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(54) Title: POLYPEPTIDES AND USES THEREOF

(57) Abstract: Disclosed are polypeptides which are pramlintide analogues and uses thereof. In particular, the present invention relates to polypeptides of SEQ ID NO 2 which are pramlintide analogues conjugated to half-life extending moieties such as albumin binding moieties and uses thereof.

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Polypeptides and uses thereof

Field of the Invention

The present invention relates to polypeptides which are pramlintide analogues and uses thereof. In particular, the present invention relates to polypeptides which are pramlintide analogues conjugated to half-life extending moieties such as albumin binding moieties and uses thereof.

Background

Pramlintide is a synthetic analogue of human amylin with three proline substitutions, at positions 25, 28 and 29. As a result of these substitutions, pramlintide has a reduced propensity to form amyloid fibrils, thereby overcoming a physicochemical liability of native human amylin (Kruger DF, Gloster MA. Pramlintide for the treatment of insulin-requiring diabetes mellitus: rationale and review of clinical data. Drugs. 2004; 64(13):1419-32).

Pramlintide is clinically used in amylin replacement therapies and simulates the important glucoregulatory actions of amylin. These glucoregulatory actions complement those of insulin by regulating the rate of appearance of glucose in the circulation, and are achieved through three primary mechanisms: slowing the rate of gastric emptying, suppression of post-meal glucagon secretion and suppression of food intake (Roth JD *et.* al. GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities. Br J Pharmacol. 2012;166(1):121-136). Pramlintide has been used as an adjunct to insulin in patients with diabetes who have failed to reach desired glucose control despite optimal insulin therapy (Pullman J, *et. al*. Pramlintide is used in the management of insulin-using patients with type 2 and type 1 diabetes. Vasc Health Risk Manag. 2006;2(3):203-212).

Pharmacokinetic studies show that the terminal half-life of amylin in rats is around 13 minutes, and the half-life for pramlintide in human is ~20-45 minutes (Roth JD *et.* al. GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities. Br J Pharmacol. 2012;166(1):121-136).

There remains a need for pramlintide analogues which retain amylin agonist activity and provide advantages such as extended half-life and reduced fibrillation tendency.

Summary of Invention

The present invention relates to polypeptides that are pramlintide analogues conjugated to albumin binding moieties (*e.g.* lipids).

Thus, in one aspect, there is provided a polypeptide, or a pharmaceutically acceptable salt thereof, comprising the amino acid sequence:

Xaa (-4) - Xaa (-3) - Xaa (-2) - Xaa (-1) - Xaa 1 - Cys 2 - Asn 3 - Xaa 4 - Ala 5 - Thr 6 - Cys 7 - Ala 8 - Thr 9 - Gln 10 - Arg 11 - Leu 12 - Ala 13 - Xaa 14 - Xaa 15 - Xaa 16 - Xaa 17 - His 18 - Ser 19 - Xaa 20 - Xaa 21 - Xaa 22 - Xaa 23 - Xaa 24 - Xaa 25 - Xaa 26 - Xaa 27 - Xaa 28 - Xaa 29 - Thr 30 - Xaa 31 - Xaa 32 - Xaa 33 - Xaa 34 - Xaa 35 - Xaa 36 - Xaa 37 - amide [SEQ ID NO:2], wherein:

Xaa (-4) is Lys(albumin binding moiety) or is absent;

Xaa (-3) is Gly or is absent;

Xaa (-2) is Gly or is absent;

Xaa (-1) is Gly, (albumin binding moiety), Lys(albumin binding moiety) or is absent;

Xaa 1 is Lys, Lys(albumin binding moiety), (albumin binding moiety) or is absent;

Xaa 4 is Thr, Ile or Ala;

Xaa 14 is Asn, His, Glu, 2,4-diaminobutanoic acid (Dab), or an alpha methyl amino acid;

Xaa 15 is Phe or Trp;

Xaa 16 is Leu or *D*-Leu (dL);

Xaa 17 is Val, Ser, Glu, Arg, (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp), Dab or an alpha methyl amino acid (*e.g.* 2-amino-2-methylpropanoic acid [Aib]);

Xaa 20 is Ser, Ile, Pro or an alpha methyl amino acid (*e.g. (S)*-2-amino-3-hydroxy-2-methylpropanoic acid [αMeSer]);

Xaa 21 is Asn, Dab, His, Pro, Ser, Arg, Lys, Gly, Glu, Ala, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 22 is Asn, His, Hyp, Dab or an alpha methyl amino acid (*e.g.* Aib);

Xaa 23 is Phe, Hyp or an alpha methyl amino acid (*e.g. (S)*-2-amino-2-methyl-3-phenylpropanoic acid [αMePhe]);

Xaa 24 is Gly, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 25 is Pro, Ala, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 26 is Ile, *D*-Ile (dI), Arg, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 27 is Leu, dL, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 28 is Pro, *D*-Pro (dP), Ser, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 29 is Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 31 is Asn, Glu, His, Arg, Pro, Dab or an alpha methyl amino acid (*e.g.* Aib);
Xaa 32 is Val, Hyp, Dab or an alpha methyl amino acid (*e.g.* Aib);
Xaa 33 is Gly, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 34 is Ser, Pro, His, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 35 is Asn, Pro, Arg, Glu, Dab, Hyp or an alpha methyl amino acid (*e.g.* Aib);
Xaa 36 is Thr, Hyp or an alpha methyl amino acid (*e.g.* Aib); and
Xaa 37 is Tyr, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib),

and wherein the polypeptide comprises at least one albumin binding moiety.

In another aspect, there is provided a lipidated polypeptide, or a pharmaceutically acceptable salt thereof, comprising the amino acid sequence:

Xaa (-4) - Xaa (-3) - Xaa (-2) - Xaa (-1) - Xaa 1 - Cys 2 - Asn 3 - Xaa 4 - Ala 5 - Thr 6 - Cys 7 - Ala 8 - Thr 9 - Gln 10 - Arg 11 - Leu 12 - Ala 13 - Xaa 14 - Xaa 15 - Xaa 16 - Xaa 17 - His 18 - Ser 19 - Xaa 20 - Xaa 21 - Xaa 22 - Xaa 23 - Xaa 24 - Xaa 25 - Xaa 26 - Xaa 27 - Xaa 28 - Xaa 29 - Thr 30 - Xaa 31 - Xaa 32 - Xaa 33 - Xaa 34 - Xaa 35 - Xaa 36 - Xaa 37 - amide [SEQ ID NO:2], wherein:

Xaa (-4) is Lys(linker-lipid) or is absent;
Xaa (-3) is Gly or is absent;
Xaa (-2) is Gly or is absent;
Xaa (-1) is Gly, (linker-lipid), Lys(linker-lipid) or is absent;
Xaa 1 is Lys, Lys(linker-lipid), (linker-lipid) or is absent;
Xaa 4 is Thr, Ile or Ala;
Xaa 14 is Asn, His, Glu, 2,4-diaminobutanoic acid (Dab), or an alpha methyl amino acid;
Xaa 15 is Phe or Trp;
Xaa 16 is Leu or D-Leu (dL);
Xaa 17 is Val, Ser, Glu, Arg, (2S,4R)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp), Dab or an alpha methyl amino acid (e.g. 2-amino-2-methylpropanoic acid [Aib]);
Xaa 20 is Ser, Ile, Pro or an alpha methyl amino acid (e.g. (S)-2-amino-3-hydroxy-2-methylpropanoic acid [αMeSer]);

Xaa 21 is Asn, Dab, His, Pro, Ser, Arg, Lys, Gly, Glu, Ala, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 22 is Asn, His, Hyp, Dab or an alpha methyl amino acid (e.g. Aib);
Xaa 23 is Phe, Hyp or an alpha methyl amino acid (e.g. (S)-2-amino-2-methyl-3-phenylpropanoic acid [αMePhe]);
Xaa 24 is Gly, Pro, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 25 is Pro, Ala, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 26 is Ile, D-Ile (dI), Arg, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 27 is Leu, dL, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 28 is Pro, D-Pro (dP), Ser, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 29 is Pro, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 31 is Asn, Glu, His, Arg, Pro, Dab or an alpha methyl amino acid (e.g. Aib);
Xaa 32 is Val, Hyp, Dab or an alpha methyl amino acid (e.g. Aib);
Xaa 33 is Gly, Pro, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 34 is Ser, Pro, His, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 35 is Asn, Pro, Arg, Glu, Dab, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 36 is Thr, Hyp or an alpha methyl amino acid (e.g. Aib);
Xaa 37 is Tyr, Pro, Hyp or an alpha methyl amino acid (e.g. Aib).

In yet another aspect, there is provided a polypeptide as set forth in Table 4.

In yet another aspect, there is provided a pharmaceutical composition comprising a polypeptide, a lipidated polypeptide or pharmaceutically acceptable salt of the invention and a pharmaceutically acceptable excipient.

In another aspect, there is provided a method for treating a disease or disorder in a subject comprising administering a polypeptide, a lipidated polypeptide, pharmaceutically acceptable salt or a pharmaceutical composition of the invention.

In a further aspect, there is provided a method for the production of a polypeptide or a lipidated polypeptide described herein.

In a further aspect, there is provided an article of manufacture comprising a polypeptide, a lipidated polypeptide, a pharmaceutically acceptable salt or a pharmaceutical composition of the invention.

In a further aspect, there is provided a kit comprising a polypeptide, a lipidated polypeptide, a pharmaceutically acceptable salt or a pharmaceutical composition of the invention, optionally further comprising instructions for use.

Aspects and embodiments of the invention are set out in the appended claims. These and other aspects and embodiments of the invention are also described herein.

Brief Description of Sequence Listing

Table 1: Compound Sequence Listing

SEQ ID NO.	Full sequence
1 (Pramlintide)	K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
3	C18diacid-γE-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
4	C18diacid-γE-γE-GGG-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
5	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
6	K(O2Oc-O2Oc-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
7	K(O2Oc-O2Oc-γE-C18diacid)GGGK[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
8	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Dab)NFPAILSPTNVGSNTY-amide
9	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGSNTY-amide
10	C18diacid-γE-[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide
11	K(γE-γE-C18diacid)[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide
12	K(γE-C18diacid)K[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide
13	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTNVGSNTY-amide
14	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTNVGSNTY-amide
15	K(γE-C18diacid)K[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTEVGSNTY-amide
16	K(γE-C18diacid)[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTEVGSNTY-amide

17	K(O2Oc-O2Oc-γE-C18diacid)[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTEVGSNTY-amide
18	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTEVGSNTY-amide
19	K(γE-C18diacid)[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide
20	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide
21	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTNVGSRTY-amide
22	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHS(αMeSer)NNFGPILPPTNVGSNTY-amide
23	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTEVGSNTY-amide
24	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTRVGSNTY-amide
25	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSSNN(αMePhe)GPILPPTRVGSNTY-amide
26	K(γE-γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSSNN(αMePhe)GPILPPTNVGSNTY-amide
27	K(γE-γE-C18diacid)[CNIATC]ATQRLANFLVHSS(Dab)NFGPILPPTNVGSRTY-amide
28	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Dab)NFGPILPPTEVGSNTY-amide
29	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Dab)NFGPILPPTNVGSNTY-amide
30	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSS(Dab)NFGPILPPTEVGSNTY-amide
31	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Dab)NFG(Aib)ILPPTNVGSNTY-amide
32	K(γE-C18diacid)K[CNTATC]ATQRLA(Dab)FLVHSSNN(αMePhe)GPILPPTEVGSNTY-amide
33	K(γE-C18diacid)K[CNTATC]ATQRLA(Dab)FLVHSSNNFGPILPPTNVGSNTY-amide
34	K(γE-C18diacid)K[CNTATC]ATQRLA(Dab)FLVHSSNNFGPILPPTEVGSNTY-amide
35	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSNTY-amide

36	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSS(Aib)NFGPILPPTNVGSNTY-amide
37	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTNVGSNTY-amide
38	K(γE-C18diacid)K[CNTATC]ATQRLAEFLVHSS(Aib)NFGPILPPTNVGSNTY-amide
39	K(γE-C18diacid)K[CNTATC]ATQRLANFLEHSS(Aib)NFGPILPPTNVGSNTY-amide
40	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTHVGSNTY-amide
41	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTEVGSNTY-amide
42	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSETY-amide
43	K(γE-C18diacid)K[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTEVGSNTY-amide
44	K(γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTEVGSNTY-amide
45	K(O2Oc-O2Oc-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTEVGSNTY-amide
46	K(γE-C18diacid)K[CNTATC]ATQRLAHFLVHSS(Aib)NFGPILPPTNVGSNTY-amide
47	K(γE-C18diacid)K[CNTATC]ATQRLAHFLVHSS(Aib)NFGPILPPTNVGSETY-amide
48	K(γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTNVGSNTY-amide
49	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTEVGSNTY-amide
50	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTEVGSNTY-amide
51	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTHVGSNTY-amide
52	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTRVGSNTY-amide
53	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTPVGSNTY-amide
54	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTNVPSNTY-amide
55	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Aib)NFGPILPPTNVGSPTY-amide
56	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSNTY-amide
57	K(γE-γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSNTY-amide
58	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSNTP-amide
59	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPI(dL)PPTNVGSNTY-amide
60	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPIL(dP)PTNVGSNTY-amide

