

**LONG ACTING GLUCAGON RECEPTOR
SELECTIVE PEPTIDES AND METHODS OF USE**

Cross-Reference to Related Applications

5 This application claims the benefit of U.S. Provisional Application No. 63/011,641, filed April 17, 2020, the disclosure of which is incorporated herein by reference in its entirety.

Field

 The present invention relates to compounds that are glucagon-receptor selective peptides and methods of preparing the same. The invention also provides pharmaceutically acceptable
10 compositions comprising compounds of the present invention and methods of using said compositions in the treatment of various disorders.

Background

 Glucagon is a peptide hormone, produced by alpha cells of the pancreas, which raises the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers
15 the glucose concentration. The pancreas releases glucagon when the concentration of glucose in the bloodstream falls too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.

20 Glucagon and glucagon-like peptide-1 (GLP-1), a neuropeptide, are derived from pre-proglucagon, a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides. These proglucagon-derived peptides which include, for example, glucagon, GLP-1, glucagon-like peptide-2 (GLP-2), and oxyntomodulin (OXM), are involved in a wide variety of physiological functions, including
25 glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. Human glucagon has the amino acid sequence:
HSQGTFTSDYSKYLDNRRAQDFVQWLMNT-OH (SEQ ID NO: 300). There exists a need for therapeutics and therapies that mimic glucagon activity.

Summary

It has now been found that compounds of this invention, and pharmaceutically acceptable compositions thereof, are effective as glucagon-receptor selective peptides (*i.e.*, glucagon analogs). Such compounds have a general formula as follows:

- 5 An isolated polypeptide, comprising the amino acid sequence of SEQ ID NO: 80: X_1 SHGTFTSX₉YSKYLX₁₅X₁₆X₁₇X₁₈AX₂₀X₂₁FX₂₃X₂₄WX₂₆EX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO: 80), or a pharmaceutically acceptable salt thereof, wherein:

- X_1 is Y or acetyl-Y;
 X_9 is D or E;
 10 X_{15} is D, E, or K;
 X_{16} is Aib or K;
 X_{17} is K;
 X_{18} is K, R, or Y;
 X_{20} is E, K, Q, or S;
 15 X_{21} is E or K;
 X_{23} is Aib or V;
 X_{24} is Aib, E, K, Q, or S;
 X_{26} is E, L, or Q;
 X_{28} is D, E, K, N, Q, S, or N-methylglutamate;
 20 Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);

wherein when X_{15} , X_{16} , X_{17} , X_{18} , X_{20} , or X_{21} are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

- 25 provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

- wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at
 30 positions X_{20} and X_{24} or at positions X_{24} and X_{28} .

Further exemplary compounds are provided in Table 3 herein.

Compounds of the present invention have been designed to attain long elimination half-lives ($t_{1/2}$) and are thus described herein as “long-acting” glucagon-receptor selective peptides (*or* glucagon analogs).

Compounds of the present invention, and pharmaceutically acceptable compositions thereof, are useful for treating a variety of diseases, disorders or conditions, associated with the human glucagon receptor. Such diseases, disorders, or conditions include metabolic diseases or disorders such as type 2 diabetes, obesity and the need to attain weight loss. In certain embodiments, the invention also relates to methods of treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH).

Brief Description of the Drawings

Figure 1 depicts a cross-sectional diagram of a representative osmotic mini-pump for drug delivery.

Figure 2 depicts activity of glucagon, exenatide, and compound A6 (SEQ ID NO: 6) against the human, cyno, and rat glucagon and GLP-1 receptors.

Figure 3 depicts the change in plasma concentration of A6 following bolus injection and intravenous infusion.

Detailed Description

General description of certain embodiments of the invention

Compounds of the present invention, and pharmaceutical compositions thereof, are useful as modulators of the glucagon receptor, particularly as modulators of the human glucagon receptor, and more particular as agonists of the human glucagon receptor. This invention also relates to methods of producing and using such compounds, i.e., long-acting glucagon analog polypeptides. These glucagon analog polypeptides are particularly useful in methods of treating metabolic diseases or disorders, such as type 2 diabetes, obesity, and in methods of providing weight loss. In certain embodiments, the invention also relates to methods of treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH).

In certain embodiments, the present disclosure provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 80:

X₁SHGTFTSX₉YSKYLYLX₁₅X₁₆X₁₇X₁₈AX₃₀X₃₁FX₂₃X₂₄WX₂₆EX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO: 80), or a pharmaceutically acceptable salt thereof.

Definitions

It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a solvent” includes a combination of two or more such solvents, reference to “a peptide” includes one or more peptides, or mixtures of peptides, reference to “a drug” includes one or more drugs, reference to “an osmotic delivery device” includes one or more osmotic delivery devices, and the like. Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive and covers both “or” and “and”.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term “about.”

Unless specifically stated or obvious from context, as used herein, the term “substantially” is understood as within a narrow range of variation or otherwise normal tolerance in the art. Substantially can be understood as within 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01% or 0.001% of the stated value.

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 the invention pertains. Although other methods and materials similar, or equivalent, to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

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

The terms “drug,” “therapeutic agent,” and “beneficial agent” are used interchangeably to refer to any therapeutically active substance that is delivered to a subject to produce a desired

beneficial effect. In one embodiment of the present invention, the drug is a polypeptide. In another embodiment of the present invention, the drug is a small molecule, for example, hormones such as androgens or estrogens. The devices and methods of the present invention are well suited for the delivery of proteins, small molecules and combinations thereof.

5 The terms "peptide," "polypeptide," and "protein" are used interchangeably herein and typically refer to a molecule comprising a chain of two or more amino acids (e.g., most typically L-amino acids, but also including, e.g., D-amino acids, modified amino acids, amino acid analogs, and amino acid mimetics).

In some embodiments, naturally-occurring L-amino acids, are represented by either
10 conventional three-letter, or capitalized one-letter, amino acid designations of Table 1. In other embodiments, naturally-occurring L-amino acids and D-amino acids, are both represented by either conventional three-letter, or capitalized one-letter, amino acid designations of Table 1. In still other embodiments, D-amino acids, are represented by lower-case one-letter amino acid designations corresponding to one-letter designations of Table 2, *i.e.*, a, l, m, f, w, k, q, e, s, p, v,
15 i, c, y, h, r, n, d, and t.

Table 1: Naturally-occurring amino acids

G	Glycine	Gly	P	Proline	Pro
A	Alanine	Ala	V	Valine	Val
L	Leucine	Leu	I	Isoleucine	Ile
M	Methionine	Met	C	Cysteine	Cys
F	Phenylalanine	Phe	Y	Tyrosine	Tyr
W	Tryptophan	Trp	H	Histidine	His
K	Lysine	Lys	R	Arginine	Arg
Q	Glutamine	Gln	N	Asparagine	Asn
E	Glutamic Acid	Glu	D	Aspartic Acid	Asp
S	Serine	Ser	T	Threonine	Thr

Table 2: Lower case designations refer to *D* stereoisomers of amino acids

			p	Proline	<i>D</i> -Pro
a	<i>D</i> -Alanine	<i>D</i> -Ala	v	<i>D</i> -Valine	<i>D</i> -Val
l	<i>D</i> -Leucine	<i>D</i> -Leu	i	<i>D</i> -Isoleucine	<i>D</i> -Ile
m	<i>D</i> -Methionine	<i>D</i> -Met	c	<i>D</i> -Cysteine	<i>D</i> -Cys
f	<i>D</i> -Phenylalanine	<i>D</i> -Phe	y	<i>D</i> -Tyrosine	<i>D</i> -Tyr

w	<i>D</i> -Tryptophan	<i>D</i> -Trp	h	<i>D</i> -Histidine	<i>D</i> -His
k	<i>D</i> -Lysine	<i>D</i> -Lys	r	<i>D</i> -Arginine	<i>D</i> -Arg
q	<i>D</i> -Glutamine	<i>D</i> -Gln	n	<i>D</i> -Asparagine	<i>D</i> -Asn
e	<i>D</i> -Glutamic Acid	<i>D</i> -Glu	d	<i>D</i> -Aspartic Acid	<i>D</i> -Asp
s	<i>D</i> -Serine	<i>D</i> -Ser	t	<i>D</i> -Threonine	<i>D</i> -Thr

Peptides may be naturally occurring, synthetically produced, or recombinantly expressed. Peptides may also comprise additional groups modifying the amino acid chain, for example, functional groups added via post-translational modification. Examples of post-translation
5 modifications include, but are not limited to, acetylation, alkylation (including, methylation), biotinylation, glutamylation, glycylation, glycosylation, isoprenylation, lipoylation, phosphopantetheinylation, phosphorylation, selenation, and C-terminal amidation. The term peptide also includes peptides comprising modifications of the amino terminus and/or the carboxy terminus. Modifications of the terminal amino group include, but are not limited to, des-amino, N-
10 lower alkyl, N-di-lower alkyl, and N-acyl modifications. Modifications of the terminal carboxy group include, but are not limited to, amide, lower alkyl amide, dialkyl amide, and lower alkyl ester modifications (e.g., wherein lower alkyl is C₁-C₄ alkyl). The term peptide also includes modifications, such as but not limited to those described above, of amino acids falling between the amino and carboxy termini. In one embodiment, a peptide may be modified by addition of a small-
15 molecule drug.

The terminal amino acid at one end of the peptide chain typically has a free amino group (i.e., the amino terminus). The terminal amino acid at the other end of the chain typically has a free carboxyl group (i.e., the carboxy terminus). Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminus and increasing in the direction of the carboxy
20 terminus of the peptide.

The phrase “amino acid residue” as used herein refers to an amino acid that is incorporated into a peptide by an amide bond or an amide bond mimetic.

The term “insulinotropic” as used herein typically refers to the ability of a compound, e.g., a peptide, to stimulate or affect the production and/or activity of insulin (e.g., an insulinotropic hormone). Such compounds typically stimulate or otherwise affect the secretion or biosynthesis of
25 insulin in a subject. Thus, an “insulinotropic peptide” is an amino acid-containing molecule capable of stimulating or otherwise affecting secretion or biosynthesis of insulin.

The term "acylated" as used herein, in relation to disclosed polypeptides, means the disclosed polypeptide is substituted with one or more lipophilic substituents each optionally via a spacer, wherein "lipophilic substituent" and "spacer" are defined herein. Certain lipophilic substituents, each optionally via a spacer, can bind albumin and confer affinity to albumin to the resulting acylated polypeptide. The extent is variable, and depending on numerous factors, to which lipophilic substituents, each optionally via a spacer, bind albumin and confer affinity to albumin to the resulting acylated polypeptide. Numerous factors include identities of the lipophilic substituent, optional spacer, polypeptide, and the site of covalent attachment to the polypeptide.

The terms "linear" or "linear polypeptide" as used herein, refer to a "non-acylated" polypeptide, in other words, a disclosed glucagon analog polypeptide without a lipophilic substituents each optionally via a spacer, wherein "lipophilic substituent" and "spacer" are defined herein.

The terms "conjugated" or "conjugated polypeptide" as used herein, refer to an "acylated" polypeptide, in other words, a disclosed glucagon analog polypeptide having one or more lipophilic substituents each optionally via a spacer, wherein "lipophilic substituent" and "spacer" are defined herein.

As used herein, the term "pharmaceutically acceptable salts" refers to derivatives of the disclosed polypeptides wherein the parent polypeptide is modified by converting an existing acid or base moiety to its salt form. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and Journal of Pharmaceutical Science, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

The term "vehicle" as used herein refers to a medium used to carry a compound, e.g., a drug or a particle containing a drug. Vehicles of the present invention typically comprise components such as polymers and solvents. The suspension vehicles of the present invention typically comprise solvents and polymers that are used to prepare suspension formulations further comprising drug particle formulations.

