGST-Hyp-NH₂.
- 25
19. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATERLA-Aad-FLQRTTF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
- 30
20. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-KD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
21. An amylin analogue according to claim 1 which is:
35 [19CD]-isoGlu-RD()GTATK()ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.

22. An amylin analogue according to claim 1 which is:
[19CD]-isoGlu-RD()GTATK()ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂.
- 5 23. A pharmaceutically acceptable salt of an amylin analogue according to any one of claims 12 to 22.
24. A pharmaceutical composition comprising an amylin analogue or pharmaceutically acceptable salt according to any one of the preceding claims in
10 combination with a pharmaceutically acceptable carrier, excipient or vehicle.
25. A method for the synthesis of an amylin analogue or pharmaceutically acceptable salt according to any one of claims 1 to 22 comprising synthesising the analogue by solid-phase or liquid-phase peptide synthesis methodology, optionally
15 isolating and/or purifying the final product, and optionally further comprising the step of forming an amide bond between the side chains at positions 2 and 7.
26. An amylin analogue or pharmaceutically acceptable salt according to any one of claims 1 to 23 for use in a method of medical treatment.
20
27. Use of an amylin analogue or pharmaceutically acceptable salt according to any one of claims 1 to 23 in the manufacture of a medicament for:
- (i) a therapeutic method of treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight;
- 25 (ii) treating obesity, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, or reproductive health complications of obesity or overweight;
- (iii) preventing or treating Alzheimer's disease, diabetes, type 1 diabetes, type 2
30 diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, hyperglycemia, hypertension, atherogenic dyslipidemia, hepatic steatosis ("fatty liver"; including non-alcoholic fatty liver disease (NAFLD), which itself includes non-alcoholic steatohepatitis (NASH)), kidney failure,
35 arteriosclerosis (e.g. atherosclerosis), macrovascular disease, microvascular disease, diabetic heart disease (including diabetic cardiomyopathy and heart failure

as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, and combinations thereof; or

(iv) a therapeutic method of lowering circulating LDL levels and/or increasing HDL/LDL ratio.