61	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGP(dl)LPPTNVGSNTY-amide
62	K(γE-C18diacid)[CNTATC]ATQRLANF(dL)VHSS(Aib)NFGPILPPTNVGSNTY-amide
63	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
64	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSRTY-amide
65	K(γE-C18diacid)K[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTNVGSNTY-amide
66	K(γE-γE-C18diacid)K[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTNVGSNTY-amide
67	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
68	(C18diacid-γE-[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
69	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
70	K(γE-γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
71	C18diacid-γE-K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTRVGSNTY-amide
72	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSRTY-amide
73	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSRTY-amide
74	K(γE-γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSRTY-amide
75	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTNVGSNTY-amide
76	K(γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTRVGSNTY-amide
77	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTRVGSNTY-amide
78	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGP(Aib)LPPTNVGSNTY-amide
79	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPI(Aib)PPTNVGSNTY-amide
80	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide
81	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNV(Aib)SNTY-amide
82	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGS(Aib)TY-amide
83	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSN(Aib)Y-amide
84	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVG(Aib)NTY-amide
85	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGSNT(Aib)-amide

86	K(γE-C18diacid)K[CNTATC]ATQRLAHFL(Aib)HSS(Aib)NFGPILPPTEVGSNTY-amide
87	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSS(Aib)NFGPILPPTNVGSNTP-amide
88	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPIL(Aib)PTNVGSNTY-amide
89	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide
90	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide
91	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPTNVGS(Aib)TY-amide
92	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLRHSS(Aib)NFGPILPPTNVGS(Aib)TY-amide
93	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSS(Dab)NFG(Aib)ILPPTNVGSNTY-amide
94	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGP(Aib)LPPTNVGSNTY-amide
95	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPI(Aib)PPTNVGSNTY-amide
96	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPIL(Aib)PTNVGSNTY-amide
97	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILP(Aib)TNVGSNTY-amide
98	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPT(Aib)VGSNTY-amide
99	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTN(Aib)GSNTY-amide
100	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNV(Aib)SNTY-amide
101	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVG(Aib)NTY-amide
102	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGS(Aib)TY-amide
103	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSSNNFGPI(dL)PPTNVGSNTY-amide
104	K(γE-C18diacid)[CNTATC]ATQRLANFLVHSSNNFGPIL(dP)PTNVGSNTY-amide
105	K(γE-C18diacid)K[CNTATC]ATQRLANFLSHSS(Dab)NFG(Aib)ILPPTNVGSNTY-amide
106	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNNFGP(Aib)LPPTNVGSNTY-amide
107	K(γE-C18diacid)K[CNTATC]ATQRLANFLRHSSNNFGP(Aib)LPPTNVGSNTY-amide
108	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNNFGPI(Aib)PPTNVGSNTY-amide
109	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSN(Aib)FGPILPPTNVGSNTY-amide

110	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNNF(Aib)PILPPTNVGSNTY-amide
111	K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNHFGPILPPTNVGSETY-amide
112	C20diacid-γE-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
113	C20diacid-γE-O2Oc-O2Oc-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide
114	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSRTY-amide
115	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSSPNFPAILSPTNVGSNTY-amide
116	K(γE-γE-C20diacid)[CNTATC]ATQRLAEFLRHSSNNFGPILPPTNVGSNTY-amide
117	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGSNTY-amide
118	K(γE-γE-C20diacid)[CNIATC]ATQRLANFLVHSIANFGPILPPTNVGSRTY-amide
119	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSPPNFPAILSPTNVGSNTY-amide
120	K(γE-γE-C20diacid)[CNAATC]ATQRLANWLVHSSPNFPAILSPTNVGSNTY-amide
121	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NF(Hyp)AILSPTNVGSNTY-amide
122	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPT(Dab)VGSNTY-amide
123	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPT(Dab)VGSNTY-amide
124	K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGS(Dab)TY-amide
125	K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGS(Dab)TY-amide
126	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSS(Dab)NFGPILPPTNVGSNTY-amide
127	K(γE-γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
128	K(γE-C18diacid)K[CNTATC]ATQRLAEFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
129	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
130	K(γE-C18diacid)K[CNTATC]ATQRLAEFL(Aib)HSSHNFGPILPPTNVGSNTY-amide
131	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSHNFGPILPPTNVGSNTY-amide
132	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSPNFGPILPPTNVGSNTY-amide
133	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSSNFGPILPPTNVGSNTY-amide
134	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTPVGSNTY-amide
135	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNHFGPILPPTNVGSNTY-amide

136	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSNTP-amide
137	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSRNFGPILPPTNVGSNTY-amide
138	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSPNFGPILPPTEVGSNTY-amide
139	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSPNFGPILPPTNVGSETY-amide
140	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTEVGSNTY-amide
141	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSRTY-amide
142	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSETY-amide
143	K(γE-C18diacid)GGK[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
144	K(γE-C18diacid)[CNTATC]ATQRLAHFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
145	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGHNTY-amide
146	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTHVGSETY-amide
147	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNHFGPILPPTNVGSETY-amide
148	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGPNTY-amide
149	K(γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTNVGSNTY-amide
150	K(γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSS(Dab)NFGPILPPTNVGSNTY-amide
151	K(γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSSNNFGPI(dL)PPTNVGSNTY-amide
152	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSNNFGPRLPPTNVGSNTY-amide
153	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSKNFGPILPPTNVGSNTY-amide
154	K(γE-C18diacid)K[CNTATC]ATQRLANFL(Aib)HSSGNFGPILPPTNVGSNTY-amide
155	K(γE-C18diacid)[CNTATC]ATQRLANFL(Aib)HSSNNFGPILPPTRVGSNTY-amide
156	K(γE-C18diacid)K[CNTATC]ATQRLA(Dab)FL(Aib)HSSNNFGPILPPTEVGSNTY-amide
157	(C18diacid)K[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide
158	K(C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide

Table 1: The square bracket [ ] between the two cysteine residues (cys 2 and cys 7) indicate the presence of an intramolecular disulphide bridge.

Detailed Description

The present inventors have observed that pramlintide conjugated to an albumin binding moiety, such as a lipid, has poor stability (*e.g.* the fibril-forming tendency of pramlintide is increased) under conditions required for drug product formulation. The present invention is based, at least in part, on the finding that the polypeptides (e.g. lipidated polypeptides) described herein may exhibit improved stability (*e.g.* reduced or no fibrillation tendency) as compared to such pramlintide conjugates.

For example, the present inventors have found that when pramlintide is conjugated to a lipid to increase the half-life, the fibril-forming tendency also increases. Accordingly, the polypeptides (e.g. lipidated polypeptides) described herein may bring the benefit of extended half-life compared to pramlintide but without the fibril-forming tendency of alternative lipidated pramlintide analogues. Peptides disclosed here can be formulated in or chemically conjugated to e.g. a protein, polymeric drug carrier or advance drug delivery system that enhances the chemical stability and or physical stability and or the circulatory exposure of the therapeutic moiety. The present inventors have further found that the polypeptides (e.g. lipidated polypeptides) described herein may exhibit improved physical and/or chemical stability as compared to human amylin or pramlintide. Furthermore, the polypeptides (e.g. lipidated polypeptides) described herein may have similar or improved selectivity to human amylin (hAMYR) compared to pramlintide.

Throughout this specification, amino acid positions of the polypeptides (e.g. lipidated polypeptides) are numbered according to the corresponding position in pramlintide having the sequence set forth in SEQ ID NO. 1.

Throughout this specification, amino acids are referred to 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, frequently employed three- or four-character codes are employed for residues thereof, including αMeSer (*(S)*-2-amino-2-methyl-3-phenylpropanoic acid), αMePhe (*(S)*-2-amino-2-methyl-3-phenylpropanoic acid), Aib (2-amino-2-methylpropanoic acid), Dab (2,4-diaminobutanoic acid) and γ-Glu (γ-glutamic acid).

In embodiments of any aspect of the invention, the polypeptides (e.g. lipidated polypeptides) of the invention are isolated polypeptides (e.g. isolated lipidated polypeptides).

Albumin binding moiety

The polypeptides of the invention comprise at least one albumin binding moiety. Without being bound by theory, it is thought that the albumin binding moiety protects the polypeptide against clearance and degradation, thereby extending the half-life of the polypeptide. As used herein, "albumin binding moiety" refers to a compound that binds to albumin. Exemplary albumin binding moieties suitable for use in the polypeptides of the invention include lipids (e.g. a fatty acid derivative), albumin-binding peptides, albumin-binding proteins, or small molecule ligands that bind to albumin. Optionally, the albumin binding moiety is a lipid, e.g. a lipid described herein.

The polypeptides of the invention may comprise one or more albumin binding moiety (e.g. lipid), *e.g.* one, two or three albumin binding moieties. In preferred embodiments, the polypeptides of the invention comprise only one albumin binding moiety (e.g. lipid).

The albumin binding moiety (e.g. lipid) may be attached to an amino acid residue of the polypeptide. In some embodiments, the albumin binding moiety (e.g. lipid) is attached to the amino acid residue through a linker. In alternative embodiments, the albumin binding moiety (e.g. lipid) is directly attached to the amino acid residue without an intervening linker. The albumin binding moiety (e.g. lipid) may be attached to the amino acid residue via an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulphonamide. Accordingly, it will be understood that the albumin binding moiety (e.g. lipid) or the linker includes an acyl group, a sulphonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulphonyl ester, thioester, amide, amine or sulphonamide. Optionally, an acyl group in the albumin binding moiety (e.g. lipid) or the linker forms part of an amide or ester with the amino acid residue. Accordingly, in preferred embodiments the albumin binding moiety (e.g. lipid) is attached to an acylation site on the amino acid residue.

The albumin binding moiety (e.g. lipid) may be attached to any residue at position Xaa -4 to Xaa 37 (e.g. to the εN of a lysine residue) of the polypeptide. In some embodiments, the albumin binding moiety (e.g. lipid) is attached to the side chain of an amino acid residue in the polypeptide, for example to the εN of a lysine residue. In some

embodiments, the albumin binding moiety (e.g. lipid) is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide).

In some embodiments, the albumin binding moiety (e.g. lipid) is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide). In some embodiments, the albumin binding moiety (e.g. lipid) is attached to the amino acid residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1 (e.g. to the εN of a lysine residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1). In preferred embodiments, the albumin binding moiety (e.g. lipid) is attached to Xaa -4, Xaa -1 or Xaa 1 (either to the N-terminus or to the side chain of Xaa -4, Xaa -1 or Xaa 1).

Lipid

In preferred embodiments, the albumin binding moiety is a lipid. Accordingly, the polypeptides of the invention may comprise at least one lipid (referred to herein as "lipidated polypeptide"). Without being bound by theory, it is thought that the lipid acts as an albumin binding moiety and protects the polypeptide against clearance and degradation, thereby extending the half-life of the polypeptide. The lipid may also modulate the potency of the compound as an agonist to the amylin (calcitonin) receptor.

In some embodiments, the polypeptide comprises at least one lipidated amino acid residue. In some embodiments, the polypeptide comprises at least two lipidated amino acid residues. In preferred embodiments, the polypeptide contains only one lipidated amino acid residue. The lipid may be attached to an amino acid residue of the polypeptide. In some embodiments, the lipid is attached to the amino acid residue through a linker (referred to herein as "linker-lipid"). In alternative embodiments, the lipid is directly attached to the amino acid residue without an intervening linker. The lipid may be attached to the amino acid residue via an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulphonamide. Accordingly, it will be understood that the lipid or the linker includes an acyl group, a sulphonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulphonyl ester, thioester, amide, amine or sulphonamide. Optionally, an acyl group in the lipid or linker forms part of an amide or ester with the amino acid residue. Accordingly, in preferred embodiments the lipid is attached to an acylation site on the amino acid residue.

The lipid may be attached to any residue at position Xaa -4 to Xaa 37 (e.g. to the εN of a lysine residue) of the polypeptide. In some embodiments, the lipid is attached to the side

chain of an amino acid residue in the polypeptide, for example to the εN of a lysine residue. In some embodiments, the lipid is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide).

In some embodiments, the lipid is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide). In some embodiments, the lipid is attached to the amino acid residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1 (e.g. to the εN of a lysine residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1). In preferred embodiments, the lipid is attached to Xaa -4, Xaa -1 or Xaa 1 (either to the N-terminus or to the side chain of Xaa -4, Xaa -1 or Xaa 1).

In embodiments of any aspect of the invention, the lipid may comprise a hydrocarbon chain having from 10 to 26 C atoms, e.g. from 14 to 24 C atoms, e.g. from 16 to 22 C atoms. For example, the hydrocarbon chain may contain 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 C atoms. In preferred embodiments, the lipid has 18 to 20 C atoms. In particular, the lipid may have 18 C atoms or 20 C atoms. The hydrocarbon chain may be linear or branched, and may be saturated or unsaturated. Furthermore, it can include a functional group at the end of the lipophilic chain, *e.g.* a carboxylic acid group which may or may not be protected during synthesis.

Optionally, the lipid comprises a dicarboxylic acid. For example, the lipid may comprise C12diacid, C14diacid, C16diacid, C17diacid, C18diacid, C19diacid or C20diacid. In preferred embodiments, the lipid comprises C18diacid or C20diacid.

Linker

The albumin binding moiety (e.g. lipid) may be attached to the polypeptide through a linker. In embodiments of any aspect of the invention, the linker may comprise one or more residues of any naturally occurring or non-naturally occurring amino acid. The linker may comprise a combination of residues, as single or repeating units. For example, the linker may comprise multiple combinations of residues, as single or repeating units, each of which may independently be a residue of Glu, y-Glu, Lys, Ɛ-Lys, Asp, ß-Asp, Gaba, ß-Ala (3-aminopropanoyl), O2Oc (2-(2-(2-aminoethoxy)ethoxy)acetic acid), PEG2 (3-(2-(2-aminoethoxy)ethoxy)propanoic acid), PEG4 (1-amino-3,6,9,12-tetraoxapentadecan-15-oic acid), PEG8 (1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid, PEG12 (1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid). y-Glu and ß-Asp refer to amino acids where the alpha-amino group and the side chain carboxyl

group participate in peptide bond formation. Ɛ-Lys refers to an amino acid where the epsilon-amino and carboxyl group of lysine participate in peptide bond formation.