The phrase "phase separation" as used herein refers to the formation of multiple phases (e.g., liquid and gel phases) in the suspension vehicle, such as when the suspension vehicle contacts the aqueous environment. In some embodiments of the present invention, the suspension vehicle

is formulated to exhibit phase separation upon contact with an aqueous environment having less than approximately 10% water.

The phrase "single-phase" as used herein refers to a solid, semisolid, or liquid homogeneous system that is physically and chemically uniform throughout.

5 The term "dispersed" as used herein refers to dissolving, dispersing, suspending, or otherwise distributing a compound, for example, a drug particle formulation, in a suspension vehicle.

10 The phrase "chemically stable" as used herein refers to formation in a formulation of an acceptable percentage of degradation products produced over a defined period of time by chemical pathways, such as deamidation (usually by hydrolysis), aggregation, or oxidation.

The phrase "physically stable" as used herein refers to formation in a formulation of an acceptable percentage of aggregates (e.g., dimers and other higher molecular weight products). Further, a physically stable formulation does not change its physical state as, for example, from liquid to solid, or from amorphous to crystal form.

15 The term "viscosity" as used herein typically refers to a value determined from the ratio of shear stress to shear rate (see, e.g., Considine, D. M. & Considine, G. D., Encyclopedia of Chemistry, 4th Edition, Van Nostrand, Reinhold, N.Y., 1984) essentially as follows:

$$F/A = \mu * V/L \quad \text{(Equation 1)}$$

where F/A =shear stress (force per unit area),

20 μ =a proportionality constant (viscosity), and

V/L =the velocity per layer thickness (shear rate).

From this relationship, the ratio of shear stress to shear rate defines viscosity. Measurements of shear stress and shear rate are typically determined using parallel plate rheometry performed under selected conditions (for example, a temperature of about 37° C.). Other methods
25 for the determination of viscosity include, measurement of a kinematic viscosity using viscometers, for example, a Cannon-Fenske viscometer, an Ubbelohde viscometer for the Cannon-Fenske opaque solution, or an Ostwald viscometer. Generally, suspension vehicles of the present invention have a viscosity sufficient to prevent a particle formulation suspended therein from settling during storage and use in a method of delivery, for example, in an implantable, drug
30 delivery device.

The term "non-aqueous" as used herein refers to an overall moisture content, for example, of a suspension formulation, typically of less than or equal to about 10 wt %, for example, less than or equal to about 7 wt %, less than or equal to about 5 wt %, and/or less than about 4 wt %. Also, a particle formulation of the present invention comprises less than about 10 wt %, for example, less than about 5 wt %, residual moisture.

The term "subject" as used herein refers to any member of the subphylum Chordata, including, without limitation, humans and other primates, including non-human primates such as rhesus macaques and other monkey species and chimpanzees and other ape species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age or gender. Thus, both adult and newborn individuals are intended to be covered.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

The term "osmotic delivery device" as used herein typically refers to a device used for delivery of a drug (e.g., a disclosed glucagon analog polypeptide) to a subject, wherein the device comprises, for example, a reservoir (made, e.g., from a titanium alloy) having a lumen that contains a suspension formulation comprising a drug (e.g., a disclosed glucagon analog polypeptide) and an osmotic agent formulation. A piston assembly positioned in the lumen isolates the suspension formulation from the osmotic agent formulation. A semi-permeable membrane is positioned at a first distal end of the reservoir adjacent the osmotic agent formulation and a diffusion moderator (which defines a delivery orifice through which the suspension formulation exits the device) is positioned at a second distal end of the reservoir adjacent the suspension formulation. Typically, the osmotic delivery device is implanted within the subject, for example, subdermally or

subcutaneously (e.g., in the inside, outside, or back of the upper arm and in the abdominal area). An exemplary osmotic delivery device is the DUROS® (ALZA Corporation, Mountain View, Calif.) delivery device. Examples of terms synonymous to “osmotic delivery device” include but are not limited to “osmotic drug delivery device”, “osmotic drug delivery system”, “osmotic device”, “osmotic delivery device”, “osmotic delivery system”, “osmotic pump”, “implantable drug delivery device”, “drug delivery system”, “drug delivery device”, “implantable osmotic pump”, “implantable drug delivery system”, and “implantable delivery system”. Other terms for “osmotic delivery device” are known in the art.

The term “continuous delivery” as used herein typically refers to a substantially continuous release of drug from an osmotic delivery device and into tissues near the implantation site, e.g., subdermal and subcutaneous tissues. For example, an osmotic delivery device releases drug essentially at a predetermined rate based on the principle of osmosis. Extracellular fluid enters the osmotic delivery device through the semi-permeable membrane directly into the osmotic engine that expands to drive the piston at a slow and consistent rate of travel. Movement of the piston forces the drug formulation to be released through the orifice of the diffusion moderator. Thus, release of the drug from the osmotic delivery device is at a slow, controlled, consistent rate.

The term “substantial steady-state delivery” as used herein typically refers to delivery of a drug at or near a target concentration over a defined period of time, wherein the amount of the drug being delivered from an osmotic delivery device is substantially zero-order delivery. Substantial zero-order delivery of an active agent (e.g., a disclosed glucagon analog polypeptide) means that the rate of drug delivered is constant and is independent of the drug available in the delivery system; for example, for zero-order delivery, if the rate of drug delivered is graphed against time and a line is fitted to the data the line has a slope of approximately zero, as determined by standard methods (e.g., linear regression).

The phrase “drug half-life” as used herein refers how long it takes a drug to be eliminated from blood plasma by one half of its concentration. A drug’s half-life is usually measured by monitoring how a drug degrades when it is administered via injection or intravenously. A drug is usually detected using, for example, a radioimmunoassay (RIA), a chromatographic method, an electrochemiluminescent (ECL) assay, an enzyme linked immunosorbent assay (ELISA) or an immunoenzymatic sandwich assay (IEMA).

The terms “μg” and “meg” and “ug” are understood to mean “micrograms”. Similarly, the terms “μl” and “uL” are understood to mean “microliter”, and the terms “μM” and “uM” are understood to mean “micromolar”.

The term “serum” is meant to mean any blood product from which a substance can be detected. Thus, the term serum includes at least whole blood, serum, and plasma. For example, “an amount of [a substance] in a subject’s serum” would cover “an amount of [the substance] in a subject’s plasma”.

Baseline is defined as the last assessment on or before the day of the initial placement of an osmotic delivery device (containing drug or placebo).

Exemplary compounds: glucagon analog polypeptides

Certain disclosed glucagon analog polypeptides, including those of Table 3 below, exhibit one or more of: excellent solubility, stability, bioavailability, biological activity and specificity, and longer half-lives than those for endogenous glucagon and known glucagon analogs. Certain disclosed glucagon analog polypeptides were developed to accommodate less frequent administration than is required for known glucagon analogs. Certain disclosed glucagon analog polypeptides were developed for administration via weekly or monthly injections. Certain disclosed glucagon analog polypeptides were developed for administration via implantation of a delivery device comprising the glucagon analog polypeptide, where the delivery device comprises a dose of the glucagon analog polypeptide of up to 3 months, 6 months, 9 months, one year, 18 months or two years.

In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of the following peptides listed in Table 3:

Table 3: Exemplary compounds: glucagon analog polypeptides covalently bound to a lipophilic substituent, optionally via a spacer.

Compound No.	Sequence	SEQ ID NO:
A1	YSHGTFTSDYSKYLK(γGlu-γGlu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)(Aib) KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 1

Compound No.	Sequence	SEQ ID NO:
A2	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 2
A3	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)Y AQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 3
A4	YSHGTFTSDYSKYLD(Aib)KK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)AQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 4
A5	YSHGTFTSDYSKYLD(Aib)KYAK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)EFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 5
A6	YSHGTFTSDYSKYLD(Aib)KYAQK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 6
A7	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEF(Aib)QWLEDEPSSGAPPPS(OH)	SEQ ID NO: 7
A8	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEF(Aib)EWLEDEPSSGAPPPS(OH)	SEQ ID NO: 8
A9	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEF(Aib)KWLEDEPSSGAPPPS(OH)	SEQ ID NO: 9
A10	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEF(Aib)SWLEDEPSSGAPPPS(OH)	SEQ ID NO: 10
A11	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAK*EFVE*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 11
A12	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*EFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 12
A13	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 13
A14	YSHGTFTSDYSKYLD(Aib)KYAQK(γ Glu- γ Glu-CO(CH ₂) ₁₈ CO ₂ H)FV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID

Compound No.	Sequence	SEQ ID NO:
		NO: 14
A15	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLEEEPPSSGAPPPS(OH)	SEQ ID NO: 15
A16	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLENEPSSGAPPPS(OH)	SEQ ID NO: 16
A17	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLEQEPSSGAPPPS(OH)	SEQ ID NO: 17
A18	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLE(N-Me-Glu)EPSSGAPPPS(OH)	SEQ ID NO: 18
A19	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 19
A20	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQEFV(Aib)WLEKEPSSGAPPPS(OH)	SEQ ID NO: 20
A21	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 21
A22	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAKEFV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 22
A23	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAEEFV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 23
A24	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYASEFV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 24
A25	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEEEPPSSGAPPPS(OH)	SEQ ID NO: 25
A26	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEKEPSSGAPPPS(OH)	SEQ ID NO: 26

Compound No.	Sequence	SEQ ID NO:
A27	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLENEPSSGAPPPS(OH)	SEQ ID NO: 27
A28	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLESEPSGAPPPS(OH)	SEQ ID NO: 28
A29	YSHGTFTSDYSKYLD(Aib)KYAQK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 29
A30	YSHGTFTSDYSKYLD(Aib)KYAQK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FVK*WLED*EPSSGAPPPS(OH)	SEQ ID NO: 30
A31	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGEPPPS(OH)	SEQ ID NO: 31
A32	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGKPPPS(OH)	SEQ ID NO: 32
A33	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGSPPPS(OH)	SEQ ID NO: 33
A34	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 34
A35	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDE(NH ₂)	SEQ ID NO: 35
A36	Acetyl-YSHGTFTSDYSKYLD(Aib)KYAQK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 36
A37	Acetyl-YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 37
A38	YSHGTFTSDYSKYLD(Aib)KYAEK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 38
A39	YSHGTFTSDYSKYLD(Aib)KYAKK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)FVK*WLEE*EPSSGAPPPS(OH)	SEQ ID

Compound No.	Sequence	SEQ ID NO:
		NO: 39
A40	Acetyl-YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 40
A41	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 41
A42	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WEEDEPSSGAPPPS(OH)	SEQ ID NO: 42
A43	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WQEDPSSGAPPPS(OH)	SEQ ID NO: 43
A44	YSHGTFTSDYSKYLEK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 44
A45	YSHGTFTSEYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 45
A46	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAEQFV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 46
A47	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAEQFV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 47
A48	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 48
A49	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)RAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 49
A50	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KAQEFV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 50
A51	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAE*EFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 51

Compound No.	Sequence	SEQ ID NO:
A52	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAK*KFVE*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 52
A53	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAQEFV(Aib)WLEDE(NH ₂)	SEQ ID NO: 53
A54	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAQEFV(Aib)WLEDE(OH)	SEQ ID NO: 54
A55	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAE*EFVK*WLEDE(NH ₂)	SEQ ID NO: 55
A56	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAE*EFVK*WLEDE(OH)	SEQ ID NO: 56
A57	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAE*KFVK*WLEDE(NH ₂)	SEQ ID NO: 57
A58	YSHGTFTSDYSKYLD(Aib)K(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)YAE*KFVK*WLEDE(OH)	SEQ ID NO: 58
A59	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQKFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 59
A60	YSHGTFTSDYSKYLDK(γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)KYAQKFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 60

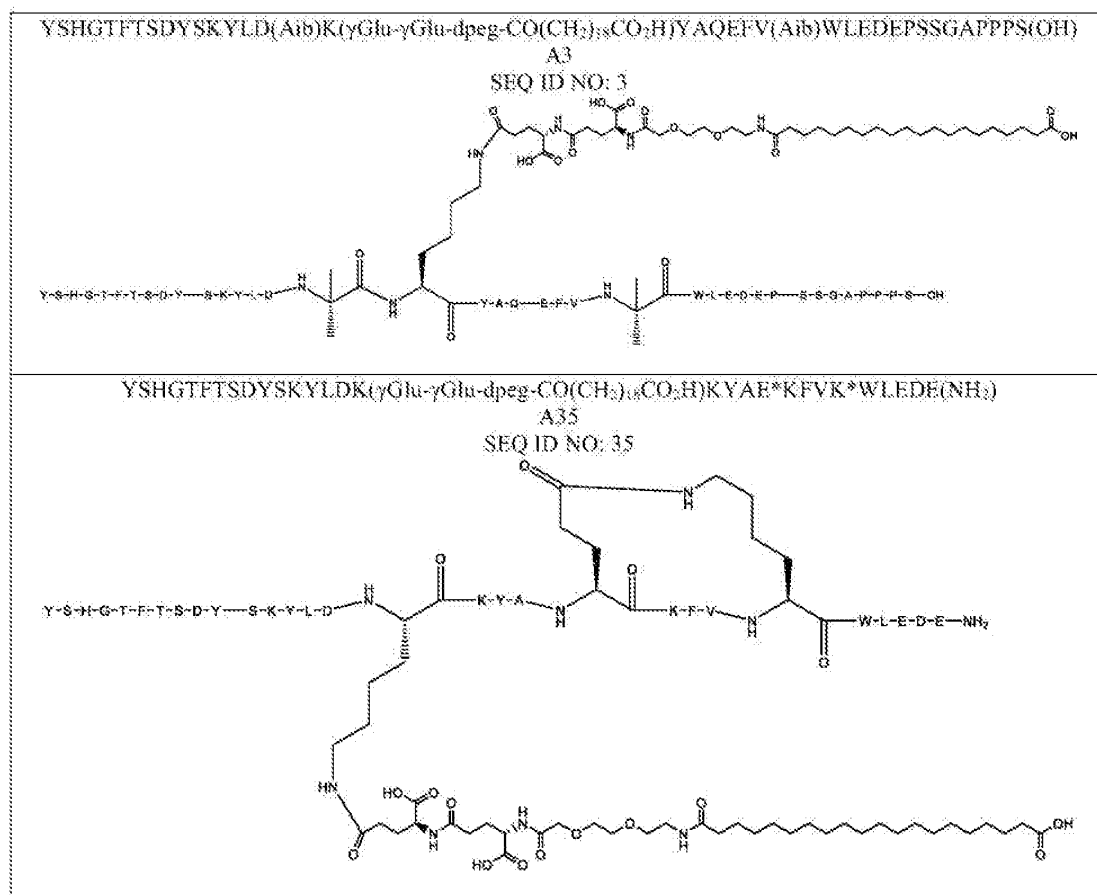
Aib is 2-Aminoisobutyric acid

Each pairing of K* and E* and each pairing of K* and D* represent a covalent amide linkage derived from the amino sidechain of K* and the carboxy sidechain of E* or the carboxy sidechain of D* (with loss of a water molecule).

Structural representations of certain peptides of Table 3 are provided below in Table 4:

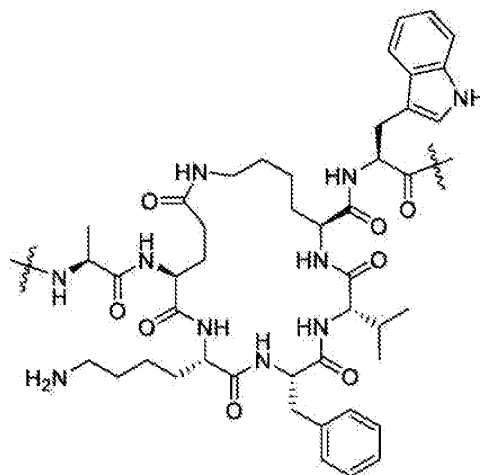
Table 4: Chemical structures of exemplary peptides: glucagon analog polypeptides comprising a lipophilic substituent via a spacer, two peptides of which further comprise a bridging moiety.

YSHGTFTSDYSKYLD(Aib)KYAQK(γGlu-γGlu-dpeg-CO(CH₂)₁₈CO₂H)FV(Aib)WLEDEPSSGAPPPS(OH) A6 SEQ ID NO: 6
YSHGTFTSDYSKYLDK(γGlu-γGlu-dpeg-CO(CH₂)₁₈CO₂H)KYAE*KFVK*WLEDEPSSGAPPPS(OH) A21 SEQ ID NO: 21
YSHGTFTSDYSKYLDK(γGlu-γGlu-dpeg-CO(CH₂)₁₈CO₂H)KYASEFV(Aib)WLESEPPSSGAPPPS(OH) A24 SEQ ID NO: 24



Notes:

- each pairing of K* and E* and each pairing of K* and D* represent a covalent amide linkage derived from the amino sidechain of K* and the carboxy sidechain of E* or the carboxy sidechain of D* (with loss of a water molecule). For example, the segment -AE*KFVK*W- represents:



- as used herein, *dpeg* represents $-\text{COCH}_2\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{NH}-$; and *dpeg-dpeg* represents $-\text{COCH}_2\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{NH}-\text{COCH}_2\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{NH}-$;
- amino terminal amino acid, *i.e.* Y_1 , shown as Acetyl- Y_1 -, depicts $\text{CH}_3\text{C}(\text{O})-\text{NH}-\text{CH}(\text{CH}_2-(p\text{-hydroxyphenyl}))- \text{C}(\text{O})-$;
- carboxy terminal amino acid, *i.e.* S_{38} , shown as $-\text{S}_{38}(\text{NH}_2)$, depicts $-\text{NH}-\text{CH}(\text{CH}_2\text{OH})-\text{CONH}_2$; and
- carboxy terminal amino acid, *i.e.* S_{38} , shown as $-\text{S}_{38}(\text{OH})$, depicts $-\text{NH}-\text{CH}(\text{CH}_2\text{OH})-\text{COOH}$.

In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 60, or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 39, or a pharmaceutically acceptable salt thereof.

In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 21, and 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated

polypeptide comprises an amino acid sequence of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the present invention provides a compound set forth in Table 3, above, or a pharmaceutically acceptable salt thereof. In some embodiments, the present invention provides an isolated polypeptide that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 60 or a pharmaceutically acceptable salt thereof. In some embodiments, the present invention provides an isolated polypeptide that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 39 or a pharmaceutically acceptable salt thereof. In some embodiments the pharmaceutically acceptable salt is an acetate salt. In some embodiments the pharmaceutically acceptable salt is a trifluoroacetic acid (TFA) salt. In some embodiments the pharmaceutically acceptable salt is a hydrochloric acid (HCl) salt.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 3. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 3. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 3.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 6. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 6. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 6.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 21. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 21. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 21.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof. In some embodiments,

the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 24. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 24. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 24.

- 5 In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of the following peptides listed in Table 5:

Table 5: Exemplary compounds; glucagon analog polypeptides optionally comprising a lipophilic substituent, optionally via a spacer; and optionally further comprising a bridging moiety.

Compound No.	Sequence	SEQ ID NO:
B1	YSHGTFTSDYSKYLK(Aib)KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 101
B2	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 102
B3	YSHGTFTSDYSKYLD(Aib)KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 103
B4	YSHGTFTSDYSKYLD(Aib)KKAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 104
B5	YSHGTFTSDYSKYLD(Aib)KYAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 105
B6	YSHGTFTSDYSKYLD(Aib)KYAQKFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 106
B7	YSHGTFTSDYSKYLDKKY AQEF(Aib)QWLEDEPSSGAPPPS(OH)	SEQ ID NO: 107
B8	YSHGTFTSDYSKYLDKKY AQEF(Aib)EWLEDEPSSGAPPPS(OH)	SEQ ID NO: 108
B9	YSHGTFTSDYSKYLDKKY AQEF(Aib)KWLEDEPSSGAPPPS(OH)	SEQ ID NO: 109
B10	YSHGTFTSDYSKYLDKKY AQEF(Aib)SWLEDEPSSGAPPPS(OH)	SEQ ID NO: 110
B11	YSHGTFTSDYSKYLDKKY AKEFVWLEDEPSSGAPPPS(OH)	SEQ ID NO: 111
B12	YSHGTFTSDYSKYLDKKY AEEFVKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 112
B15	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLEEPSSGAPPPS(OH)	SEQ ID NO: 115
B16	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLENEPSSGAPPPS(OH)	SEQ ID NO: 116
B17	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLEQEPSSGAPPPS(OH)	SEQ ID NO: 117
B18	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLE(N-Me-Glu)EPSSGAPPPS(OH)	SEQ ID NO: 118
B19	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 119
B20	YSHGTFTSDYSKYLDKKY AQEFV(Aib)WLEKEPSSGAPPPS(OH)	SEQ ID NO: 120

Compound No.	Sequence	SEQ ID NO:
B21	YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 121
B22	YSHGTFTSDYSKYLDKKYAEKFEV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 122
B23	YSHGTFTSDYSKYLDKKYAEFEV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 123
B24	YSHGTFTSDYSKYLDKKYAEFEV(Aib)WLESEPSSGAPPPS(OH)	SEQ ID NO: 124
B25	YSHGTFTSDYSKYLDKKYAEKFKWLEEEPSSGAPPPS(OH)	SEQ ID NO: 125
B26	YSHGTFTSDYSKYLDKKYAEKFKWLEKEPSSGAPPPS(OH)	SEQ ID NO: 126
B27	YSHGTFTSDYSKYLDKKYAEKFKWLENEPSSGAPPPS(OH)	SEQ ID NO: 127
B28	YSHGTFTSDYSKYLDKKYAEKFKWLESEPSSGAPPPS(OH)	SEQ ID NO: 128
B29	YSHGTFTSDYSKYLD(Aib)KYAQKFKWLEEEPSSGAPPPS(OH)	SEQ ID NO: 129
B30	YSHGTFTSDYSKYLD(Aib)KYAQKFKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 130
B31	YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGEPPPS(OH)	SEQ ID NO: 131
B32	YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGKPPPS(OH)	SEQ ID NO: 132
B33	YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSQSPPPS(OH)	SEQ ID NO: 133
B34	YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 134
B35	YSHGTFTSDYSKYLDKKYAEKFKWLEDE(NH ₂)	SEQ ID NO: 135
B36	Acetyl-YSHGTFTSDYSKYLD(Aib)KYAQKFKWLEEEPSSGAPPPS(OH)	SEQ ID NO: 136
B37	Acetyl-YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 137
B38	YSHGTFTSDYSKYLD(Aib)KYAEKFKWLEEEPSSGAPPPS(OH)	SEQ ID NO: 138
B39	YSHGTFTSDYSKYLD(Aib)KYAKKFKWLEEEPSSGAPPPS(OH)	SEQ ID NO: 139
B40	Acetyl-YSHGTFTSDYSKYLDKKYAEKFKWLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 140
B42	YSHGTFTSDYSKYLDKKYAEKFKWEEDEPSSGAPPPS(OH)	SEQ ID NO: 142
B43	YSHGTFTSDYSKYLDKKYAEKFKWQEDEPSSGAPPPS(OH)	SEQ ID NO: 143
B44	YSHGTFTSDYSKYLEKKYAEKFKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 144
B45	YSHGTFTSEYSKYLDKKYAEKFKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 145
B46	YSHGTFTSDYSKYLD(Aib)KYAQFEV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 146

Compound No.	Sequence	SEQ ID NO:
B47	YSHGTFTSDYSKYLD(Aib)KYAQKFV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 147
B49	YSHGTFTSDYSKYLD(Aib)KRAQEFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 149
B50	YSHGTFTSDYSKYLD(Aib)KKAQEFV(Aib)WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 150
B51	YSHGTFTSDYSKYLD(Aib)KYAEEFVKWLEDEPSSGAPPPS(OH)	SEQ ID NO: 151
B52	YSHGTFTSDYSKYLD(Aib)KYAKKFVEWLEDEPSSGAPPPS(OH)	SEQ ID NO: 152
B53	YSHGTFTSDYSKYLD(Aib)KYAQEFV(Aib)WLEDE(NH ₂)	SEQ ID NO: 153
B54	YSHGTFTSDYSKYLD(Aib)KYAQEFV(Aib)WLEDE(OH)	SEQ ID NO: 154
B55	YSHGTFTSDYSKYLD(Aib)KYAEEFVKWLEDE(NH ₂)	SEQ ID NO: 155
B56	YSHGTFTSDYSKYLD(Aib)KYAEEFVKWLEDE(OH)	SEQ ID NO: 156
B57	YSHGTFTSDYSKYLD(Aib)KYAEKFVKWLEDE(NH ₂)	SEQ ID NO: 157
B58	YSHGTFTSDYSKYLD(Aib)KYAEKFVKWLEDE(OH)	SEQ ID NO: 158
B59	YSHGTFTSDYSKYLDKKY AQKFV(Aib)WLEDEPSSGAPPPS(OH)	SEQ ID NO: 159
B60	YSHGTFTSDYSKYLDKKY AQKFV(Aib)WLEEEPPSSGAPPPS(OH)	SEQ ID NO: 160

In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142-147, and 149-160, or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 101 to 112 and 115 to 139, or a pharmaceutically acceptable salt thereof.