In some embodiments, the linker comprises a residue of γ-Glu, *e.g.* γGlu, γGlu-γGlu, γGlu-(O2Oc)-(O2Oc) or γGlu-(PEG2)-(PEG2). In some embodiments, the linker consists of γGlu, γGlu-γGlu, γGlu-(O2Oc)-(O2Oc) or γGlu-(PEG2)-(PEG2).

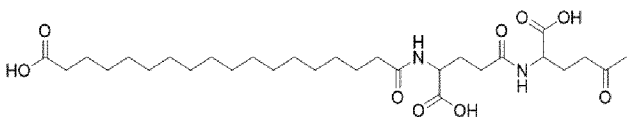
In some embodiments of any aspect of the invention, the polypeptide comprises any one of the linker and lipid combinations set forth in any one of the rows in Table 2.

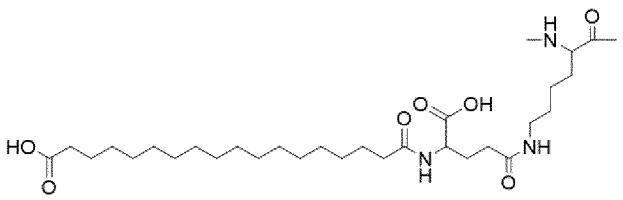
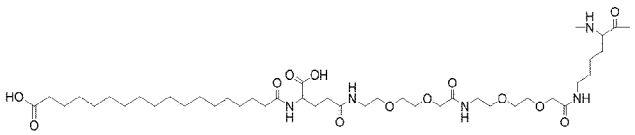
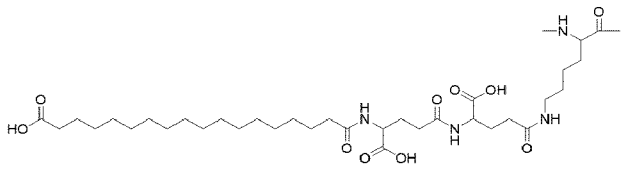
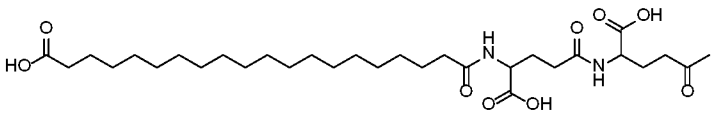
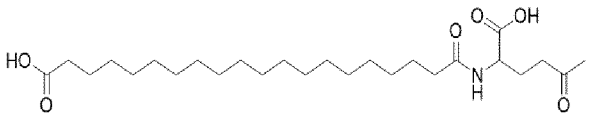
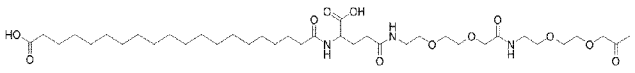
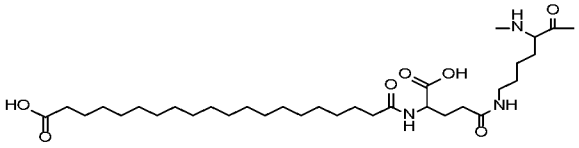
The linker may be attached to the amino acid residue via an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulphonamide. Accordingly it will be understood that optionally the linker includes an acyl group, a sulphonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulphonyl ester, thioester, amide, amine or sulphonamide. Optionally, an acyl group in the linker forms part of an amide or ester with the amino acid residue. Accordingly, in preferred embodiments the linker is attached to an acylation site on the amino acid residue.

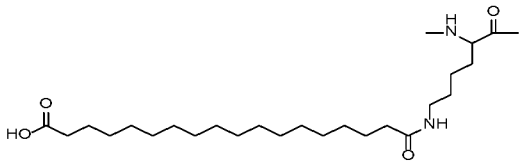
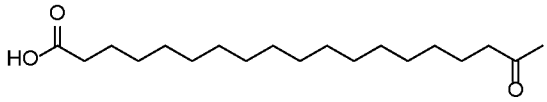
The linker may be attached to a site (*e.g.* an acylation site) at the N-terminus of the lipidated polypeptide or to the ε amino group "εN" of a residue in the lipidated polypeptide, *e.g.* to εN of a lysine residue.

In some embodiments, the polypeptide comprises a combination of linker, lipid and acylation site set forth in any one of the rows of Table 2.

Table 2: Combinations of linker, lipid and polypeptide acylation site

Lipid	Linker	Acylation site	Formula
C18diacid	**γE-γE**	**N-terminal**	

C18diacid	γE	(εN)K	
C18diacid	γE-(O2Oc)-(O2Oc)	(εN)K	
C18diacid	γE-γE	(εN)K	
C20diacid	γE-γE	N-terminal	
C20diacid	γE	N-terminal	
C20diacid	γE-(O2Oc)-(O2Oc)	N-terminal	
C20diacid	γE-γE	(εN)K	

C18diacid	**Nil**	**(εN)K**	
C18diacid	**Nil**	**N-terminal**	

The linker may be attached to any residue at position Xaa -4 to Xaa 37 (e.g. to the εN of a lysine residue) of the polypeptide. In some embodiments, the linker is attached to the side chain of an amino acid residue in the polypeptide, for example to the εN of a lysine residue. In some embodiments, the linker is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide).

In some embodiments, the linker is attached to the N-terminus of the polypeptide, (*e.g.* to a lysine at the N-terminus of the polypeptide). In some embodiments, the linker is attached to the amino acid residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1 (e.g. to the εN of a lysine residue at Xaa -4, Xaa -3, Xaa -2, Xaa -1 or Xaa 1). In preferred embodiments, the linker is attached to Xaa -4, Xaa -1 or Xaa 1 (either to the N-terminus or to the side chain of Xaa -4, Xaa -1 or Xaa 1).

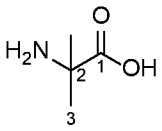
In some embodiments, the linker is attached to a site (*e.g.* an acylation site) selected from the N-terminus of the polypeptide, εN of a lysine at position Xaa (1) "1K", the εN of a lysine at position Xaa (-1) "-1K", or the εN of a lysine at position Xaa (-4) "-4K".

Amino acid substitutions and modifications

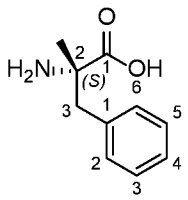
The polypeptides (e.g. lipidated polypeptides) of the invention may comprise one or more amino acid modifications or substitutions compared to the pramlintide sequence [SEQ ID NO: 1].

In some embodiments, the polypeptides (e.g. lipidated polypeptides) comprises one or more non-proteinogenic amino acids. Non-proteinogenic amino acids may include alpha methyl amino acids, *D*-enantiomers of naturally occurring amino acids, 2,4-diaminobutanoic acid (Dab), and (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp). In

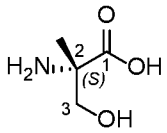
some embodiments, the polypeptide (e.g. lipidated polypeptide) comprises one or more non-proteinogenic amino acids between positions 14-37, optionally at one or more of 14, 17 or 20-37.n some embodiments, the polypeptide (e.g. lipidated polypeptide) comprises one or more alpha methyl amino acids between positions 14-37, optionally at one or more of alpha methyl amino acids at positions 14, 17 or 20-37. Polypeptides (e.g. lipidated polypeptides) comprising one or more alpha methyl amino acids at positions 17, 21 or 23 are particularly preferred. Representative examples of alpha methyl amino acids include 2-amino-2-methylpropanoic acid (Aib), alpha-methyl glutamine (αMeGlu), alpha methyl phenylalanine (αMePhe or αMeF), alpha-methyl leucine (αMeLeu) and alpha-methyl serine (αMeSer). Thus, in certain embodiments, the alpha methyl amino acid can be Aib, αMeGlu, αMePhe, αMeLeu or αMeSer, or any combination thereof. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) comprises at least one alpha methyl amino acid, optionally selected from Aib, αMePhe and αMeSer. The reference to αMePhe and αMeF herein refers to (*S*)-2-amino-2-methyl-3-phenylpropanoic acid. The reference to αMeSer herein refers to (*S*)-2-amino-3-hydroxy-2-methylpropanoic acid. In preferred embodiments, the alpha methyl amino acid is Aib, αMePhe or αMeSer.



2-amino-2-methylpropanoic acid
(Aib)

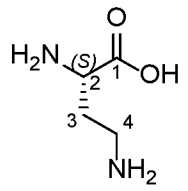


(*S*)-2-amino-2-methyl-3-phenylpropanoic acid
($^{\alpha}$MePhe)

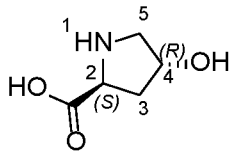


(*S*)-2-amino-3-hydroxy-2-methylpropanoic acid
($^{\alpha}$MeSer)

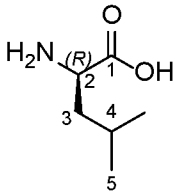
In some embodiments, the polypeptide (e.g. lipidated polypeptide) comprises one or more non-proteinogenic amino acids between positions 14-37 selected from the group consisting of: 2,4-diaminobutanoic acid (Dab), (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp), D-leucine (dL), *D*-isoleucine (dI) and *D*-proline (dP).



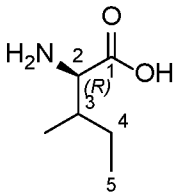
2,4-diaminobutanoic acid (Dab)



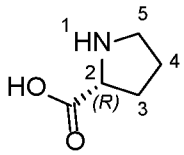
(2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp)



D-leucine (dL)



D-isoleucine (dI)



D-proline (dP)

In some embodiments, the polypeptide (e.g. lipidated polypeptide) does not comprise (2S)-2-aminohexanedioic acid) (Aad) and/or does not comprise Aad at positions 14-37.

In some embodiments, the polypeptide (e.g. lipidated polypeptide) does not comprise Aib at one or more of positions 15, 16, 17, 19 or 20. In alternative embodiments, the polypeptide (e.g. lipidated polypeptide) comprises Aib at one or more of positions 15, 16, 17, 19 or 20 and at least one different non-proteinogenic amino acid (*e.g.* an alpha methyl amino acid that is not Aib) at positions 14-37.

In some embodiments, the polypeptide (e.g. lipidated polypeptide) comprises one or more natural amino acid substitutions or modifications compared to the pramlintide sequence [SEQ ID NO: 1].

In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) comprises one or more of the following natural amino acid substitutions or modifications: deleted 1K (Δ1K), Ile 4, Ala 4, Glu 14, His 14, Trp 15, Arg 17, Ser 17, Glu 17, , Pro 20, Ile 20, His 21, Ala 21, Glu 21, Gly 21, Lys 21, Pro 21, Arg 21, Ser 21, His 22, Pro 24, Ala 25, Arg 26, Ser 28, His 31, Glu 31, Pro 31, Arg 31, His 34, Pro 33, Pro 34, Glu 35, Arg 35, Pro 35 and Pro 37.

It will be understood that the polypeptide (e.g. lipidated polypeptides) may comprise a combination of non-proteinogenic amino acids and natural amino acid substitutions or modifications compared to the pramlintide sequence [SEQ ID NO: 1].

In some aspects, there is provided a polypeptide (e.g. lipidated polypeptide) that is a pramlintide analogue, or a pharmaceutically acceptable salt thereof, comprising any of the amino acid sequence modification combinations set forth in Table 3.

Table 3: Amino acid modifications to pramlintide sequence

Sequence modification with respect to pramlintide
-1G, -2G, 17Aib
4A, 15W, 21P, 24P, 25A, 28S
4I, 20I, 21A, 35R
4I, 21Dab, 35R
14Dab
14Dab, 17Aib, 31E
14Dab, 23αMePhe, 31E
14Dab, 31E
14E, 17Aib
14E, 17Aib, 21H
14E, 17R
14E, 17R, 23αMePhe
14E, 21Aib
14H, 17Aib
14H, 17Aib, 21Aib, 31E
14H, 21Aib
14H, 21Aib, 35E
16dL, 21Aib
17Aib
17Aib, 37P
17Aib, 21Aib
17Aib, 21Aib, 37P
17Aib, 21G
17Aib, 21H
17Aib, 21K
17Aib, 21P
17Aib, 21P, 31E
17Aib, 21P, 35E

17Aib, 21R
17Aib, 21S
17Aib, 21Dab
17Aib, 21Dab, 31E
17Aib, 22H
17Aib, 22H, 35E
17Aib, 23αMePhe
17Aib, 26Aib
17Aib, 26R
17Aib, 27Aib
17Aib, 27dL
17Aib, 28Aib
17Aib, 29Aib
17Aib, 31Aib
17Aib, 31E
17Aib, 31H, 35E
17Aib, 31P
17Aib, 31R
17Aib, 32Aib
17Aib, 33Aib
17Aib, 34Aib
17Aib, 34H
17Aib, 34P
17Aib, 35Aib
17Aib, 35E
17Aib, 35R
17E, 21Aib
17R, 21Aib
17R, 21Aib, 31Aib
17R, 21Aib, 31E
17R, 21Aib, 31R
17R, 21Aib, 35Aib
17R, 23αMePhe
17R, 23αMePhe, 31E

17R, 26Aib
17S, 21Aib
17S, 21Aib, 31H
17S, 21Aib, 31P
17S, 21Aib, 31R
17S, 21Aib, 33P
17S, 21Aib, 35P
20αMeSer
20P, 21P, 24P, 25A, 28S
21Aib
21Aib, 24P, 25A, 28S
21Aib, 24P, 25A, 28S, 31Dab
21Aib, 24P, 25A, 28S, 35Dab
21Aib, 26dI
21Aib, 26Aib
21Aib, 27Aib
21Aib, 27dL
21Aib, 28Aib
21Aib, 28dP
21Aib, 31Aib
21Aib, 31E
21Aib, 31H
21Aib, 31R
21Aib, 33Aib
21Aib, 34Aib
21Aib, 35Aib
21Aib, 35E
21Aib, 35R
21Aib, 36Aib
21Aib, 37Aib
21Aib, 37P
21Aib, 24Hyp, 25A, 28S
21Dab, 24P, 25A, 28S
21Dab, 25Aib

21Dab, 31E
21P, 24P, 25A, 28S
22Aib
22H, 35E
23αMePhe
23αMePhe, 31E
23αMePhe, 31R
23αMePhe, 35R
24Aib
26Aib
27Aib
27dL
28dP
35R
Δ1K, 4I, 21Dab, 35R
Δ1K, 14E, 17R, 23αMePhe
Δ1K, 21Aib, 31R,

In one aspect, there is provided a polypeptide (e.g. lipidated polypeptide) that is a pramlintide analogue, or a pharmaceutically acceptable salt thereof, having an alpha methyl amino acid at position 23. In preferred embodiments, the alpha methyl amino acid is αMePhe.