In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103, 106, 121, and 124 or a pharmaceutically acceptable salt thereof.

In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 103 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 106 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 121 or a pharmaceutically acceptable salt thereof. In some embodiments,

the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 124 or a pharmaceutically acceptable salt thereof.

In some embodiments, the present invention provides a compound set forth in the Table 5, above, or a pharmaceutically acceptable salt thereof. In some embodiments, the present invention provides an isolated polypeptide that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, and 149 to 160, or a pharmaceutically acceptable salt thereof. In some embodiments, the present invention provides an isolated polypeptide that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 101 to 112 and 115 to 139 or a pharmaceutically acceptable salt thereof. In some
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embodiments the pharmaceutically acceptable salt is an acetate salt. In some embodiments the pharmaceutically acceptable salt is a trifluoroacetic acid (TFA) salt. In some embodiments the pharmaceutically acceptable salt is a hydrochloric acid (HCl) salt.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 103 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of
15
SEQ ID NO: 103. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 103. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 103.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid
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sequence of SEQ ID NO: 106 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 106. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 106. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 106.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid
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sequence of SEQ ID NO: 121 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 121. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 121. In some embodiments, the compound is
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the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 121.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 124 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 124. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 124. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 124.

In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of the following peptides listed in Table 6:

Table 6: Exemplary compounds: glucagon analog polypeptides optionally covalently bound to a lipophilic substituent, optionally via a spacer

Compound No.	Sequence	SEQ ID NO:
C11	YSHGTFTSDYSKYLDKKYAK*EFVE*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 211
C12	YSHGTFTSDYSKYLDKKYAE*EFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 212
C21	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 221
C25	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEEPSSGAPPPS(OH)	SEQ ID NO: 225
C26	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEKEPSSGAPPPS(OH)	SEQ ID NO: 226
C27	YSHGTFTSDYSKYLDKKYAE*KFVK*WLENEPSSGAPPPS(OH)	SEQ ID NO: 227
C28	YSHGTFTSDYSKYLDKKYAE*KFVK*WLESEPSSGAPPPS(OH)	SEQ ID NO: 228
C29	YSHGTFTSDYSKYLD(Aib)KYAQKFVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 229
C30	YSHGTFTSDYSKYLD(Aib)KYAQKFVK*WLED*EPSSGAPPPS(OH)	SEQ ID NO: 230
C31	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGEPPPS(OH)	SEQ ID NO: 231
C32	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGKPPPS(OH)	SEQ ID NO: 232
C33	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGSPPPS(OH)	SEQ ID NO: 233
C34	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 234
C35	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDE(NH ₂)	SEQ ID NO: 235
C38	YSHGTFTSDYSKYLD(Aib)KYAEKFVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 238
C39	YSHGTFTSDYSKYLD(Aib)KYAKKFVK*WLEE*EPSSGAPPPS(OH)	SEQ ID NO: 239

Compound No.	Sequence	SEQ ID NO:
C40	Acetyl-YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 240
C41	YSHGTFTSDYSKYLDKKYAE*KFVK*WLEDEPSSGAPPPS(NH ₂)	SEQ ID NO: 241
C42	YSHGTFTSDYSKYLDKKYAE*KFVK*WEEDEPSSGAPPPS(OH)	SEQ ID NO: 242
C43	YSHGTFTSDYSKYLDKKYAE*KFVK*WQEDEPSSGAPPPS(OH)	SEQ ID NO: 243
C44	YSHGTFTSDYSKYLEKKYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 244
C45	YSHGTFTSEYSKYLDKKYAE*KFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 245
C51	YSHGTFTSDYSKYLD(Aib)KYAE*EFVK*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 251
C52	YSHGTFTSDYSKYLD(Aib)KYAK*KFVE*WLEDEPSSGAPPPS(OH)	SEQ ID NO: 252
C55	YSHGTFTSDYSKYLD(Aib)KYAE*EFVK*WLEDE(NH ₂)	SEQ ID NO: 255
C56	YSHGTFTSDYSKYLD(Aib)KYAE*EFVK*WLEDE(OH)	SEQ ID NO: 256
C57	YSHGTFTSDYSKYLD(Aib)KYAE*KFVK*WLEDE(NH ₂)	SEQ ID NO: 257
C58	YSHGTFTSDYSKYLD(Aib)KYAE*KFVK*WLEDE(OH)	SEQ ID NO: 258

Note:

- Each pairing of K* and E* and each pairing of K* and D* represent a covalent amide linkage derived from the amino sidechain of K* and the carboxy sidechain of E* or the carboxy sidechain of D* (with loss of a water molecule).

5

In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255 to 258, or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 211, 212, 221, 225 to 235, 238, and 239 or a pharmaceutically acceptable salt thereof.

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In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 211 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 212 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 221 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 225 or a

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pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 226 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 227 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 228 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 229 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 230 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 231 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 232 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 233 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 234 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 235 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 238 or a pharmaceutically acceptable salt thereof. In some embodiments, the isolated polypeptide comprises an amino acid sequence of SEQ ID NO: 239 or a pharmaceutically acceptable salt thereof.

In some embodiments the pharmaceutically acceptable salt is an acetate salt. In some embodiments the pharmaceutically acceptable salt is a trifluoroacetic acid (TFA) salt. In some embodiments the pharmaceutically acceptable salt is a hydrochloric acid (HCl) salt.

In one embodiment, the compound is the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 221 or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is the acetate salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 221. In some embodiments, the compound is the TFA salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 221. In some embodiments, the compound is the HCl salt of the isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 221.

Description of exemplary embodiments

In certain embodiments, the present disclosure provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 80:

X₁SHGTFTSX₉YSKYLX₁₅X₁₆X₁₇X₁₈AX₂₀X₂₁FX₂₃X₂₄WX₂₆EX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO: 80), or a pharmaceutically acceptable salt thereof, wherein:

5 X₁ is Y or acetyl-Y;

X₉ is D or E;

X₁₅ is D, E, or K;

X₁₆ is Aib or K;

X₁₇ is K;

10 X₁₈ is K, R, or Y;

X₂₀ is E, K, Q, or S;

X₂₁ is E or K;

X₂₃ is Aib or V;

X₂₄ is Aib, E, K, Q, or S;

15 X₂₆ is E, L, or Q;

X₂₈ is D, E, K, N, Q, S, or N-methylglutamate;

Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);

20 wherein when X₁₅, X₁₆, X₁₇, X₁₈, X₂₀, or X₂₁ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

25 wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₂₀ and X₂₄ or at positions X₂₄ and X₂₈.

In some embodiments, one of X₁₆, X₁₇, or X₂₁ is lysine bound to a lipophilic substituent, optionally via a spacer.

30 In some embodiments, X₁₆ is Aib or K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X_{20} is Q, then X_{16} is K, wherein the lysine at residue X_{16} is covalently bound to a lipophilic substituent, optionally via a spacer; or X_{21} is K, wherein the lysine residue at X_{21} is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

5 In some embodiments, when X_{20} is Q, then X_{21} is K, wherein the lysine residue at X_{21} is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X_{21} is E, then X_{20} is E, K, or S.

In some embodiments, when X_{20} is Q, then X_{21} is not E.

In some embodiments, X_{24} is Aib or K.

10 In some embodiments, X_{28} is D or S.

In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and a glutamic acid at positions X_{20} and X_{24} , respectively. In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a glutamic acid and a lysine at positions X_{20} and X_{24} ,
15 respectively.

In some embodiments, X_1 is Y. In some embodiments, X_1 is acetyl-Y.

In some embodiments, X_9 is D. In some embodiments, X_9 is E.

In some embodiments, X_{15} is D. In some embodiments, X_{15} is E. In some embodiments, X_{15} is K. In some embodiments, X_{15} is K covalently bound to a lipophilic substituent, optionally
20 via a spacer.

In some embodiments, X_{16} is Aib. In some embodiments, X_{16} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer.

As used herein, Aib refers to 2-aminoisobutyric acid, alternately known as α -aminoisobutyric acid, α -methylalanine, or 2-methylalanine.

25 In some embodiments, X_{17} is K. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X_{18} is K. In some embodiments, X_{18} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{18} is R. In some embodiments, X_{18} is Y.

In some embodiments, X₂₀ is E. In some embodiments, X₂₀ is K. In some embodiments, X₂₀ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₂₀ is Q. In some embodiments, X₂₀ is S.

In some embodiments, X₂₁ is E. In some embodiments, X₂₁ is K. In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X₂₃ is Aib. In some embodiments, X₂₃ is V.

In some embodiments, X₂₄ is Aib. In some embodiments, X₂₄ is E. In some embodiments, X₂₄ is K. In some embodiments, X₂₄ is Q. In some embodiments, X₂₄ is S.

In some embodiments, X₂₆ is E. In some embodiments, X₂₆ is L. In some embodiments, X₂₆ is Q.

In some embodiments, X₂₈ is D. In some embodiments, X₂₈ is E. In some embodiments, X₂₈ is K. In some embodiments, X₂₈ is N. In some embodiments, X₂₈ is Q. In some embodiments, X₂₈ is S. In some embodiments, X₂₈ is N-methylglutamate.

In some embodiments, Z-tail is absent. In some embodiments, Z-tail is PSSGAPPPS (SEQ ID NO: 90). In some embodiments, Z-tail is PSSGEPPPS (SEQ ID NO: 91). In some embodiments, Z-tail is PSSGKPPPS (SEQ ID NO: 92). In some embodiments, Z-tail is PSSGSPPPS (SEQ ID NO: 93).

Certain embodiments concerning particular amino acids represented by the consensus sequence of SEQ ID NO: 80 are as follows:

In some embodiments, X₁₆ is Aib, and X₁₇ is K. In some embodiments, X₁₆ is Aib, and X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, and X₂₁ is E. In some embodiments, X₁₆ is Aib, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, and X₂₄ is Aib. In some embodiments, X₁₆ is Aib and X₂₈ is D.

In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₁₇ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is S. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally

via a spacer, and X_{24} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is Aib. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is D. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is S.