In preferred embodiments of any aspect in which the polypeptide (e.g. lipidated polypeptide) comprises an alpha methyl amino acid (*e.g.* αMePhe) at position 23, the polypeptide (e.g. lipidated polypeptide) comprises any one of the following combinations of modifications:

14E, 17R, 23αMePhe;

Δ1K, 14E, 17R, 23αMePhe;

14Dab, 23αMePhe, 31E;

17Aib, 23αMePhe;

17R, 23αMePhe, 31E;

23αMePhe, 31E;

23αMePhe, 31R; or

23αMePhe, 35R.

In one aspect, there is provided a polypeptide (e.g. lipidated polypeptide) that is a pramlintide analogue, or a pharmaceutically acceptable salt thereof having at least two Aib residues. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) comprises Aib at at least two of positions 17 and 20-37. In particularly preferred embodiments, the polypeptide (e.g. lipidated polypeptide) comprises Aib at positions 21, 26, 27, 28, 29, 31, 32, 33, 34 and 35.

In preferred embodiments of any aspect in which the polypeptide (e.g. lipidated polypeptide) comprises at least two Aib residues, the polypeptide (e.g. lipidated polypeptide) comprises any one of the following combinations of modifications:
14H, 17Aib, 21Aib, 31E;
17Aib, 21Aib;
17Aib, 21Aib, 37P;
17Aib, 26Aib;
17Aib, 27Aib;
17Aib, 28Aib;
17Aib, 29Aib;
17Aib, 31Aib;
17Aib, 32Aib;
17Aib, 33Aib;
17Aib, 34Aib;
17Aib, 35Aib;
17R, 21Aib, 31Aib;
17R, 21Aib, 35Aib;
21Aib, 26Aib;
21Aib, 27Aib;
21Aib, 28Aib;
21Aib, 31Aib;
21Aib, 33Aib;
21Aib, 34Aib;
21Aib, 35Aib;
21Aib, 36Aib; or
21Aib, 37Aib.

In one aspect, there is provided a polypeptide (e.g. lipidated polypeptide) that is a pramlintide analogue, or a pharmaceutically acceptable salt thereof, having an alpha methyl amino acid at position 21. In preferred embodiments, the alpha methyl amino acid is Aib.

In preferred embodiments of any aspect in which the polypeptide (e.g. lipidated polypeptide) comprises an alpha methyl amino acid (*e.g.* Aib) at position 21, the polypeptide (e.g. lipidated polypeptide) comprises any one of the following combinations of modifications:

14E, 21Aib;
14H, 17Aib, 21Aib, 31E;
14H, 21Aib, 35E;
14H, 21Aib;
16dL, 21Aib;
17Aib, 21Aib, 37P;
17Aib, 21Aib;
17E, 21Aib;
17R, 21Aib, 31Aib;
17R, 21Aib, 31E;
17R, 21Aib, 31R;
17R, 21Aib, 35Aib;
17R, 21Aib;
17S, 21Aib, 31H;
17S, 21Aib, 31P;
17S, 21Aib, 31R;
17S, 21Aib, 33P;
17S, 21Aib, 35P;
17S, 21Aib;
21Aib, 24Hyp, 25A, 28S
21Aib, 24P, 25A, 28S, 31Dab;
21Aib, 24P, 25A, 28S, 35Dab.
21Aib, 24P, 25A, 28S;
21Aib, 26Aib;
21Aib, 26dl;
21Aib, 27Aib;

21Aib, 27dL;
21Aib, 28Aib;
21Aib, 28dP;
21Aib, 31Aib;
21Aib, 31E;
21Aib, 31H;
21Aib, 31R; Δ1K
21Aib, 33Aib;
21Aib, 34Aib;
21Aib, 35Aib;
21Aib, 35E;
21Aib, 35R;
21Aib, 36Aib;
21Aib, 37Aib;
21Aib, 37P; or
21Aib.

In one aspect, there is provided a polypeptide (e.g. lipidated polypeptide) that is a pramlintide analogue, or a pharmaceutically acceptable salt thereof, having an alpha methyl amino acid at position 17. In preferred embodiments, the alpha methyl amino acid is Aib.

In preferred embodiments of any aspect in which the polypeptide (e.g. lipidated polypeptide) comprises an alpha methyl amino acid (*e.g.* Aib) at position 17, the polypeptide (e.g. lipidated polypeptide) comprises any one of the following combinations of modifications:
14H, 17Aib;
-1G, -2G, 17Aib;
17Aib, 23αMePhe;
17Aib, 21Dab, 31E;
17Aib, 21Dab;
17Aib, 21Aib;
14H, 17Aib, 21Aib, 31E;
17Aib, 21Aib, 37P;
17Aib, 26Aib;

17Aib, 27Aib;
17Aib, 28Aib;
17Aib, 29Aib;
17Aib, 31Aib;
17Aib, 32Aib;
17Aib, 33Aib;
17Aib, 34Aib; or
17Aib, 35Aib.

Pharmacokinetics

The polypeptides (e.g. lipidated polypeptides) of the invention may exhibit favourable pharmacokinetic properties as compared to pramlintide. For example, the polypeptides (e.g. lipidated polypeptides) of the invention may have an extended half-life as compared to pramlintide.

As used herein, the term "half-life" is used to refer to the time taken for the concentration of isolated polypeptide in plasma to decline to 50% of its original level. Methods to determine the half-life of proteins are known in the art and are described in Example 4.

It will be recognised that an extended half-life is advantageous, as it permits the therapeutic proteins to be administered according to a safe and convenient dosing schedule, *e.g.* lower doses that can be administered less frequently. Moreover, the achievement of lower doses may provide further advantages such as the provision of an improved safety profile. To the contrary, pramlintide requires frequent and inconvenient administration.

The present inventors have shown that the polypeptides (e.g. lipidated polypeptides) of the invention may have a half-life of at least 4 hours in rat models (see Example 4). In embodiments, the polypeptide (e.g. lipidated polypeptide) has a half-life of at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours or at least 14 hours in rat models. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) has a half-life of at least 14 hours.

Reduced fibrillation

The polypeptides (e.g. lipidated polypeptides) of the invention may exhibit reduced tendency to undergo fibrillation in pharmaceutically relevant aqueous media, especially at pH values in the range from 4 to 7, as compared to lipidated pramlintide. In some embodiments, the polypeptide (e.g. lipidated polypeptide) exhibits reduced tendency to undergo fibrillation in pharmaceutically relevant aqueous media, especially at pH values in the range from 4 to 7, as compared to pramlintide which is lipidated in a similar manner e.g. the same lipid is attached, the lipid is attached through the same linker and/or the lipid is attached at the same position. Exemplary lipidated pramlintide molecules are given in Table 1, for example SEQ ID NO. 3, 4, 5, 6, 7, 112 and 113.

Accordingly, the polypeptides (e.g. lipidated polypeptides) of the invention may be suited for formulation in acidic media (e.g. pH 4) and in neutral or near-neutral media (e.g. pH 7 or 7.4). Such polypeptides (e.g. lipidated polypeptides) may be 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 some embodiments, the polypeptide (e.g. lipidated polypeptide) shows no detectable fibrillation after about 5 hours, after about 7 hours, after about 9 hours, after about 11 hours, after about 13 hours, after about 15 hours, after about 17 hours or after about 20 hours at pH 4 and 37°C, *e.g.* under the conditions described in Example 3.

In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) shows no detectable fibrillation after about 48 hours, after about 72 hours, after about 96 hours, after about 108 hours, after about 120 hours, after 132 about hours or after about 144 hours at pH 4 and 37°C, *e.g.* under the conditions described in Example 3. In particularly preferred embodiments, the polypeptide (e.g. lipidated polypeptide) shows no detectable fibrillation after 144 hours at pH 4 and 37°C, *e.g.* under the conditions described in Example 3.

In some embodiments, the formation of fibrils is detected by an increase in fluorescence intensity in a Thioflavin T fibrillation assay, *e.g.* as described in Example 3.

In preferred embodiments, the polypeptides (e.g. lipidated polypeptides) of the invention are soluble at concentrations required for therapeutic efficacy. In some embodiments, the lipidated polypeptides of the invention are soluble at a concentration of at least 1 mg/mL under the conditions described in Example 3.

Efficacy

The polypeptides (e.g. lipidated polypeptides) of the invention are amylin receptor agonists, *i.e.* they are capable of binding to, and inducing signalling by, one or more receptors or receptor complexes regarded as physiological receptors for human amylin. These include the human calcitonin receptor hCTR, as well as complexes comprising the human calcitonin receptor hCTR and at least one of the human receptor activity modifying proteins designated hRAMP1, hRAMP2 and hRAMP3. Complexes between hCTR and hRAMP1, hRAMP2 and hRAMP3 are designated hAMYR1, hAMYR2 and hAMYR3 (i.e. human amylin receptors 1, 2 and 3) respectively. In some embodiments, a compound is considered an amylin receptor agonist if it has agonist activity at one or more of hAMYR1, hAMYR2 and hAMYR3. For example, a compound may be considered an amylin receptor agonist if it has agonist activity at hAMYR3.

The ability to induce cAMP formation 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 readouts for amylin receptor agonist activity. These may include calcium release, arrestin recruitment, receptor internalization, kinase activation or inactivation, lipase activation, inositol phosphate release, diacylglycerol release or nuclear transcription factor translocation.

EC50 values may be used as a 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, for example a cAMP assay as described in Example 2. In Example 2, the present inventors have shown that certain polypeptides (e.g. lipidated polypeptides) disclosed herein exhibit greater or similar selectivity to hAMYR over hCTR as pramlintide, as measured using cAMP release from binding to hAMYR and hCTR. Pramlintide exhibits at least 10-fold selectivity to hAMYR as compared to hCTR.

The polypeptides (e.g. lipidated polypeptides) of the invention may exhibit improved efficacy, *e.g.* as amylin receptor agonists, as compared to lipidated pramlintide.

In some embodiments, the polypeptide (e.g. lipidated polypeptide) has at least about 1-fold selectivity to hAMYR over hCTR, optionally at least about 2-fold, at least about 4-fold, at least about 6-fold, at least about 8-fold, at least about 10-fold, at least about 12-fold, at least about 14-fold, at least about 16-fold, at least about 18-fold, at least about 20-fold, at

least about 50-fold, at least about 75-fold, or at least about 100-fold selectivity to hAMYR over hCTR. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) has at least about 10-fold selectivity to hAMYR over hCTR.

In some embodiments, the polypeptide (e.g. lipidated polypeptide) has around 12-20 fold, around 14-18 fold, optionally around 16-fold selectivity to hAMYR over hCTR.

n some embodiments, the isolated polypeptide has an EC50 measured under the conditions described in Example 2 (i.e. containing 0.1% bovine serum albumin (BSA)) of below about 1.4 nM, below about 1.2 nM, below about 1 nM, below about 0.8 nM, below about 0.6 nM, below about 0.4nM, below about 0.3 nM, or below about 0.2 nM.

Chemical stability

The polypeptides (e.g. lipidated polypeptides) of the invention may be chemically stable, *e.g.* they may form in a formulation an acceptable percentage of degradation products produced over a defined period of time by chemical pathways, such as deamidation, aggregation, or oxidation.

The polypeptides (e.g. lipidated polypeptides) of the invention may be chemically conjugated to a protein or polymeric drug carrier, or formulated in an advance drug delivery system, that enhances the chemical stability and/or physical stability and/or the circulatory exposure of the polypeptide.

In some aspects, there is provided a polypeptide or a pharmaceutically acceptable salt thereof, wherein the polypeptide comprises any one of the lipid linkers as set forth in Table 2 and any one of the sequence modifications as set forth in Table 3.

In some aspects, there is provided a polypeptide or a pharmaceutically acceptable salt thereof, wherein the polypeptide comprises the lipid linker and amino acid sequence modification combinations set forth in Table 4.