5 In some embodiments, X_{17} is K, and X_{20} is Q. In some embodiments, X_{17} is K, and X_{20} is E. In some embodiments, X_{17} is K, and X_{20} is S. In some embodiments, X_{17} is K, and X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{17} is K, and X_{21} is K. In some embodiments, X_{17} is K, and X_{21} is E. In some embodiments, X_{17} is K, and X_{24} is Aib. In some embodiments, X_{17} is K, and X_{24} is K. In some embodiments, X_{17} is K, and
10 X_{28} is D. In some embodiments, X_{17} is K, and X_{28} is S.

In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{20} is Q. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{21} is E. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is Aib. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is D.
15

In some embodiments, X_{20} is Q, and X_{21} is E. In some embodiments, X_{20} is Q, and X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{20} is Q, and X_{24} is Aib. In some embodiments, X_{20} is Q, and X_{28} is D.

In some embodiments, X_{20} is E, and X_{21} is K. In some embodiments, X_{20} is E, and X_{24} is K. In some embodiments, X_{20} is E, and X_{28} is D.
20

In some embodiments, X_{20} is S, and X_{21} is E. In some embodiments, X_{20} is S, and X_{24} is Aib. In some embodiments, X_{20} is S, and X_{28} is S.

In some embodiments, X_{21} is E, and X_{24} is Aib. In some embodiments, X_{21} is E, and X_{28} is D. In some embodiments, X_{21} is E, and X_{28} is S.

25 In some embodiments, X_{21} is K, and X_{24} is K. In some embodiments, X_{21} is K, and X_{28} is D.

In some embodiments, X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is Aib. In some embodiments, X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is D.

30 In some embodiments, X_{24} is Aib, and X_{28} is D. In some embodiments, X_{24} is Aib, and X_{28} is S.

In some embodiments, X₂₃ is K, and X₂₈ is D.

Certain embodiments concerning particular amino acids represented by the consensus sequence of SEQ ID NO: 80 are as follows:

In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is E. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₈ is D.

In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is S. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is D. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is S.

In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₁ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₄ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₁ is E. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₈ is S.

In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₁ is E. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₃ is Aib. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₈ is D.

5 In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₃ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₈ is D.

In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₃ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

10 In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is K. In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is D.

In some embodiments, X₂₀ is S, X₂₁ is E and X₂₄ is Aib. In some embodiments, X₂₀ is S, X₂₁ is E and X₂₈ is S.

In certain embodiments, the present disclosure provides an isolated polypeptide
15 comprising the amino acid sequence of SEQ ID NO: 81:
X₁SHGTFSTSDYSKYLX₁₅X₁₆X₁₇X₁₈AX₂₀X₂₁FX₂₃X₂₄WLEX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO:
81), or a pharmaceutically acceptable salt thereof, wherein:

X₁ is Y or acetyl-Y;

X₁₅ is D or K;

20 X₁₆ is Aib or K;

X₁₇ is K;

X₁₈ is K or Y;

X₂₀ is E, K, Q, or S;

X₂₁ is E or K;

25 X₂₃ is Aib or V;

X₂₄ is Aib, E, K, Q, or S;

X₂₈ is D, E, K, N, Q, S, or N-methylglutamate;

Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90),
PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO:
30 93);

wherein when X_{15} , X_{16} , X_{17} , X_{18} , X_{20} , or X_{21} are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

5 wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X_{20} and X_{24} or at positions X_{24} and X_{28} ,

10 In some embodiments, one of X_{16} , X_{17} , or X_{21} is lysine bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X_{16} is Aib or K covalently bound to a lipophilic substituent, optionally via a spacer.

15 In some embodiments, when X_{20} is Q, then X_{16} is K, wherein the lysine at residue X_{16} is covalently bound to a lipophilic substituent, optionally via a spacer; or X_{21} is K, wherein the lysine residue at X_{21} is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X_{20} is Q, then X_{21} is K, wherein the lysine residue at X_{21} is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X_{21} is E, then X_{20} is E, K, or S.

20 In some embodiments, when X_{20} is Q, then X_{21} is not E.

In some embodiments, X_{24} is Aib or K.

In some embodiments, X_{28} is D or S.

In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and a glutamic acid at positions X_{20} and X_{24} ,

25 respectively. In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a glutamic acid and a lysine at positions X_{20} and X_{24} , respectively.

In some embodiments, X_1 is Y. In some embodiments, X_1 is acetyl-Y.

30 In some embodiments, X_{15} is D. In some embodiments, X_{15} is K. In some embodiments, X_{15} is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X_{16} is Aib. In some embodiments, X_{16} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X_{17} is K. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer.

5 In some embodiments, X_{18} is K. In some embodiments, X_{18} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{18} is Y.

In some embodiments, X_{20} is E. In some embodiments, X_{20} is K. In some embodiments, X_{20} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{20} is Q. In some embodiments, X_{20} is S.

10 In some embodiments, X_{21} is E. In some embodiments, X_{21} is K. In some embodiments, X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X_{23} is Aib. In some embodiments, X_{23} is V.

In some embodiments, X_{24} is Aib. In some embodiments, X_{24} is E. In some embodiments, X_{24} is K. In some embodiments, X_{24} is Q. In some embodiments, X_{24} is S.

15 In some embodiments, X_{28} is D. In some embodiments, X_{28} is E. In some embodiments, X_{28} is K. In some embodiments, X_{28} is N. In some embodiments, X_{28} is Q. In some embodiments, X_{28} is S. In some embodiments, X_{29} is N-methylglutamate.

In some embodiments, Z-tail is absent. In some embodiments, Z-tail is PSSGAPPPS (SEQ ID NO: 90). In some embodiments, Z-tail is PSSGEPPPS (SEQ ID NO: 91). In some
20 embodiments, Z-tail is PSSGKPPPS (SEQ ID NO: 92). In some embodiments, Z-tail is PSSGSPPPS (SEQ ID NO: 93).

Certain embodiments concerning particular amino acids represented by the consensus sequence of SEQ ID NO: 81 are as follows:

In some embodiments, X_{16} is Aib, and X_{17} is K. In some embodiments, X_{16} is Aib, and X_{17}
25 is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{16} is Aib, and X_{20} is Q. In some embodiments, X_{16} is Aib, and X_{21} is E. In some embodiments, X_{16} is Aib, and X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{16} is Aib, and X_{24} is Aib. In some embodiments, X_{16} is Aib and X_{28} is D.

In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via
30 a spacer, and X_{17} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{20} is E. In some embodiments, X_{16} is K covalently bound to a

lipophilic substituent, optionally via a spacer, and X_{20} is S. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{21} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{21} is E. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is K. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is Aib. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is D. In some embodiments, X_{16} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is S.

In some embodiments, X_{17} is K, and X_{20} is Q. In some embodiments, X_{17} is K, and X_{20} is E. In some embodiments, X_{17} is K, and X_{20} is S. In some embodiments, X_{17} is K, and X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{17} is K, and X_{21} is K. In some embodiments, X_{17} is K, and X_{21} is E. In some embodiments, X_{17} is K, and X_{24} is Aib. In some embodiments, X_{17} is K, and X_{24} is K. In some embodiments, X_{17} is K, and X_{28} is D. In some embodiments, X_{17} is K, and X_{28} is S.

In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{20} is Q. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{21} is E. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{24} is Aib. In some embodiments, X_{17} is K covalently bound to a lipophilic substituent, optionally via a spacer, and X_{28} is D.

In some embodiments, X_{20} is Q, and X_{21} is E. In some embodiments, X_{20} is Q, and X_{21} is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X_{20} is Q, and X_{24} is Aib. In some embodiments, X_{20} is Q, and X_{28} is D.

In some embodiments, X_{20} is E, and X_{21} is K. In some embodiments, X_{20} is E, and X_{24} is K. In some embodiments, X_{20} is E, and X_{28} is D.

In some embodiments, X_{20} is S, and X_{21} is E. In some embodiments, X_{20} is S, and X_{24} is Aib. In some embodiments, X_{20} is S, and X_{28} is S.

In some embodiments, X_{21} is E, and X_{24} is Aib. In some embodiments, X_{21} is E, and X_{28} is D. In some embodiments, X_{21} is E, and X_{28} is S.

In some embodiments, X_{21} is K, and X_{24} is K. In some embodiments, X_{21} is K, and X_{28} is D.

In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

5 In some embodiments, X₂₄ is Aib, and X₂₈ is D. In some embodiments, X₂₄ is Aib, and X₂₈ is S.

In some embodiments, X₂₄ is K, and X₂₈ is D.

Certain embodiments concerning particular amino acids represented by the consensus sequence of SEQ ID NO: 81 are as follows:

10 In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is E. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is Aib. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

15 In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₈ is Aib. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₈ is D.

20 In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is S. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is D. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is S.

In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₁ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₄ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₁ is E. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₈ is S.

In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₁ is E. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₄ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₈ is D.

In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is K. In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is D.

In some embodiments, X₂₀ is S, X₂₁ is E and X₂₄ is Aib. In some embodiments, X₂₀ is S, X₂₁ is E and X₂₈ is S.

In certain embodiments, the present disclosure provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 82:

X₁SHGTFTSDYSKYLDX₁₆X₁₇YAX₂₀X₂₁FX₂₃X₂₄WLEX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO:

82), or a pharmaceutically acceptable salt thereof, wherein:

X₁ is Y or acetyl-Y;

X₁₆ is Aib or K;

X₁₇ is K;

X₂₀ is E, K, Q, or S;

X₂₁ is E or K;

X₂₃ is Aib or V;

X₂₄ is Aib, E, K, Q, or S;

X₂₈ is D, E, K, N, Q, S, or N-methylglutamate;

Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);

wherein when X₁₆, X₁₇, or X₂₁ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₂₀ and X₂₄ or at positions X₂₄ and X₂₈.

In some embodiments, X₁₆ is Aib or K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X₂₀ is Q, then X₁₆ is K, wherein the lysine at residue X₁₆ is covalently bound to a lipophilic substituent, optionally via a spacer; or X₂₁ is K, wherein the lysine residue at X₂₁ is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X₂₀ is Q, then X₂₁ is K, wherein the lysine residue at X₂₁ is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, when X₂₁ is E, then X₂₀ is E, K, or S.

In some embodiments, when X₂₀ is Q, then X₂₁ is not E.

In some embodiments, X₂₄ is Aib or K.

In some embodiments, X₂₈ is D or S.

In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and a glutamic acid at positions X₂₀ and X₂₄,

respectively. In some embodiments, the peptide further comprises a lactam bridge formed via an amide bond between the side chains of a glutamic acid and a lysine at positions X₂₀ and X₂₄,

respectively.

In some embodiments, X₁ is Y. In some embodiments, X₁ is acetyl-Y.

In some embodiments, X₁₆ is Aib. In some embodiments, X₁₆ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X₁₇ is K. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer.

5 In some embodiments, X₂₀ is E. In some embodiments, X₂₀ is K. In some embodiments, X₂₀ is Q. In some embodiments, X₂₀ is S.

In some embodiments, X₂₁ is E. In some embodiments, X₂₁ is K. In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer.

In some embodiments, X₂₃ is Aib. In some embodiments, X₂₃ is V.

10 In some embodiments, X₂₄ is Aib. In some embodiments, X₂₄ is E. In some embodiments, X₂₄ is K. In some embodiments, X₂₄ is Q. In some embodiments, X₂₄ is S.

In some embodiments, X₂₈ is D. In some embodiments, X₂₈ is E. In some embodiments, X₂₈ is K. In some embodiments, X₂₈ is N. In some embodiments, X₂₈ is Q. In some embodiments, X₂₈ is S. In some embodiments, X₂₈ is N-methylglutamate.

15 In some embodiments, Z-tail is absent. In some embodiments, Z-tail is PSSGAPPPS (SEQ ID NO: 90). In some embodiments, Z-tail is PSSGEPPPS (SEQ ID NO: 91). In some embodiments, Z-tail is PSSGKPPPS (SEQ ID NO: 92). In some embodiments, Z-tail is PSSGSPPPS (SEQ ID NO: 93).