Table 4: Lipidated polypeptides

ID	Lipid	Linker	Acylation site	Sequence modification with respect to pramlintide
8	C18diacid	γE-γE	1K	21Dab, 24P, 25A, 28S
9	C18diacid	γE-γE	1K	21Aib, 24P, 25A, 28S
10	C18diacid	γE	N-terminal	14E, 17R, 23αMePhe, and Δ1K
11	C18diacid	γE-γE	1K	14E, 17R, 23αMePhe

12	C18diacid	γE	-1K	14E, 17R, 23αMePhe
13	C18diacid	γE-γE	1K	23αMePhe
14	C18diacid	γE	-1K	23αMePhe
15	C18diacid	γE	-1K	17R, 23αMePhe, 31E
16	C18diacid	γE	1K	17R, 23αMePhe, 31E
17	C18diacid	γE-(O2Oc)-(O2Oc)	1K	17R, 23αMePhe, 31E
18	C18diacid	γE-γE	1K	17R, 23αMePhe, 31E
19	C18diacid	γE	1K	17R, 23αMePhe
20	C18diacid	γE-γE	1K	17R, 23αMePhe
21	C18diacid	γE-γE	1K	23αMePhe, 35R
22	C18diacid	γE-γE	1K	20αMeS
23	C18diacid	γE-γE	1K	23αMePhe, 31E
24	C18diacid	γE-γE	1K	23αMePhe, 31R
25	C18diacid	γE	1K	23αMePhe, 31R
26	C18diacid	γE-γE	1K	17Aib, 23αMePhe
27	C18diacid	γE-γE	1K	4I, 21Dab, 35R
28	C18diacid	γE	-1K	21Dab, 31E
29	C18diacid	γE	-1K	21Dab
30	C18diacid	γE	-1K	17Aib, 21Dab, 31E
31	C18diacid	γE	-1K	21Dab, 25Aib
32	C18diacid	γE	-1K	14Dab, 23αMePhe, 31E
33	C18diacid	γE	-1K	14Dab
34	C18diacid	γE	-1K	14Dab, 31E
35	C18diacid	γE	-1K	21Aib
36	C18diacid	γE	-1K	17Aib, 21Aib
37	C18diacid	γE	-1K	17S, 21Aib
38	C18diacid	γE	-1K	14E, 21Aib
39	C18diacid	γE	-1K	17E, 21Aib
40	C18diacid	γE	-1K	21Aib, 31H
41	C18diacid	γE	-1K	21Aib, 31E
42	C18diacid	γE	-1K	21Aib, 35E
43	C18diacid	γE	-1K	17R, 21Aib, 31E
44	C18diacid	γE	1K	17R, 21Aib, 31E
45	C18diacid	γE-(O2Oc)-(O2Oc)	1K	17R, 21Aib, 31E
46	C18diacid	γE	-1K	14H, 21Aib
47	C18diacid	γE	-1K	14H, 21Aib, 35E
48	C18diacid	γE	1K	17R, 21Aib
49	C18diacid	γE-γE	1K	21Aib, 31E
50	C18diacid	γE-γE	1K	17R, 21Aib, 31E
51	C18diacid	γE	-1K	17S, 21Aib, 31H
52	C18diacid	γE	-1K	17S, 21Aib, 31R
53	C18diacid	γE	-1K	17S, 21Aib, 31P
54	C18diacid	γE	-1K	17S, 21Aib, 33P

55	C18diacid	γE	-1K	17S, 21Aib, 35P
56	C18diacid	γE	1K	21Aib
58	C18diacid	γE	-1K	21Aib, 37P
59	C18diacid	γE	1K	21Aib, 27dL
60	C18diacid	γE	1K	21Aib, 28dP
61	C18diacid	γE	1K	21Aib, 26dI
62	C18diacid	γE	1K	16dL, 21Aib
63	C18diacid	γE	1K	21Aib, 31R
64	C18diacid	γE	1K	21Aib, 35R
65	C18diacid	γE	-1K	17R, 21Aib
66	C18diacid	γE-γE	-1K	17R, 21Aib
67	C18diacid	γE-γE	1K	21Aib, 31R
68	C18diacid	γE	N-terminal	21Aib, 31R; Δ1K
69	C18diacid	γE	-1K	21Aib, 31R
70	C18diacid	γE-γE	-1K	21Aib, 31R
71	C18diacid	γE	-1K	21Aib, 31R
72	C18diacid	γE-γE	1K	21Aib, 35R
73	C18diacid	γE	-1K	21Aib, 35R
74	C18diacid	γE-γE	-1K	21Aib, 35R
75	C18diacid	γE-γE	1K	17R, 21Aib
76	C18diacid	γE	1K	17R, 21Aib, 31R
77	C18diacid	γE-γE	1K	17R, 21Aib, 31R
78	C18diacid	γE	-1K	21Aib, 26Aib
79	C18diacid	γE	-1K	21Aib, 27Aib
80	C18diacid	γE	-1K	21Aib, 31Aib
81	C18diacid	γE	-1K	21Aib, 33Aib
82	C18diacid	γE	-1K	21Aib, 35Aib
83	C18diacid	γE	-1K	21Aib, 36Aib
84	C18diacid	γE	-1K	21Aib, 34Aib
85	C18diacid	γE	-1K	21Aib, 37Aib
86	C18diacid	γE	-1K	14H, 17Aib, 21Aib, 31E
87	C18diacid	γE	-1K	17Aib, 21Aib, 37P
88	C18diacid	γE	-1K	21Aib, 28Aib
89	C18diacid	γE-γE	1K	21Aib, 31Aib
90	C18diacid	γE-γE	1K	17R, 21Aib, 31Aib
91	C18diacid	γE-γE	1K	21Aib, 35Aib
92	C18diacid	γE-γE	1K	17R, 21Aib, 35Aib
94	C18diacid	γE	-1K	17Aib, 26Aib
95	C18diacid	γE	-1K	17Aib, 27Aib
96	C18diacid	γE	-1K	17Aib, 28Aib
97	C18diacid	γE	-1K	17Aib, 29Aib
98	C18diacid	γE	-1K	17Aib, 31Aib
99	C18diacid	γE	-1K	17Aib, 32Aib
100	C18diacid	γE	-1K	17Aib, 33Aib

101	C18diacid	γE	-1K	17Aib, 34Aib
102	C18diacid	γE	-1K	17Aib, 35Aib
103	C18diacid	γE	1K	27dL
104	C18diacid	γE	1K	28dP
106	C18diacid	γE	-1K	26Aib
107	C18diacid	γE	-1K	17R, 26Aib
108	C18diacid	γE	-1K	27Aib
109	C18diacid	γE	-1K	22Aib
110	C18diacid	γE	-1K	24Aib
111	C18diacid	γE	-1K	22H, 35E
114	C20diacid	γE-γE	1K	35R
115	C20diacid	γE-γE	1K	21P, 24P, 25A, 28S
116	C20diacid	γE-γE	1K	14E, 17R
117	C20diacid	γE-γE	1K	21Aib, 24P, 25A, 28S
118	C20diacid	γE-γE	1K	4I, 20I, 21A, 35R
119	C20diacid	γE-γE	1K	20P, 21P, 24P, 25A, 28S
120	C20diacid	γE-γE	1K	4A, 15W, 21P, 24P, 25A, 28S
121	C20diacid	γE-γE	1K	21Dab, 24Hyp, 25A, 28S
122	C18diacid	γE-γE	1K	21Aib, 24P, 25A, 28S, 31Dab
123	C20diacid	γE-γE	1K	21Aib, 24P, 25A, 28S, 31Dab
124	C18diacid	γE-γE	1K	21Aib, 24P, 25A, 28S, 35Dab
125	C20diacid	γE-γE	1K	21Aib, 24P, 25A, 28S, 35Dab
126	C18diacid	γE	-1K	17Aib, 21Dab
127	C18diacid	γE-γE	1K	17Aib
128	C18diacid	γE	-1K	14E, 17Aib
129	C18diacid	γE	-1K	17Aib
130	C18diacid	γE	-1K	14E, 17Aib, 21H
131	C18diacid	γE	-1K	17Aib, 21H
132	C18diacid	γE	-1K	17Aib, 21P
133	C18diacid	γE	-1K	17Aib, 21S
134	C18diacid	γE	-1K	17Aib, 31P
135	C18diacid	γE	-1K	17Aib, 22H
136	C18diacid	γE	-1K	17Aib, 37P
137	C18diacid	γE	-1K	17Aib, 21R
138	C18diacid	γE	-1K	17Aib, 21P, 31E
139	C18diacid	γE	-1K	17Aib, 21P, 35E
140	C18diacid	γE	-1K	17Aib, 31E
141	C18diacid	γE	-1K	17Aib, 35R
142	C18diacid	γE	-1K	17Aib, 35E
143	C18diacid	γE	-3K	-1G, -2G, 17Aib
144	C18diacid	γE	1K	14H, 17Aib
145	C18diacid	γE	-1K	17Aib, 34H
146	C18diacid	γE	-1K	17Aib, 31H, 35E
147	C18diacid	γE	-1K	17Aib, 22H, 35E

148	C18diacid	γE	-1K	17Aib, 34P
149	C18diacid	γE	1K	17Aib
150	C18diacid	γE	1K	17Aib, 21Dab
151	C18diacid	γE	1K	17Aib, 27dL
152	C18diacid	γE	-1K	17Aib, 26R
153	C18diacid	γE	-1K	17Aib, 21K
154	C18diacid	γE	-1K	17Aib, 21G
155	C18diacid	γE	1K	17Aib, 31R
156	C18diacid	γE	-1K	14Dab, 17Aib, 31E
157	C18diacid	Nil	N-terminal	21Aib, 31Aib
158	C18diacid	Nil	1K	21Aib, 31Aib

Process

The polypeptides (e.g. lipidated polypeptides) of the invention may be produced by any method known in the art. The production of polypeptides such as amylin or analogues thereof is well known in the art. The polypeptide (e.g. lipidated polypeptides) of the invention can thus be produced by chemical synthesis, e.g. solid phase polypeptide synthesis using t-Boc or Fmoc chemistry, or other well-established techniques. They may alternatively be produced by recombinant expression of a nucleic acid molecule encoding a fusion polypeptide in a host cell. Following synthesis, the polypeptides (e.g. lipidated polypeptides) of the invention may optionally be isolated or purified.

Therapeutic Methods

In further aspects, the polypeptides (e.g. lipidated polypeptides) of the invention are provided in a pharmaceutical composition.

The pharmaceutical compositions of the invention may comprise one or more excipient(s). Pharmaceutically acceptable excipients are known in the art, see for instance Remington's Pharmaceutical Sciences (by Joseph P. Remington, 18th ed., Mack Publishing Co., Easton, PA), which is incorporated herein in its entirety.

The present invention encompasses therapies which involve administering the polypeptides (e.g. lipidated polypeptides) of the invention to an animal, in particular a mammal, for instance a human, for preventing, treating, or ameliorating symptoms associated with a disease, disorder, or infection.

Accordingly, the polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention may be used in therapy, for example for treating a disease or disorder. Also provided is a method of treating a disease or disorder comprising administering to a subject or patient in need thereof a therapeutically effective amount of the polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention. The use or method may comprise administering a therapeutically effective schedule that has less frequent doses of the polypeptides (e.g. lipidated polypeptides) of the invention than the therapeutically effective dosing schedule of pramlintide.

It will be understood that the polypeptides (e.g. lipidated polypeptides) of the invention may be used in the treatment and/or prevention of obesity, metabolic diseases such as diabetes (e.g. type 1 or type 2 diabetes), and/or obesity-related conditions.

Accordingly, the polypeptides (e.g. lipidated polypeptides) of the invention may be used in a method of treating obesity, overweight, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease, sleep apnea and respiratory problems, hyperlipidemia, degeneration of cartilage, osteoarthritis, or reproductive health complications of obesity or overweight such as infertility in a subject, the method comprising administering a therapeutically effective amount of the polypeptide (e.g. lipidated polypeptide) to the subject.

This is also provided a method of inhibiting or reducing weight gain, promoting weight loss, reducing food intake, and/or reducing excess body weight, the method comprising administering the polypeptide (e.g. lipidated polypeptide) of the invention to the subject.

Metabolic diseases that may be treated by the polypeptide (e.g. lipidated polypeptide) of the invention include diabetes, type 1 diabetes, type 2 diabetes, gestational diabetes, pre-diabetes, insulin resistance, impaired glucose tolerance (IGI), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, or hyperglycemia *e.g.* abnormal postprandial hyperglycemia. .

In preferred embodiments, the polypeptides (e.g. lipidated polypeptides) of the invention are used for the treatment of type 1 diabetes or type 2 diabetes.

The polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention may be used for treating, inhibiting or reducing weight gain, promoting weight loss, reducing food intake, and/or reducing excess body weight.

The polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention may be used in the treatment and/or prevention of an eating disorder, Alzheimer's disease, hepatic steatosis ("fatty liver"), kidney failure, arteriosclerosis (e.g. atherosclerosis), cardiovascular disease, macrovascular disease, microvascular disease, diabetic heart (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, cancer, dumping syndrome, hypertension e.g. pulmonary hypertension, or dyslipidemia e.g. atherogenic dyslipidemia, cholescystitis, or short bowel syndrome.

The route of administration of polypeptides (e.g. lipidated polypeptides) of the invention, or pharmaceutical compositions thereof, can be, for example, oral, parenteral, by inhalation or topical. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) or pharmaceutical composition thereof is administered by parenteral administration to a subject or patient. The term “parenteral” as used herein includes, *e.g.,* intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. In preferred embodiments, the polypeptide (e.g. lipidated polypeptide) or pharmaceutical composition thereof is administered by injection, such as by intravenous, subcutaneous or intramuscular injection, to a subject or patient. In particularly preferred embodiments, the polypeptide (e.g. lipidated polypeptide) or pharmaceutical composition thereof is administered by subcutaneous injection. Administration by injection, such as by subcutaneous injection, offers the advantage of better comfort for the subject or patient and the opportunity to administer to a subject or patient outside of a hospital setting. In some embodiments, the polypeptide (e.g. lipidated polypeptide) or pharmaceutical composition thereof is administered by self-administration.

In some embodiments the subject or patient is a mammal, in particular a human.

In some embodiments, the polypeptide or pharmaceutical composition is administered to the subject in combination with insulin.

Articles of Manufacture and Kits

In other aspects, the present invention provides an article of manufacture comprising the polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention.

In yet other aspects, the present invention provides a kit comprising the polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention. The kit may comprise a package containing the polypeptide (e.g. lipidated polypeptide) or

pharmaceutical composition, optionally with instructions. In some embodiments, the polypeptides (e.g. lipidated polypeptides) or pharmaceutical compositions of the invention are formulated in single dose vials or a container closure system (e.g. pre- filled syringe). Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 20 ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide the skilled person with a general dictionary of many of the terms used in this disclosure.