Certain embodiments concerning particular amino acids represented by the consensus
20 sequence of SEQ ID NO: 82 are as follows:

In some embodiments, X₁₆ is Aib, and X₁₇ is K. In some embodiments, X₁₆ is Aib, and X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, and X₂₁ is E. In some embodiments, X₁₆ is Aib, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In
25 some embodiments, X₁₆ is Aib, and X₂₄ is Aib. In some embodiments, X₁₆ is Aib and X₂₈ is D.

In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₁₇ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is S. In some embodiments, X₁₆ is K
30 covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and

X₂₁ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is S.

In some embodiments, X₁₇ is K, and X₂₀ is Q. In some embodiments, X₁₇ is K, and X₂₀ is E. In some embodiments, X₁₇ is K, and X₂₀ is S. In some embodiments, X₁₇ is K, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₇ is K, and X₂₁ is K. In some embodiments, X₁₇ is K, and X₂₁ is E. In some embodiments, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₇ is K, and X₂₄ is K. In some embodiments, X₁₇ is K, and X₂₈ is D. In some embodiments, X₁₇ is K, and X₂₈ is S.

In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is Q. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is E. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₂₀ is Q, and X₂₁ is E. In some embodiments, X₂₀ is Q, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₂₀ is E, and X₂₁ is K. In some embodiments, X₂₀ is E, and X₂₄ is K. In some embodiments, X₂₀ is E, and X₂₈ is D.

In some embodiments, X₂₀ is S, and X₂₁ is E. In some embodiments, X₂₀ is S, and X₂₄ is Aib. In some embodiments, X₂₀ is S, and X₂₈ is S.

In some embodiments, X₂₁ is E, and X₂₄ is Aib. In some embodiments, X₂₁ is E, and X₂₈ is D. In some embodiments, X₂₁ is E, and X₂₈ is S.

In some embodiments, X₂₁ is K, and X₂₄ is K. In some embodiments, X₂₁ is K, and X₂₈ is D.

In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₂₄ is Aib, and X₂₈ is D. In some embodiments, X₂₄ is Aib, and X₂₈ is S.

In some embodiments, X₂₄ is K, and X₂₈ is D.

Certain embodiments concerning particular amino acids represented by the consensus
5 sequence of SEQ ID NO: 82 are as follows:

In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₁ is E. In some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is Aib. In
10 some embodiments, X₁₆ is Aib, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₀ is Q. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₆ is Aib, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₆ is Aib, X₁₇ is
15 K, and X₂₈ is D.

In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₀ is S. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is K. In some
20 embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₁ is E. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is K. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₄ is Aib. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is
25 D. In some embodiments, X₁₆ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₁₇ is K, and X₂₈ is S.

In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₁ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₄ is K. In some embodiments, X₁₇ is K, X₂₀ is E, and X₂₈ is D.

In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₁ is E. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₄ is Aib. In some embodiments, X₁₇ is K, X₂₀ is S, and X₂₈ S.

In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₁ is E. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₄ is Aib. In some embodiments, X₁₇ is K covalently bound to a lipophilic substituent, optionally via a spacer, X₂₀ is Q, and X₂₈ is D.

In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₄ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is E, and X₂₈ is D.

In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₄ is Aib. In some embodiments, X₂₀ is Q, X₂₁ is K covalently bound to a lipophilic substituent, optionally via a spacer, and X₂₈ is D.

In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is K. In some embodiments, X₂₀ is E, X₂₁ is K, and X₂₄ is D.

In some embodiments, X₂₀ is S, X₂₁ is E and X₂₄ is Aib. In some embodiments, X₂₀ is S, X₂₁ is E and X₂₈ is S.

Conjugation of a lipophilic substituent to any of the peptides, optionally via a spacer

In some embodiments, any of the disclosed polypeptides is optionally substituted with one or more lipophilic substituents each optionally via a spacer, wherein "lipophilic substituent" and "spacer" are defined herein. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NO: 1 through SEQ ID NO: 60 either comprises one or more lipophilic substituents each optionally via a spacer, or can be modified, or further modified, by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NO: 1 through SEQ ID NO: 39 either comprises one or more lipophilic substituents each optionally via a spacer, or can be modified, or further modified, by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of

SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, or 149-160 can be modified by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NO: 101 to SEQ ID NO: 112 or SEQ ID NO: 115 to SEQ ID NO: 139 can be modified by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NOs: 211, 212, 221, 225 to 235, 238 to 245, 251, 252, or 255 to 258 can be modified by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NOs: 211, 212, 221, 225 to 235, 238, or 239 can be modified by covalent attachment of one or more lipophilic substituents each optionally via a spacer. In some embodiments, the lipophilic substituent may be attached to an amino group of the polypeptide (e.g., an ϵ -amino group of a lysine residue) by means of a carboxyl group of the lipophilic substituent, or optionally an amino group of the spacer, wherein a carboxyl group of the spacer forms an amide bond with an ϵ -amino group of a lysine residue.

Lipophilic substituent

Conjugation of one or more "lipophilic substituents", each optionally via a "spacer," to any of the disclosed polypeptides of this invention is intended to prolong the action of the polypeptide by facilitating binding to serum albumin and delayed renal clearance of the conjugated polypeptide. As used herein, a "lipophilic substituent" comprises a substituent comprising 4 to 40 carbon atoms, 8 to 25 carbon atoms, 12 to 22 carbon atoms, or 6 to 20 carbon atoms. The lipophilic substituent may be attached to an amino group of the polypeptide (e.g., an ϵ -amino group of a lysine residue) by means of a carboxyl group of the lipophilic substituent, or optionally an amino group of the spacer, which carboxyl group of the spacer in turn forms an amide bond with an amino group of the amino acid (e.g., lysine) residue to which it is attached. In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with or without an optional spacer, which is defined in greater detail below.

In some embodiments, the lipophilic substituent comprises a straight-chain or branched alkyl group. In some embodiments, the lipophilic substituent is an acyl group of a straight-chain or branched fatty acid. In some embodiments, the lipophilic substituent is an acyl group of a straight-chain or branched fatty acid, further substituted with one or more carboxylic acid and/or hydroxamic acid groups.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituents each without an optional spacer. In some embodiments, the lipophilic substituent is $-\text{CO}(\text{CH}_2)_{16}\text{CO}_2\text{H}$. In some embodiments, the lipophilic substituent is $-\text{CO}(\text{CH}_2)_{18}\text{CO}_2\text{H}$. In some embodiments, the lipophilic substituent is $-\text{CO}(\text{CH}_2)_{20}\text{CO}_2\text{H}$.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituents each without an optional spacer. In some embodiments, the lipophilic substituent is a monovalent group of Formula I:



Formula I

wherein

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$; and

m is from 4 to 24,

which lipophilic substituent forms an amide bond between an amino group (e.g., ϵ -amino group of a lysine) of the disclosed polypeptide and a $\text{CO}-$ group of the lipophilic substituent.

In some embodiments, Z is $-\text{CO}_2\text{H}$. In some embodiments, m is from 14 to 20. In some embodiments, the lipophilic substituent is covalently bound to the isolated polypeptide via a spacer. In some embodiments, the lipophilic substituent, $-\text{CO}-(\text{CH}_2)_m-\text{Z}$, is linked to an amino group of isolated polypeptide via the spacer, wherein the spacer forms a bridge between the amino group of the isolated polypeptide and a $\text{CO}-$ group of the lipophilic substituent.

In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20.

In some embodiments, Z is $-\text{CO}_2\text{H}$, and the lipophilic substituent has the formula $-\text{CO}-(\text{CH}_2)_m-\text{CO}_2\text{H}$. In some embodiments, $-\text{CO}-(\text{CH}_2)_m-\text{Z}$ is selected from the group consisting of $-\text{CO}-(\text{CH}_2)_4-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_5-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_6-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_7-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_8-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_9-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{10}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{11}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{12}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{13}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{14}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{15}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{16}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{17}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{18}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{19}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{20}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{21}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{22}-\text{CO}_2\text{H}$, $-\text{CO}-(\text{CH}_2)_{23}-\text{CO}_2\text{H}$, and $-\text{CO}-(\text{CH}_2)_{24}-\text{CO}_2\text{H}$.

(CH₂)₁₃-CO₂H, -CO-(CH₂)₁₄-CO₂H, -CO-(CH₂)₁₅-CO₂H, -CO-(CH₂)₁₆-CO₂H, -CO-(CH₂)₁₇-CO₂H, -CO-(CH₂)₁₈-CO₂H, -CO-(CH₂)₁₉-CO₂H, -CO-(CH₂)₂₀-CO₂H.

In some embodiments, the lipophilic substituent is -CO-(CH₂)₁₄-CO₂H. In some embodiments, the lipophilic substituent is -CO-(CH₂)₁₆-CO₂H. In some embodiments, the lipophilic substituent is -CO-(CH₂)₁₈-CO₂H. In some embodiments, the lipophilic substituent is -CO-(CH₂)₂₀-CO₂H.

In some embodiments, Z is -CH₃, and the lipophilic substituent has the formula -CO-(CH₂)_m-CH₃. In some embodiments, -CO-(CH₂)_m-Z is selected from the group consisting of -CO-(CH₂)₄-CH₃, -CO-(CH₂)₅-CH₃, -CO-(CH₂)₆-CH₃, -CO-(CH₂)₇-CH₃, -CO-(CH₂)₈-CH₃, -CO-(CH₂)₉-CH₃, -CO-(CH₂)₁₀-CH₃, -CO-(CH₂)₁₁-CH₃, -CO-(CH₂)₁₂-CH₃, -CO-(CH₂)₁₃-CH₃, -CO-(CH₂)₁₄-CH₃, -CO-(CH₂)₁₅-CH₃, -CO-(CH₂)₁₆-CH₃, -CO-(CH₂)₁₇-CH₃, -CO-(CH₂)₁₈-CH₃, -CO-(CH₂)₁₉-CH₃, and -CO-(CH₂)₂₀-CH₃.

Lipophilic substituent & Spacer

In some embodiments, the lipophilic substituent is attached to the parent peptide by means of a "spacer." In some embodiments, provided herein is any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NO:1 to 60, 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, or 255 to 258, comprising a lipophilic substituent, wherein the lipophilic substituent is linked to the ε-amino group of a lysine via a spacer, which spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of the lipophilic substituent. In some embodiments, provided herein is any of the disclosed polypeptides, comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by any of the consensus sequences of SEQ ID NO:1 to 39, 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, or 239, comprising a lipophilic substituent, wherein the lipophilic substituent is linked to the ε-amino group of a lysine via a spacer, which spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of the lipophilic substituent.

In some embodiments, the spacer comprises one or more amino acids, for example, single amino acid such as Glu, Asp, Gly or Lys, dipeptide such as 2(Glu), Glu-Gly, or polypeptide such as 3(Glu), 4(Glu), 2(Glu)-Gly etc. In some embodiments, when the spacer comprises one or more

amino acids, e.g., Glu, Asp, Gly or Lys, one carboxyl group of the spacer may form an amide bond with an amino group of the disclosed polypeptide, and an amino group of the spacer may form an amide bond with a carboxyl group of the lipophilic substituent.

In some embodiments, when the spacer comprises Glu or Asp, that further include a carboxylic acid-terminating sidechain, the terminal carboxyl group of the sidechain of the Glu or Asp-containing spacer may form an amide bond with an amino group of the disclosed polypeptide, and an amino group of the Glu or Asp-containing spacer may form an amide bond with a carboxyl group of the lipophilic substituent, i.e., γ Glu or β Asp.

In some embodiments, the spacer is $-\gamma$ Glu- γ Glu-dpeg-. In some embodiments, the spacer is $-\gamma$ Glu- γ Glu-dpeg- γ Glu- γ Glu-. In some embodiments, the spacer is $-\text{[COCH}_2\text{(OCH}_2\text{CH}_2\text{)}_2\text{NH]}_n\text{-}\gamma$ Glu-.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some non-limiting embodiments, the lipophilic substituent and spacer form a monovalent group selected from the group consisting of those listed in Table 7:

Table 7: representative lipophilic substituent and spacer moieties

$-\gamma$ Glu- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-CO(CH ₂) ₁₇ CO ₂ H
$-\gamma$ Glu- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg- γ Glu- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg- γ Glu- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-dpeg-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-dpeg-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu-dpeg-dpeg- γ Glu-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu- γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu- γ Glu- γ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H
$-\gamma$ Glu-dpeg-CO(CH ₂) ₁₆ CO ₂ H
$-\gamma$ Glu-dpeg-CO(CH ₂) ₁₈ CO ₂ H

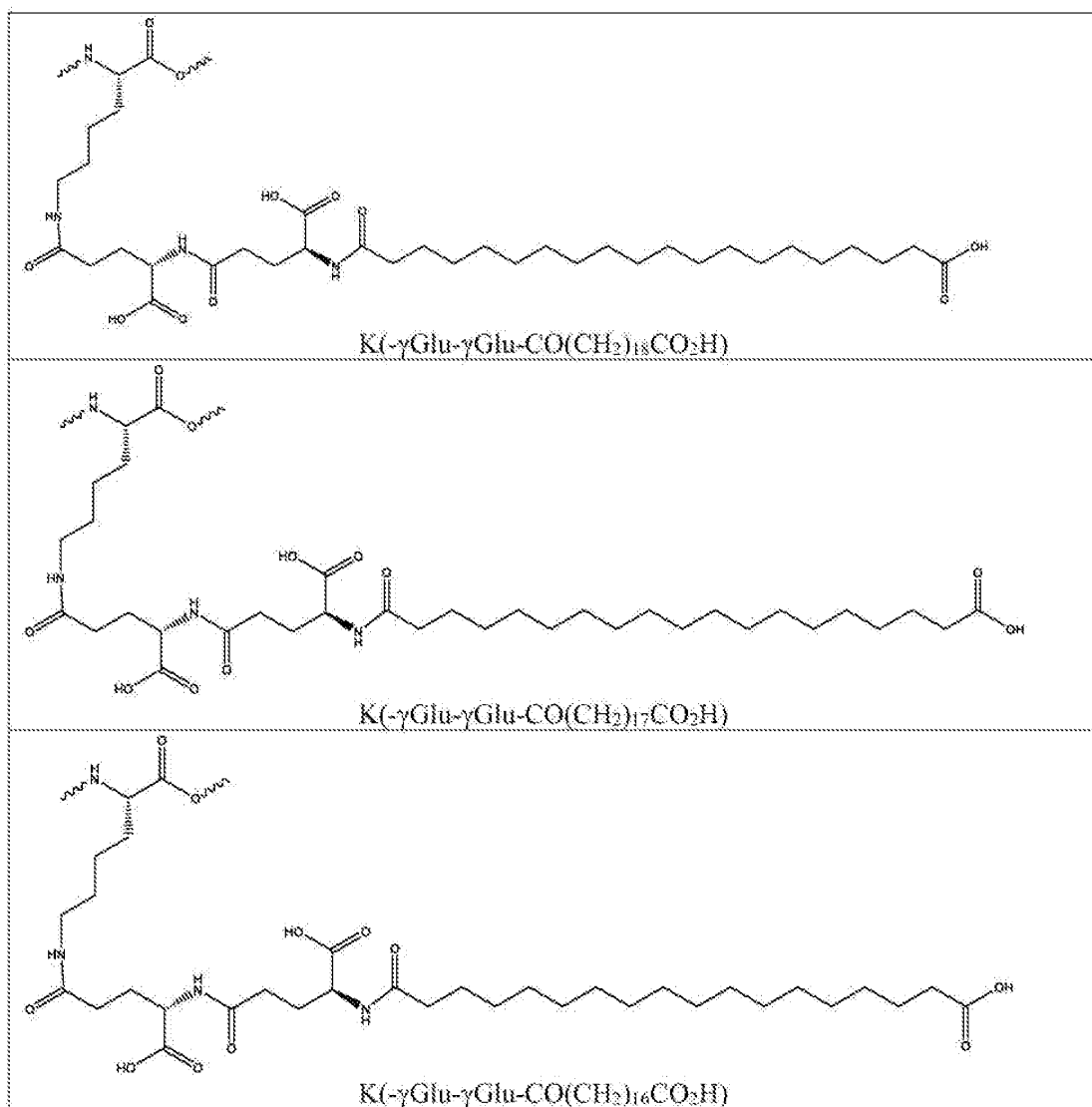


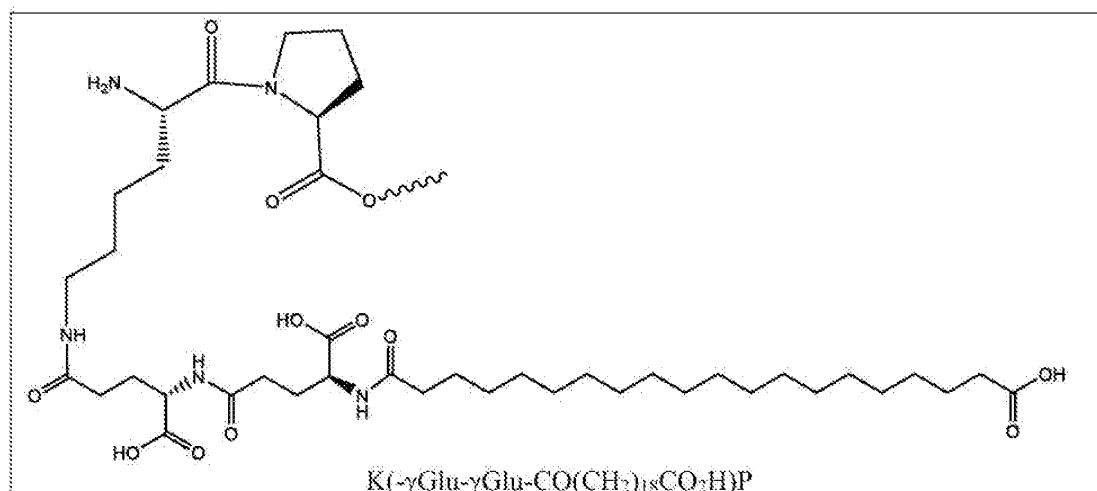
Preferably, the lipophilic substituent and spacer are attached to an amino group of the polypeptide. In particular, a carboxyl group of the lipophilic substituent, or optionally a carboxyl group of the spacer, forms an amide bond with an ϵ -amino group of a lysine residue. The lysine residue bound to the lipophilic substituent, optionally via a spacer, may be L-lysine or D-lysine.

5 Structural representations of representative spacer moieties and lipophilic substituents are provided in Table 8:

Table 8: Structures of Representative Spacers Moieties & Lipophilic Substituents Bound to Lysine

k(-γGlu-dpeg-dpeg-γGlu-CO(CH ₂) ₁₈ CO ₂ H)
K(-γGlu-dpeg-dpeg-γGlu-CO(CH ₂) ₁₈ CO ₂ H)
K(-dpeg-dpeg-γGlu-CO(CH ₂) ₁₆ CO ₂ H)
K(-γGlu-γGlu-dpeg-CO(CH ₂) ₁₈ CO ₂ H)





In some embodiments, the lipophilic substituent and spacer form a monovalent group of Formula II:



Formula II

wherein

Y is selected from the group consisting of γGlu , Asp, Lys and Gly;

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24; and

n is from 1 to 10.

In some embodiments, Z is $-\text{CO}_2\text{H}$. In some embodiments, m is from 14 to 20. In some embodiments, Y is γGlu . In some embodiments, n is from 1 to 5.

In some embodiments, Y is selected from the group consisting of γGlu and Gly. In some embodiments, Y is γGlu . In some embodiments, Y is Gly.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula III:



Formula III

wherein,

V is $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_l\text{NH]}-$;

Y is selected from the group consisting of γ Glu, Asp, and Gly;

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24;

n is from 1 to 10;

5 r is from 1 to 6; and

t is from 1 to 6.

In some embodiments, Z is $-\text{CO}_2\text{H}$. In some embodiments, Z is $-\text{CH}_3$.

In some embodiments, Y is γ Glu. In some embodiments, Y is Asp. In some embodiments, Y is Gly.

10 In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20. In some embodiments, m is from 14 to 20.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n is from 1 to 5. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

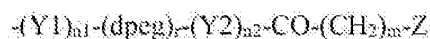
15 In some embodiments, r is from 1 to 3. In some embodiments, r is 1. In some embodiments, r is 2. In some embodiments, r is 3. In some embodiments, r is 4. In some embodiments, r is 5.

In some embodiments, t is from 1 to 3. In some embodiments, t is selected from the group consisting of 1, 2, 3, 4, 5 and 6.

In some embodiments, Y is γ Glu; Z is $-\text{CO}_2\text{H}$; m is 16; n is 1; r is 2; and t is 2.

20 In an embodiment, $-(V)_t-(Y)_n-$ is $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_3\text{NH}]_t-\gamma\text{Glu}-$.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula IV:



25 Formula IV

wherein

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24;

Y1 is selected from the group consisting of γ Glu, Asp, and Gly;

30 Y2 is selected from the group consisting of γ Glu, Asp, and Gly;

dpeg is $-\text{[CO}(\text{CH}_2)\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)\text{NH}]_r-$;

r is from 1 to 8;

n1 is from 0 to 10; and

n2 is from 0 to 10.

In some embodiments, Z is $-\text{CO}_2\text{H}$. In some embodiments, Z is $-\text{CH}_3$.

5 In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20. In some embodiments, m is from 14 to 20.

In some embodiments, Y1 is γGlu . In some embodiments, Y1 is Asp. In some embodiments, Y1 is Gly.

10 In some embodiments, Y2 is γGlu . In some embodiments, Y2 is Asp. In some embodiments, Y2 is Gly.

In some embodiments, n1 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n1 is from 0 to 3. In some embodiments, n1 is 0. In some embodiments, n1 is 1. In some embodiments, n1 is 2. In some embodiments, n1 is 3. In some embodiments, n1 is 4. In some embodiments, n1 is 5.

15 In some embodiments, n2 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n2 is from 0 to 3. In some embodiments, n2 is 0. In some embodiments, n2 is 1. In some embodiments, n2 is 2. In some embodiments, n2 is 3. In some embodiments, n2 is 4. In some embodiments, n2 is 5.

20 In some embodiments, r is from 1 to 3. In some embodiments, r is 1. In some embodiments, r is 2. In some embodiments, r is 3. In some embodiments, r is 4. In some embodiments, r is 5. In some embodiments, r is 6. In some embodiments, r is 7. In some embodiments, r is 8.

In some embodiments, r is 1, n1 is 2, and n2 is 0.

In some embodiments, r is 1, n1 is 2, and n2 is 2.

In some embodiments, Y1 is γGlu and Y2 is γGlu .

25 In some embodiments, Y1 is γGlu and n2 is 0.

In some embodiments, Y1 is γGlu , r is 1, n1 is 2, and n2 is 0.

In some embodiments, $-(\text{Y1})_{m1}-(\text{dpeg})_r-(\text{Y2})_{n2}-$ is selected from the group consisting of $-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-$, $-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-\gamma\text{Glu}-\gamma\text{Glu}-$, $-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-\gamma\text{Glu}-$, $-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-\text{dpeg}-$, $-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-\text{dpeg}-\gamma\text{Glu}-$, $-\text{dpeg}-\text{dpeg}-\gamma\text{Glu}-$, $-\gamma\text{Glu}-\gamma\text{Glu}-\gamma\text{Glu}-\text{dpeg}-$, and $-\gamma\text{Glu}-\text{dpeg}-$.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula V:



Formula V

wherein

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24; and

n is from 1 to 10.