This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure.

Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an agent” includes a plurality of such agents and reference to “the agent” includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth.

“About” may generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values. Optionally, the term “about” shall be understood herein as plus or minus (±) 5%, optionally ± 4%, ± 3%, ± 2%, ± 1%, ± 0.5%, ± 0.1%, of the numerical value of the number with which it is being used.

Embodiments described herein as "comprising" one or more features may also be considered as disclosure of the corresponding embodiments "consisting of" such features.

The term "pharmaceutically acceptable" as used herein means approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Concentrations, amounts, volumes, percentages and other numerical values may be presented herein in a range format. It is also to be understood that such range format is used merely for convenience and brevity and should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited.

The above embodiments are to be understood as illustrative examples. Further embodiments are envisaged. It is to be understood that any feature described in relation to any one embodiment may be used alone, or in combination with other features described, and may also be used in combination with one or more features of any other of the embodiments, or any combination of any other of the embodiments. Furthermore, equivalents and modifications not described above may also be employed without departing from the scope of the invention, which is defined in the accompanying claims.

In the context of the present disclosure other examples and variations of the polypeptides (e.g. lipidated polypeptides) and methods described herein will be apparent to a person of skill in the art.

Other examples and variations are within the scope of the disclosure, as set out in the appended claims.

All documents cited herein are each entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited documents.

Examples

Example 1: Generation of lipidated pramlintide analogue peptides

Lipidated pramlintide analogue peptides were synthesized as C-terminal carboxamides using rink amide MBHA resin (100-200 mesh). All peptides were prepared by automated synthesis using a Liberty Blue™ microwave solid phase peptide synthesizer (CEM

Corporation, NC, USA) using the Fmoc/tBu protocol. Manufacturer-supplied protocols were applied for coupling of amino acids in DMF and deprotection of Fmoc protecting group using piperidine in DMF (20% v/v). Asparagine, cysteine, glutamine and histidine were incorporated as their sidechain trityl (Trt) derivatives. Lysine was incorporated as the sidechain tert-butyloxycarbonyl (Boc) derivative. Serine, threonine and tyrosine were incorporated as sidechain tert-butyl (tBu) ethers, and aspartate and glutamate as their sidechain OtBu esters. Arginine was incorporated as the sidechain 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) derivative.

Boc-Lys(Fmoc) was incorporated when a subsequent chemical modification of the N-terminal lysine side chain was required. Upon completion of the peptide chain elongation, coupling of an albumin binding moiety, such as a lipid, was performed manually using HATU as a coupling reagent in the presence of DIPEA.

Peptides were cleaved from the solid support by treatment with a mixture of TFA:TIS:EDT:thioanisole:water (90:2.5:2.5:2.5:2.5 v/v) for 4 h with agitation at room temperature. Thereafter, the cleavage mixtures were filtered, concentrated in vacuo, precipitated and washed with diethyl ether and solids were isolated by centrifugation. The linear crude peptides were dried under a flow of nitrogen and dissolved in 20% MeCN in water (v/v) with 1% TFA (v/v) and filtered. The crude linear peptides were purified using a preparative RP-HPLC on a Varian SD-1 Prep Star binary pump system, monitoring by UV absorption at 210 nm using an Xbridge C18-A stationary phase (19.0 x 250 mm, 5 micron) column eluting a linear solvent gradient of 25 – 70% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 25 min.

The linear purified peptides were cyclised by treatment with iodine (1% w/v in methanol) for 10 min at room temperature and excess iodine was reduced by treatment with ascorbic acid (1% w/v in water). The cyclic crude peptides were re-purified as described above. The purified fractions were pooled, frozen and lyophilised.

LC/MS characterisation of purified peptides were performed on a Waters MassLynx 3100 platform using a XBridge C18 stationary phase (4.6 x 100 mm, 3 micron) eluting a linear binary gradient of 10 – 90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 10 minutes at 1.5 mL/min at ambient temperature. Analytes were detected by both UV absorption at 210 nm and ionization using a Waters 3100 mass detector (ESI+ mode). Analytical RP-HPLC characterisation was performed on an Agilent 1260 Infinity system using an Agilent Polaris C8-A stationary phase (4.6 x 100 mm, 3 micron) eluting a linear binary gradient of

10 – 90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) at 1.5 mL/min over 15 minutes at 40 °C.

Example 2: *in vitro* potency of lipidated pramlintide analogue peptides in human or rat amylin or calcitonin receptor cells

The functional activities of lipidated pramlintide analogue peptides, such as cAMP production, were tested in 1321N1 cell line with stable recombinant expression of human calcitonin receptor (hCTR) or human amylin receptor (calcitonin receptor co-expressed with receptor activity modifying protein, RAMP3) (hAMYR3) or HEK cells with stable recombinant expression of rat calcitonin receptor (rat CTR) or rat amylin receptor (calcitonin receptor co-expressed with receptor activity modifying protein, RAMP3) (Rat AMYR3).

Cryopreserved cell stock was thawed rapidly in a water-bath, suspended in assay buffer (0.1% BSA (Sigma # A3059) in HBSS (Sigma # H8264) with 25mM HEPES, pH 7.4 and containing 0.5mM IB MX (Sigma# 17018)) and spun at 240xg for 5 minutes. Cells were re-suspended in assay buffer at a batch-dependent optimized concentration (e.g. hCTR cells at $0.125x10^5$ cells/mL, hAMYR3 cells at $0.125x10^5$ cells/mL, rat CTR cells at at $1x10^5$ cells/mL, rat AMYR3 at 2 $x10^5$ cells/mL).

The test peptide stock was prepared in DMSO and diluted in assay buffer to reach stated concentrations and transferred in duplicates into a 384-black shallow well microtitre assay plate (Corning # 3676). Cells were added to the assay plate, incubated at room temperature for 30 minutes and the cAMP level measured using cAMP dynamic 2 HTRF kit (Cisbio, Cat # 62AM4PEJ), following the two step protocol as per manufacturer's recommendations. The plates were read on an Envision (Perkin Elmer) using excitation wavelength of 320nm and emission wavelengths of 620nm & 665nm.

Data was transformed to % Delta F as described in the manufacturer's guidelines and analyzed as percent activation of maximal amylin or calcitonin effect by 4-parameter logistic fit to determine EC_{50} values. The selectivity of a peptide to hAMYR vs hCTR is defined as a ratio of EC50 values at the two receptors.

All tested compounds show measurable potency in hAMYR and hCTR. Analogues that show >10 fold selectivity for hAMYR over hCTR are preferred.

Table 5: in vitro potency of lipidated pramlintide analogues at human amylin3 and calcitonin receptors

Peptide	EC50 (pM)		Ratio
	hAMYR3	hCTR	
1	**10**	**160**	**16**
3	246	1353	5
4	248	7532	30
5	174	3605	21
6	68	310	5
7	173	2159	12
8	375	20228	54
9	320	13521	42
10	183	2728	15
11	177	3640	21
12	158	1224	8
13	196	2043	10
14	220	1350	6
15	522	5461	10
16	221	2018	9
17	365	7083	19
18	319	4686	15
19	278	4041	15
20	174	4593	26
21	136	3408	25
22	162	6539	40
23	238	589	2
24	127	1857	15
25	105	1248	12
26	409	2797	7
27	134	731	5
28	390	9142	23
29	243	8211	34
30	873	10549	12
31	349	1280	4
32	742	11149	15
33	598	15830	26
34	877	17733	20
35	259	14153	55
36	331	6394	19
37	249	1962	8
38	190	1049	6
39	1048	10362	10
40	228	3329	15
41	178	1184	7

42	470	2954	6
43	647	19046	29
44	284	6262	22
45	588	16891	29
46	109	362	3
47	218	468	2
48	126	5273	42
49	334	4142	12
50	349	18927	54
51	126	1645	13
52	89	1104	12
53	98	235	2
54	7587	1507	0.2
55	678	1874	3
56	70	493	7
58	71	65	1
59	157	809	5
60	96	1027	11
61	99	654	7
62	167	7249	43
63	76	375	5
64	149	1278	9
65	207	6400	31
66	418	7011	17
67	132	4006	30
68	192	1774	9
69	164	1572	10
70	235	5405	23
71	211	1385	7
72	227	4004	18
73	256	1821	7
74	356	4574	13
75	161	15998	99
76	209	22291	107
77	231	2520	11
78	288	3147	11
79	659	4799	7
80	407	2970	7
81	404	2764	7
82	514	4277	8
83	599	6342	11
84	118	401	3
85	806	3104	4
86	119	311	3
87	71	74	1

88	281	1172	4
89	447	8239	18
90	1056	15595	15
91	200	2357	12
92	341	44414	130
94	322	1903	6
95	691	1172	2
96	556	859	2
97	684	944	1
98	942	972	1
99	3798	1825	0.5
100	364	459	1
101	177	441	2
102	479	1630	3
103	161	1881	12
104	79	1816	23
106	473	3420	7
107	1071	26264	25
108	801	6808	8
109	82	176	2
110	204	541	3
111	403	1875	5
112	861	1335	2
113	1173	18495	16
114	1317	15796	12
115	703	7645	11
116	1207	9557	8
117	770	3190	4
118	417	792	2
119	1461	34657	24
120	1769	10717	6
121	1328	6074	5
122	655	6181	9
123	873	4793	5
124	1129	6633	6
125	1201	10197	8
126	445	8453	19
127	364	7127	19.6
128	118	568	4.8
129	553	5750	10.4
130	446	6686	15
131	737	15143	20.5
132	416	3848	9.3
133	378	2578	6.8
134	281	2223	7.9

135	296	345	1.2
136	122	101	0.8
137	238	1504	6.3
138	612	2506	4.1
139	285	1043	3.7
140	656	6046	9.2
141	414	3950	9.5
142	730	2270	3.1
143	592	6937	11.7
144	138	725	5.3
145	417	1876	4.5
146	2533	4874	1.9
147	802	1508	1.9
148	191	728	3.8
149	130	1200	9.2
150	179	4181	23.4
151	61	898	14.7
154	574	1540	2.7
155	329	3930	11.9
156	3749	83771	22.3

Table 6: in vitro potency of lipidated pramlintide analogues at rat amylin3 and calcitonin receptors

Peptide	**EC50 (pM)**	
	Rat AMYR3	**Rat CTR**
1	**0.4**	**70.0**
3	**2.9**	**186.0**
8	**25.6**	**19856.1**
9	**17.8**	**9724.4**
10	**12.1**	**2781.5**
12	**5.0**	**106.8**
15	**10.3**	**1821.8**
18	**16.5**	**3086.9**
20	**10.4**	**1854.2**
24	**17.7**	**754.9**
35	**5.9**	**514.1**
38	**5.1**	**20.3**
40	**12.0**	**782.1**
41	**8.2**	**924.8**
43	**14.5**	**3650.9**
44	**18.5**	**2237.3**
48	**4.8**	**2431.0**
112	**16.0**	**258.5**

113	**32.6**	**10375.7**
114	**12.2**	**4873.7**
115	**22.6**	**22552.0**
116	**23.7**	**9052.7**
129	**11.2**	**2869.2**
140	**19.5**	**1218.4**
149	**5.6**	**521.8**

Example 3: Thioflavin T fibrillation assay

Peptide aggregation that form fibrils is an indication of physical instability. Fibril formation in solution poses a significant risk for the stability of injectable peptide drug products. Thioflavin T (ThT) fibrillation assay is a useful tool to assess the aggregation kinetics of a peptide or protein under accelerated and stressed conditions that can be used to forecast the long-term viability of a compound in solution.

ThT can selectively bind amyloid fibrils and the resultant complex emits strong fluorescence signal at 482 nm when excited at 450 nm (Anal Biochem. 1989 Mar;177(2):244-9). Monitoring of the change in fluorescence signal is an established method to study the fibril forming potential of peptides and proteins.

ThT (purchased from Sigma Aldrich) stock solution is prepared by dissolving the ThT powder in Milli-Q water and filtered to obtain a 0.25 mM solution. The concentration of the solution is measured at 412 nm using an extinction coefficient of 36 $mM^{-1}cm^{-1}$. Test peptides were dissolved at 1 mg/mL in 25 mM sodium acetate buffer pH 4.0.

100 µL aliquot of peptide solution and 5 µL aliquot of ThT solution were placed in a clear bottom black fluorescence 96-well plate. 5 replicates of each test samples were placed in the same row of the plate. Buffer was placed in control wells for baseline correction. All empty wells were filled with water to prevent evaporation. The plate was sealed with aluminium seal and placed in fluorescence plate reader and incubated for 6 days at 37 °C with intermittent orbital shaking at 500 to 750 rpm. The fluorescence intensity was measured every 30 min using excitation at 444 nm and emission at 480 nm.

The fibril forming potential of the test peptides was determined by measuring the average time taken to detect an increase in baseline corrected fluorescence intensity. A time >144

h indicates no increase in fluorescence intensity, relative to baseline, during the course of the experiment.

Conjugating pramlintide to a lipid (for example, as in SEQ ID NO. 3, 4, 5, 6, 112, 113, increases the fibril-forming tendency as seen in Table 7.

Table 7: Tht fibrillation assay of lipidated pramlintide analogues

Peptide	Time taken to detect increase in fluorescence intensity (h)
3	<5
4	<5
5	7
6	15
8	7
9	7
10	>144
11	>144
12	>144
13	25
15	>144
17	>144
18	>144
19	>144
20	>144
21	>144
24	>144
25	>144
28	45
33	>144
40	>144
44	>144
48	>144
66	>144
70	>144
80	>144
103	<5
104	<5
112	<5
113	<5
114	<5
115	<5
129	>144
156	>144

Example 4: Pharmacokinetic determination via IV and SC Administration in Sprague Dawley Rats

The objective of the pharmacokinetic (PK) studies were to determine the plasma pharmacokinetic profile of lipidated pramlintide analogue peptides in fasted male SD rats after single intravenous (IV) and subcutaneous (SC) administration. PK studies were performed to determine the half-life ($T_{1/2}$) of test peptides. $T_{1/2}$ describes the time taken for the maximum plasma concentration (Cmax) of a test substance to halve its steady-state concentration when in circulation.

Male SD rats were purchased from Si Bei Fu Laboratory Animal Technology Co. Ltd (China). The animals were 6 – 8 weeks old with body weights of 200 – 300 g on the dosing date. The animals were housed in a 12-hour light/12-hour dark cycle environment and were fasted overnight before dosing. The body weight of the animals were recorded before dosing, 24 h and 48h post dosing . Animals had free access to food and drinks, and the food consumption was quantified every day.

Test articles were administered at 20 nmol/kg. Blood samples were collected from each animal via Jugular vein. The sampling timepoints are as below.

Blood Samples per test article;

Group	Route	Animals			Time points
1	**IV**	1	2	3	0, 0.033, 0.1, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48 h
2	**SC**	4	5	6	0, 0.033, 0.1, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 h

The blood samples were transferred into eppendorf low binding tube containing K_2EDTA. Above 0.150 mL blood were collected at each time point. Blood samples were centrifuged at 4,000 g for 5 minutes at 4°C to obtain plasma. The plasma samples were stored frozen at -75±15°C until analysis.

Concentrations of the test articles in the plasma samples were analyzed using a LC-MS/MS method. Data acquisition was performed by LabSolution version 5.89 software (Shimadzu, Kyoto, Japan). Data statistics were performed using Excel 97-2003 software. The pharmacokinetic parameters of test articles were calculated using a non-compartmental approach with Phoenix™ WinNonlin®6.1.

The following pharmacokinetic parameters were calculated, whenever possible from the plasma concentration versus time data:

- IV administration: $T_{1/2}$, C_0, AUC_{last}, AUC_{inf}, MRT_{inf}, Cl, Vss, Number of Points for Regression.
- SC administration: T_{max}, C_{max}, AUC_{last}, AUC_{inf}, MRT_{inf}, F, Number of Points for Regression.

Table 8: Half-life of lipidated pramlintide analogues in rats

	Rat $T_{1/2}$ (h)	
ID	**IV**	**SC**
3	14	-
4	12	12
5	10.5	9.4
6	9.5	17.6
8	4.2	6.2
9	5.9	7.8
10	10.1	8.8
12	9.2	9.7
15	10.4	11.5
18	12	13.6
19	6.6	9.3
20	10	10.8
21	8	8.1
24	9.4	10.5
25	6.9	8
35	5.4	8.1
36	7.2	11.6
38	6.6	9
40	7.4	8.1
41	8.3	10.1
43	9.3	10
44	8.9	11
48	5.6	8.1
64	6	5
65	6	9.3
66	9.4	12.2
69	6.3	8.5
70	10.3	12.5
80	9.7	9.9
112	11.8	19
113	11.2	18.2

114	13	21.6
115	14.9	20.3
117	9.8	12.9
129	10.3	9
140	10.6	12.9
149	6.9	9.3
155	7.7	8.9

Pharmacokinetic studies show that the terminal half-life of amylin in rats is around 13 minutes, and the half-life for pramlintide in human is ~20-45 minutes (Roth JD et. al. GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities. Br J Pharmacol. 2012;166(1):121-136). The lipidated polypeptides show marked improvement in prolonging circulatory T1/2 compared to pramlintide.

Example 6: Rat acute food intake study

Male Sprague Dawley rats were obtained from Taconic Denmark, ApS at approximately 7 weeks of age.. Rats were implanted with a microchip for identification, housed 4/cage with enrichment, free access to food and water, and allowed one week acclimatisation while non-invasive characterization was performed. Rats were on a 12:12 light:dark cycle that switches at 1pm:1am. Food intake was monitored via the HM2 system (Lafayette Instrument) that allows for monitoring in a home cage. As each rat enters an access tunnel to feed, an IR beam is broken, and the implanted microchip is read. Resulting changes to food weight is then assigned to the specific animal. Social order has shown no impact to overall feeding patterns and amounts.

Rats were sorted into groups based on Day -1 body weight and 24-hour accumulated food intake (n=7 per group). On Day 0 rats were weighed, then fasted for 6 hours. Thirty (30) minutes prior to the reintroduction of food, rats were dosed subcutaneously (5mL/kg) with 20 nmol/kg of test compound or 60 nmol/kg peptide 1 (pramlintide) diluted in an appropriate vehicle, after which food was returned, and lights went out. Automated food intake was monitored for the following 3 days, and rats were weighed once per day.

Food intake per rat was batched into 1-hour intervals and integrated into Gubra's GubraView data management system. Discrete food intake data was exported into MS Excel from which cumulative food intake data was generated. Cumulative food intake data was then transposed into GraphPad Prism (v8.0.1) for analysis of dark period feeding

The lipidated polypeptides show marked suppression of food intake compared to pramlintide.

Table 9: Effect of lipidated pramlintide analogues on food uptake in lean rats

Peptide	Cummulative food intake (% vehicle treated intake))		
	At 12h	At 24h	At 48h
1	71.6	85.6	98.7
3	13.4	14.4	30.3
8	28.1	42.7	68.8
9	21.9	30.8	56.5
10	18.8	24.6	46.6
12	9.4	7.5	23.6
15	45.2	49.1	51
18	45.8	46.9	68.8
20	27	26.2	49.7
24	18.7	19.4	40.6
35	49.8	44.8	72
38	6.9	5.5	13.7
40	60.7	51.8	72.4
41	44.6	38.5	58.8
43	60.8	55.6	46.6
44	33.5	39.2	60.7
48	27.5	32.6	57.1
112	15.8	15.9	22.1
113	69.5	65.8	77.9
114	42.3	45.1	53.8
115	42.4	41.3	52.2
116	51.7	43.1	50.2
129	29	32.4	51
140	62.4	54.4	56.5
149	31	31.8	17.5

Claims

1. A polypeptide, or a pharmaceutically acceptable salt thereof, comprising the amino acid sequence:

Xaa (-4) - Xaa (-3) - Xaa (-2) - Xaa (-1) - Xaa 1 - Cys 2 - Asn 3 - Xaa 4 - Ala 5 - Thr 6 - Cys 7 - Ala 8 - Thr 9 - Gln 10 - Arg 11 - Leu 12 - Ala 13 - Xaa 14 - Xaa 15 - Xaa 16 - Xaa 17 - His 18 - Ser 19 - Xaa 20 - Xaa 21 - Xaa 22 - Xaa 23 - Xaa 24 - Xaa 25 - Xaa 26 - Xaa 27 - Xaa 28 - Xaa 29 - Thr 30 - Xaa 31 - Xaa 32 - Xaa 33 - Xaa 34 - Xaa 35 - Xaa 36 - Xaa37 - amide [SEQ ID NO:2], wherein:

Xaa (-4) is Lys(albumin binding moiety) or is absent;

Xaa (-3) is Gly or is absent;

Xaa (-2) is Gly or is absent;

Xaa (-1) is Gly, (albumin binding moiety), Lys(albumin binding moiety) or is absent;

Xaa 1 is Lys, Lys(albumin binding moiety), (albumin binding moiety) or is absent;

Xaa 4 is Thr, Ile or Ala;

Xaa 14 is Asn, His, Glu, 2,4-diaminobutanoic acid (Dab), or an alpha methyl amino acid;

Xaa 15 is Phe or Trp;

Xaa 16 is Leu or *D*-Leu (dL);

Xaa 17 is Val, Ser, Glu, Arg, (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid (Hyp), Dab or an alpha methyl amino acid (*e.g.* 2-amino-2-methylpropanoic acid [Aib]);

Xaa 20 is Ser, Ile, Pro or an alpha methyl amino acid (*e.g. (S)*-2-amino-3-hydroxy-2-methylpropanoic acid [αMeSer]);

Xaa 21 is Asn, Dab, His, Pro, Ser, Arg, Lys, Gly or Glu, Ala, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 22 is Asn, His, Hyp, Dab or an alpha methyl amino acid (*e.g.* Aib);

Xaa 23 is Phe, Hyp or an alpha methyl amino acid (*e.g. (S)*-2-amino-2-methyl-3-phenylpropanoic acid [αMePhe]);

Xaa 24 is Gly, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 25 is Pro, Ala, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 26 is Ile, *D*-Ile (dI), Arg, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 27 is Leu, dL, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 28 is Pro, *D*-Pro (dP), Ser, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 29 is Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 31 is Asn, Glu, His, Arg, Pro, Dab or an alpha methyl amino acid (*e.g.* Aib);

Xaa 32 is Val, Hyp, Dab or an alpha methyl amino acid (*e.g.* Aib);

Xaa 33 is Gly, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 34 is Ser, Pro, His, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 35 is Asn, Pro, Arg, Glu, Dab, Hyp or an alpha methyl amino acid (*e.g.* Aib);

Xaa 36 is Thr, Hyp or an alpha methyl amino acid (*e.g.* Aib); and

Xaa 37 is Tyr, Pro, Hyp or an alpha methyl amino acid (*e.g.* Aib),

and wherein the polypeptide comprises at least one albumin binding moiety.

2. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein the polypeptide comprises Dab, Hyp or an alpha methyl amino acid at least one of positions 14, 17 or 20-37, optionally wherein the alpha methyl amino acid is Aib, αMePhe or αMeSer.

3. The polypeptide or pharmaceutically acceptable salt of claim 1 or 2, wherein Xaa 14 is Dab.

4. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 16 is dL.

5. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 17 is an alpha methyl amino acid, optionally Aib.

6. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 20 is an alpha methyl amino acid, optionally αMeS.

7. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 21 is an alpha methyl amino acid, optionally Aib.

8. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 6, wherein Xaa

21 is Dab.

9. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 22 is an alpha methyl amino acid, optionally Aib.

10. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 23 is an alpha methyl amino acid, optionally αMePhe.

11. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 24 is an alpha methyl amino acid, optionally Aib.

12. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 10, wherein Xaa 24 is Hyp.

13. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 25 is an alpha methyl amino acid, optionally Aib.

14. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 26 is an alpha methyl amino acid, optionally Aib.

15. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 13, wherein Xaa 26 is dI.

16. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 27 is an alpha methyl amino acid, optionally Aib.

17. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 15, wherein Xaa 27 is dL.

18. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 28 is an alpha methyl amino acid, optionally Aib.

19. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 17, wherein Xaa 28 is dP.

20. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims,

wherein Xaa 29 is an alpha methyl amino acid, optionally Aib.

21. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 31 is an alpha methyl amino acid, optionally Aib.

22. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 20, wherein Xaa 31 is Dab.

23. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 32 is an alpha methyl amino acid, optionally Aib.

24. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 33 is an alpha methyl amino acid, optionally Aib.

25. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 34 is an alpha methyl amino acid, optionally Aib.

26. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 35 is an alpha methyl amino acid, optionally Aib.

27. The polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 25, wherein Xaa 35 is Dab.

28. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 36 is an alpha methyl amino acid, optionally Aib.

29. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa 37 is an alpha methyl amino acid, optionally Aib.

30. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein the polypeptide comprises any one of the following combinations of amino acid modifications:

-1G, -2G, 17Aib

4A, 15W, 21P, 24P, 25A, 28S

4I, 20I, 21A, 35R

4I, 21Dab, 35R

14Dab

14Dab, 17Aib, 31E

14Dab, 23αMePhe, 21E

14Dab, 31E

14E, 17Aib

14E, 17Aib, 21H

14E, 17R

14E, 17R, 23αMePhe

14E, 21Aib

14H, 17Aib

14H, 17Aib, 21Aib, 31E

14H, 21Aib

14H, 21Aib, 35E

16dL, 21Aib

17Aib

17Aib, 37P

17Aib, 21Aib

17Aib, 21Aib, 37P

17Aib, 21G

17Aib, 21H

17Aib, 21K

17Aib, 21P

17Aib, 21P, 31E

17Aib, 21P, 35E

17Aib, 21R

17Aib, 21S

17Aib, 21Dab

17Aib, 21Dab, 31E

17Aib, 22H

17Aib, 22H, 35E

17Aib, 23αMePhe

17Aib, 26Aib

17Aib, 26R

17Aib, 27Aib

17Aib, 27dL

17Aib, 28Aib

17Aib, 29Aib

17Aib, 31Aib

17Aib, 31E

17Aib, 31H, 35E

17Aib, 31P

17Aib, 31R

17Aib, 32Aib

17Aib, 33Aib

17Aib, 34Aib

17Aib, 34H

17Aib, 34P

17Aib, 35Aib

17Aib, 35E

17Aib, 35R

17E, 21Aib

17R, 21Aib

17R, 21Aib, 31Aib

17R, 21Aib, 31E

17R, 21Aib, 31R

17R, 21Aib, 35Aib

17R, 23αMePhe

17R, 23αMePhe, 31E

17R, 26Aib

17S, 21Aib

17S, 21Aib, 31H

17S, 21Aib, 31P

17S, 21Aib, 31R

17S, 21Aib, 33P

17S, 21Aib, 35P

20αMeSer

20P, 21P, 24P, 25A, 28S

21Aib

21Aib, 24P, 25A, 28S

21Aib, 24P, 25A, 28S, 31Dab

21Aib, 24P, 25A, 28S, 35Dab

21Aib, 26dI

21Aib, 26Aib

21Aib, 27Aib

21Aib, 27dL

21Aib, 28Aib

21Aib, 28dP

21Aib, 31Aib

21Aib, 31E

21Aib, 31H

21Aib, 31R

21Aib, 33Aib

21Aib, 34Aib

21Aib, 35Aib

21Aib, 35E

21Aib, 35R

21Aib, 36Aib

21Aib, 37Aib

21Aib, 37P

21Dab, 24Hyp, 25A, 28S

21Dab, 24P, 25A, 28S

21Dab, 25Aib

21Dab, 31E

21P, 24P, 25A, 28S

22Aib

22H, 35E

23αMePhe

23αMePhe, 31E

23αMePhe, 31R

23αMePhe, 35R

24Aib

26Aib

27Aib

27dL

28dP

35R

Δ1K, 4I, 21Dab, 35R

Δ1K, 14E, 17R, 23αMePhe.

31. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein:

Xaa (-1) is (albumin binding moiety), Lys(albumin binding moiety) or is absent;

Xaa 4 is Thr;

Xaa 14 is Asn, Glu or Dab;

Xaa 15 is Phe;

Xaa 16 is Leu;

Xaa 17 is Val, Arg or Aib;

Xaa 20 is Ser;

Xaa 21 is Asn -

Xaa 22 is Asn;

Xaa 23 is Phe or αMePhe;

Xaa 24 is Gly;

Xaa 25 is Pro;

Xaa 26 is Ile;

Xaa 27 is Leu;

Xaa 28 is Pro;

Xaa 29 is Pro;

Xaa 31 is Asn, Glu or Arg;

Xaa 32 is Val;

Xaa 33 is Gly;

Xaa 34 is Ser; and

Xaa 35 is Asn or Arg;

Xaa 36 is Thr; and

Xaa 37 is Tyr.

32. The polypeptide or pharmaceutically acceptable salt of claim 31, wherein Xaa 23 is αMePhe.

33. The polypeptide or pharmaceutically acceptable salt of claim 31 or 32, wherein Xaa 14 is Glu.

34. The polypeptide or pharmaceutically acceptable salt of claim 31 or 32, wherein Xaa 14 is Dab.

35. The polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 34, wherein Xaa 17 is Arg.

36. The polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 34, wherein Xaa 17 is Aib.

37. The polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 36, wherein Xaa 31 is Glu.

38. The polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 36, wherein Xaa 31 is Arg.

39. The polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 38, wherein Xaa 35 is Arg.

40. The lipidated polypeptide or pharmaceutically acceptable salt of any one of claims 31 to 39, wherein Xaa(1) is absent.

41. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein:
Xaa (-1) is Gly, Lys(albumin binding moiety) or (albumin binding moiety);
Xaa 4 is Thr;
Xaa 14 is Asn or His;
Xaa 15 is Phe;
Xaa 16 is Leu;
Xaa 17 is Val, Arg or Aib;
Xaa 20 is Ser;
Xaa 21 is Asn, Dab or Aib;
Xaa 22 is Asn;
Xaa 23 is Phe or αMePhe;
Xaa 24 is Gly;
Xaa 25 is Pro;
Xaa 26 is Ile or Aib;
Xaa 27 is Leu or Aib;
Xaa 28 is Pro or Aib;

Xaa 29 is Pro or Aib;

Xaa 31 is Asn, Glu or Aib;

Xaa 32 is Val or Aib;

Xaa 33 is Gly or Aib;

Xaa 34 is Ser or Aib;

Xaa 35 is Asn or Aib;

Xaa 36 is Thr or Aib; and

Xaa 37 is Tyr, Pro or Aib,

wherein the polypeptide comprises at least 2 Aib residues.

42. The polypeptide or pharmaceutically acceptable salt of claim 41, wherein Xaa 17 is Aib.

43. The polypeptide or pharmaceutically acceptable salt of claim 42, wherein at least one of Xaa 21, 26, 27, 28, 29, 31, 32, 33, 34 or 35 is Aib.

44. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 43, wherein Xaa 21 is Aib.

45. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 44, wherein at least one of Xaa 26, 27, 28, 31, 32, 33, 34, 35, 36, or 37 is Aib.

46. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 45, wherein Xaa 14 is His.

47. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 or 43 to 46, wherein Xaa 17 is Arg.

48. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 47, wherein Xaa 31 is Glu.

49. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 47, wherein Xaa 31 is Aib.

50. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 49, wherein Xaa 35 is Aib.

51. The polypeptide or pharmaceutically acceptable salt of any one of claims 41 to 50, wherein Xaa 37 is Pro.

52. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein:
Xaa (-1) is Gly, Lys(albumin binding moiety), or (albumin binding moiety);
Xaa 14 is Asn, Glu or His;
Xaa 15 is Phe
Xaa 17 is Val, Ser, Glu, Arg or Aib;
Xaa 20 is Ser;
Xaa 21 is Asn or Aib;
Xaa 22 is Asn;
Xaa 23 is Phe;
Xaa 24 is Gly, Hyp or Pro;
Xaa 25 is Pro or Ala;
Xaa 26 is Ile, dI, or Aib;
Xaa 27 is Leu, dL, or Aib;
Xaa 28 is Pro, dP, Ser, or Aib;
Xaa 29 is Pro
Xaa 32 is Val;
Xaa 33 is Gly, Pro or Aib;
Xaa 34 is Ser or Aib;
Xaa 35 is Asn, Pro, Arg, Glu, Dab, or Aib;
Xaa 36 is Thr, or Aib; and

Xaa 37 is Tyr, Pro or Aib.

53. The polypeptide or pharmaceutically acceptable salt of claim 52, wherein Xaa 21 is Aib.

54. The polypeptide or pharmaceutically acceptable salt of claim 52 or 53, wherein Xaa 17 is Aib.

55. The polypeptide or pharmaceutically acceptable salt of claim 52 or 53, wherein Xaa 17 is Arg.

56. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 55, wherein Xaa 24 is Pro.

57. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 56, wherein Xaa 25 is Ala.

58. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 57, wherein Xaa 28 is Ser.

59. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 58, wherein Xaa 31 is Glu.

60. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 58, wherein Xaa 31 is Arg.

61. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 58, wherein Xaa 31 is His.

62. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 58, wherein Xaa 31 is Aib.

63. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 62, wherein Xaa 35 is Aib.

64. The polypeptide or pharmaceutically acceptable salt of any one of claims 52 to 62, wherein Xaa 35 is Arg.

65. The polypeptide or pharmaceutically acceptable salt of claim 1, wherein:
Xaa (-1) is (albumin binding moiety), Lys(albumin binding moiety), or is absent;
Xaa 4 is Thr;
Xaa 15 is Phe;
Xaa 16 is Leu;
Xaa 17 is Val or Aib;
Xaa 20 is Ser;
Xaa 21 is Asn, His, Pro, Ser, Arg, Dab, Lys or Gly;
Xaa 22 is Asn or His;
Xaa 23 is Phe;
Xaa 24 is Gly;
Xaa 25 is Pro;
Xaa 26 is Ile or Arg;
Xaa 27 is Leu or dL;
Xaa 28 is Pro;
Xaa 29 is Pro;
Xaa 31 is Asn, Glu, His, Arg or Pro;
Xaa 32 is Val;
Xaa 33 is Gly;
Xaa 34 is Ser, Pro or His;
Xaa 35 is Asn, Glu or Arg;Xaa 36 is Thr; and
Xaa 37 is Tyr.

66. The polypeptide or pharmaceutically acceptable salt of claim 65, wherein Xaa 17 is Aib.

67. The polypeptide or pharmaceutically acceptable salt of claim 65 or 66, wherein Xaa 14 is His.

68. The polypeptide or pharmaceutically acceptable salt of any one of claims 65 to 67, wherein Xaa 21 is Dab.

69. The polypeptide or pharmaceutically acceptable salt of any one of claims 65 to 71, wherein Xaa 31 is Glu.

70. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein Xaa (-4) is Lys(albumin binding moiety), Xaa (-1) is Lys(albumin binding moiety) or (albumin binding moiety), or Xaa 1 is Lys(albumin binding moiety).

71. The polypeptide or pharmaceutically acceptable salt of claim 70, wherein the albumin binding moiety comprises a lipid, optionally wherein the lipid is selected from C12diacid, C14diacid, C16diacid, C17diacid, C18diacid, C19diacid or C20diacid.

72. The polypeptide or pharmaceutically acceptable salt of claim 71, wherein the lipid is C18diacid or C20diacid.

73. The polypeptide or pharmaceutically acceptable salt of any one of the preceding claims, wherein the albumin binding moiety is attached to an amino acid residue of the polypeptide.

74. The polypeptide or pharmaceutically acceptable salt of claim 73, wherein the albumin binding moiety is attached to the amino acid residue by a linker.

75. The polypeptide or pharmaceutically acceptable salt of claim 74, wherein the linker comprises a residue of γ-Glu, optionally wherein the linker comprises γGlu, γGlu-γGlu, γGlu-(O2Oc)-(O2Oc) or γGlu-(PEG2)-(PEG2).

76. A polypeptide selected from the group consisting of:

C18diacid-γE-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide

C18diacid-γE-γE-GGG-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide

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K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Dab)NFPAILSPTNVGSNTY-amide

K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGSNTY-amide

C18diacid-γE-[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide

K(γE-γE-C18diacid)[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide

K(γE-C18diacid)K[CNTATC]ATQRLAEFLRHSSNN(αMePhe)GPILPPTNVGSNTY-amide

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K(γE-C18diacid)K[CNTATC]ATQRLANFLVHSSNHFGPILPPTNVGSETY-amide

C20diacid-γE-K[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSNTY-amide

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K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSSNNFGPILPPTNVGSRTY-amide

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K(γE-γE-C20diacid)[CNTATC]ATQRLAEFLRHSSNNFGPILPPTNVGSNTY-amide

K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGSNTY-amide

K(γE-γE-C20diacid)[CNIATC]ATQRLANFLVHSIANFGPILPPTNVGSRTY-amide

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K(γE-γE-C20diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPT(Dab)VGSNTY-amide

K(γE-γE-C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFPAILSPTNVGS(Dab)TY-amide

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K(C18diacid)[CNTATC]ATQRLANFLVHSS(Aib)NFGPILPPT(Aib)VGSNTY-amide

77. A pharmaceutical composition comprising the polypeptide or pharmaceutically acceptable salt of any one of the preceding claims and a pharmaceutically acceptable excipient.

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78. A method for treating and/or preventing a disease or disorder in a subject comprising

administering the polypeptide or pharmaceutically acceptable salt of any one of the preceding claims or the pharmaceutical composition of claim 77.

79. The method of claim 78, wherein the disease or disorder is obesity, metabolic disease, an obesity-related condition, eating disorder, Alzheimer's disease, hepatic steatosis ("fatty liver"), kidney failure, arteriosclerosis (e.g. atherosclerosis), cardiovascular disease, macrovascular disease, microvascular disease, diabetic heart (including diabetic cardiomyopathy and heart failure as a diabetic complication), coronary heart disease, peripheral artery disease or stroke, cancer, dumping syndrome, hypertension e.g. pulmonary hypertension, or dyslipidemia e.g. atherogenic dyslipidemia, cholescystitis, or short bowel syndrome.

80. The method according to claim 79, wherein the obesity-related condition is overweight, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease, sleep apnea and respiratory problems, hyperlipidemia, degeneration of cartilage, osteoarthritis, or reproductive health complications of obesity or overweight such as infertility.

81. The method according to claim 79, wherein the metabolic disease is diabetes, type 1 diabetes, type 2 diabetes, gestational diabetes, pre-diabetes, insulin resistance, impaired glucose tolerance (IGI), disease states associated with elevated blood glucose levels, metabolic disease including metabolic syndrome, or hyperglycemia *e.g.* abnormal postprandial hyperglycemia.

82. The method of any one of claims 78 to 81, wherein the polypeptide, pharmaceutically acceptable salt or pharmaceutical composition is administered to the subject by subcutaneous injection.

83. The method of any one of claims 78 to 82, wherein the polypeptide, pharmaceutically acceptable salt or pharmaceutical composition is administered to the subject by self-administration.

84. A method for the production of the polypeptide of any one of claims 1 to 76.

85. The method of claim 84, comprising synthesizing the polypeptide by solid-phase or liquid-

phase methodology, and optionally isolating and purifying the final product.

86. An article of manufacture comprising the polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 76 or the pharmaceutical composition of claim 77.

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87. A kit comprising the polypeptide or pharmaceutically acceptable salt of any one of claims 1 to 76 or the pharmaceutical composition of claim 77, optionally further comprising instructions for use.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/086034

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/575 A61P3/10 A61P3/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/225781 A1 (AUCKLAND UNISERVICES LTD [NZ]) 12 November 2020 (2020-11-12) claims 1, 26, 32-43 page 1, lines 3-4 page 7, line 6 - page 8, line 11 page 15, line 18 - page 20, line 7 page 15, lines 15-16 page 32, line 11 - page 33, line 21 page 42, line 18 - page 51, line 11 page 53, lines 4-5	1-87

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2022	18/05/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bladier, Cecile

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/046357 A1 (NOVO NORDISK AS [DK]; SCHAEFFER LAUGE [DK] ET AL.) 29 April 2010 (2010-04-29) claims 9-14 page 14, line 26 - page 15, line 20 page 23, line 4 - page 27, line 30 page 27, line 31 - page 35, line 25 page 35, line 27 - page 37, line 2 page 40 onwards	1-87
X	WO 2007/104789 A2 (NOVO NORDISK AS [DK]; HANSEN THOMAS KRUSE [DK] ET AL.) 20 September 2007 (2007-09-20) claims 1b,8,12,16,22-27 page 6, line 23 - page 7, line 5 page 57 onwards	1-87
A	WO 2016/083499 A1 (NOVO NORDISK AS [DK]) 2 June 2016 (2016-06-02) abstract page 12, line 5 - line 13	1-87
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/086034

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2021/086034

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