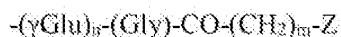
In some embodiments, Z is $-\text{CH}_3$. In some embodiments, Z is $-\text{CO}_2\text{H}$.

In some embodiments, m is from 14 to 20.

In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula VI:



Formula VI

wherein

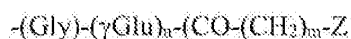
Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24; and

n is from 1 to 10.

In some embodiments, $(\gamma\text{Glu})_n$ is selected from the group consisting of γGlu ; $2(\gamma\text{Glu})$; $3(\gamma\text{Glu})$; $4(\gamma\text{Glu})$; and $5(\gamma\text{Glu})$. In some embodiments, $-(\gamma\text{Glu})_n\text{-(Gly)-}$ is selected from the group consisting of $2(\gamma\text{Glu})\text{,Gly}$; and $3(\gamma\text{Glu})\text{,Gly}$.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula VII:



Formula VII

wherein

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

5 m is from 4 to 24; and

n is from 1 to 10.

In some embodiments, certain variables represented in certain of the preceding Formulae include the following:

In some embodiments, Z is $-\text{CH}_3$. In some embodiments, Z is $-\text{CO}_2\text{H}$.

10 In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

15 In some embodiments, n is 1 and Z is $-\text{CO}_2\text{H}$. In some embodiments, n is 1 and Z is $-\text{CH}_3$. In some embodiments, n is 2 and Z is $-\text{CO}_2\text{H}$. In some embodiments, n is 2 and Z is $-\text{CH}_3$. In some embodiments, n is 3 and Z is $-\text{CO}_2\text{H}$. In some embodiments, n is 3 and Z is $-\text{CH}_3$. In some embodiments, n is 4 and Z is $-\text{CO}_2\text{H}$. In some embodiments, n is 4 and Z is $-\text{CH}_3$. In some embodiments, n is 5 and Z is $-\text{CO}_2\text{H}$. In some embodiments, n is 5 and Z is $-\text{CH}_3$.

20 In some embodiments, n is 1, Z is $-\text{CO}_2\text{H}$, and m is 14-20. In some embodiments, n is 1, Z is $-\text{CO}_2\text{H}$, and m is 14. In some embodiments, n is 1, Z is $-\text{CO}_2\text{H}$, and m is 16. In some embodiments, n is 1, Z is $-\text{CO}_2\text{H}$, and m is 18.

In some embodiments, n is 1, Z is $-\text{CH}_3$, and m is 14-20. In some embodiments, n is 1, Z is $-\text{CH}_3$ and m is 14. In some embodiments, n is 1, Z is $-\text{CH}_3$, and m is 16. In some embodiments, 25 n is 1, Z is $-\text{CH}_3$, and m is 18.

In some embodiments, n is 2, Z is $-\text{CO}_2\text{H}$, and m is 14-20. In some embodiments, n is 2, Z is $-\text{CO}_2\text{H}$, and m is 14. In some embodiments, n is 2, Z is $-\text{CO}_2\text{H}$, and m is 16. In some embodiments, n is 2, Z is $-\text{CO}_2\text{H}$, and m is 18.

In some embodiments, n is 2, Z is $-\text{CH}_3$, and m is 14-20. In some embodiments, n is 2, Z is $-\text{CH}_3$ and m is 14. In some embodiments, n is 2, Z is $-\text{CH}_3$, and m is 16. In some embodiments, 30 n is 2, Z is $-\text{CH}_3$, and m is 18.

In some embodiments, n is 3, Z is -CO₂H, and m is 14-20. In some embodiments, n is 3, Z is -CO₂H, and m is 14. In some embodiments, n is 3, Z is -CO₂H, and m is 16. In some embodiments, n is 3, Z is -CO₂H, and m is 18.

In some embodiments, n is 3, Z is -CH₃, and m is 14-20. In some embodiments, n is 3, Z is -CH₃ and m is 14. In some embodiments, n is 3, Z is -CH₃, and m is 16. In some embodiments, n is 3, Z is -CH₃, and m is 18.

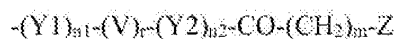
In some embodiments, n is 4, Z is -CO₂H, and m is 14-20. In some embodiments, n is 4, Z is -CO₂H, and m is 14. In some embodiments, n is 4, Z is -CO₂H, and m is 16. In some embodiments, n is 4, Z is -CO₂H, and m is 18.

In some embodiments, n is 4, Z is -CH₃, and m is 14-20. In some embodiments, n is 4, Z is -CH₃ and m is 14. In some embodiments, n is 4, Z is -CH₃, and m is 16. In some embodiments, n is 4, Z is -CH₃, and m is 18.

In some embodiments, n is 5, Z is -CO₂H, and m is 14-20. In some embodiments, n is 5, Z is -CO₂H, and m is 14. In some embodiments, n is 5, Z is -CO₂H, and m is 16. In some embodiments, n is 5, Z is -CO₂H, and m is 18.

In some embodiments, n is 5, Z is -CH₃, and m is 14-20. In some embodiments, n is 5, Z is -CH₃ and m is 14. In some embodiments, n is 5, Z is -CH₃, and m is 16. In some embodiments, n is 5, Z is -CH₃, and m is 18.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula VIII:



Formula VIII

wherein

Z is -CH₃ or -CO₂H;

m is from 4 to 24;

Y1 is selected from the group consisting of γGlu, Asp, and Gly;

Y2 is selected from the group consisting of γGlu, Asp, and Gly;

V is -[COCH₂(OCH₂CH₂)_nNH]-;

r is from 1 to 6;

n1 is from 0 to 10;

n₂ is from 0 to 10; and

i is from 1 to 6,

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula IX:



Formula IX

wherein

10 Z is -CH₃ or -CO₂H;

m is from 4 to 24;

Y is selected from the group consisting of γGlu, Asp, and Gly;

V is -[COCH₂(OCH₂CH₂)₃NH]-;

r is from 1 to 6; and

15 n is from 1 to 10; and

i is from 1 to 6,

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula X:



Formula X

wherein

Z is -CH₃ or -CO₂H;

m is from 4 to 24;

25 dpeg is -[CO(CH₂)O(CH₂)₂O(CH₂)NH]-;

Y2 is selected from the group consisting of γGlu, Asp, and Gly;

r is from 1 to 8; and

n₂ is from 0 to 10.

In some embodiments, -(dpeg)_r-(Y2)_{n2}- is selected from the group consisting of dpeg, γGlu, and dpeg, dpeg, γGlu.

In an embodiment, -(dpeg)_r-(Y2)_{n2}- is -dpeg-dpeg-γGlu-.

In some embodiments, the polypeptide comprises three, two, or preferably one lipophilic substituent each with a spacer. In some embodiments, the lipophilic substituent and spacer are a monovalent group of Formula XI:



wherein

Z is -CH_3 or $\text{-CO}_2\text{H}$;

m is from 4 to 24;

Y1 is selected from the group consisting of γGlu , Asp, and Gly;

dpeg is $\text{-[CO(CH}_2\text{)O(CH}_2\text{)}_2\text{O(CH}_2\text{)NH]-}$;

r is from 1 to 8; and

$n1$ is from 0 to 10.

In some embodiments, Z is $\text{-CO}_2\text{H}$.

In some embodiments, m is selected from the group consisting of 4-20, 8-20, 12-20, 14-20, 16-20, 14, 16, 18, and 20. In some embodiments, m is from 14 to 20.

In some embodiments, Y1 is γGlu . In some embodiments, Y1 is Asp. In some embodiments, Y1 is Gly.

In some embodiments, $n1$ is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, $n1$ is from 0 to 3. In some embodiments, $n1$ is 0. In some embodiments, $n1$ is 1. In some embodiments, $n1$ is 2. In some embodiments, $n1$ is 3. In some embodiments, $n1$ is 4. In some embodiments, $n1$ is 5.

In some embodiments, r is from 1 to 3. In some embodiments, r is 1. In some embodiments, r is 2. In some embodiments, r is 3. In some embodiments, r is 4. In some embodiments, r is 5. In some embodiments, r is 6. In some embodiments, r is 7. In some embodiments, r is 8.

In some embodiments, r is 1 and $n1$ is 2.

In some embodiments, Y1 is γGlu , r is 1, and $n1$ is 2.

In some embodiments, $\text{-(Y1)}_{n1}\text{-(dpeg)}_r\text{-}$ is selected from the group consisting of $\text{-}\gamma\text{Glu-}\gamma\text{Glu-dpeg-}$, $\text{-}\gamma\text{Glu-}\gamma\text{Glu-dpeg-dpeg-}$, $\text{-}\gamma\text{Glu-}\gamma\text{Glu-}\gamma\text{Glu-dpeg-}$, and $\text{-}\gamma\text{Glu-dpeg-}$.

Further exemplary spacers

In some embodiments, the spacer comprises a bivalent group of Formula XII:



Formula XII

wherein

5 each R_1 and R_3 is hydrogen or C_1 - C_4 alkyl;

each R_2 is H or CO_2H ;

p is 1, 2, 3, 4, 5 or 6;

q is 1, 2 or 3;

r is 0 or 1.

10 which spacer forms a bridge between an amino group of the disclosed polypeptide and a $CO-$ group of the lipophilic substituent.

In some embodiments, the spacer comprises a bivalent group of Formula XIII:



Formula XIII

15 wherein

each R_3 is hydrogen or C_1 - C_4 alkyl;

q is 1, 2 or 3;

r is 0 or 1.

20 which spacer forms a bridge between an amino group of the disclosed polypeptide and a $CO-$ group of the lipophilic substituent.

In some embodiments, certain variables represented in certain Formulae include the following:

In some embodiments, each R_1 is hydrogen. In some embodiments, each R_3 is hydrogen.

In some embodiments, each R_1 and each R_3 are hydrogen.

25 In some embodiments, at least one R_2 is CO_2H . In some embodiments, one R_2 is CO_2H .

In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3.

In some embodiments, p is 4. In some embodiments, p is 5. In some embodiments, p is 6.

In some embodiments, q is 1. In some embodiments, q is 2. In some embodiments, q is 3.

30 In some embodiments, r is 0. In some embodiments, r is 1.

In some embodiments, the spacer is γ -glutamyl, i.e., $-\text{NH}(\text{CH}(\text{CO}_2\text{H})(\text{CH}_2)_2\text{CO}-)$. In some embodiments, the spacer is γ -aminobutanoyl, i.e., $-\text{NH}(\text{CH}_2)_3\text{CO}-$. In some embodiments, the spacer is β -asparagyl, i.e., $-\text{NH}(\text{CH}(\text{CO}_2\text{H})(\text{CH}_2)\text{CO}-)$. In some embodiments, the spacer is $-\text{NH}(\text{CH}_2)_2\text{CO}-$. In some embodiments, the spacer is glycyl. In some embodiments, the spacer is β -alanyl.

In some embodiments, the spacer is $-\text{NHCH}(\text{CO}_2\text{H})(\text{CH}_2)_2\text{CO}-[\text{N}(\text{R}_3)((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_q(\text{CH}_2)\text{CO}-]_r$. In some embodiments, the spacer is $-\text{NH}(\text{CH}_2)_3\text{CO}-[\text{N}(\text{R}_3)((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_q(\text{CH}_2)\text{CO}-]_r$. In some embodiments, the spacer is $-\text{NHCH}(\text{CO}_2\text{H})(\text{CH}_2)_2\text{CO}-\text{NH}((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_2(\text{CH}_2)\text{CO}-$. In some embodiments, the spacer is $-\text{NH}(\text{CH}_2)_3\text{CO}-\text{NH}((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_2(\text{CH}_2)\text{CO}-$. In some embodiments, the spacer is $-\text{NHCH}(\text{CO}_2\text{H})\text{CH}_2\text{CO}-[\text{N}(\text{R}_3)((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_q(\text{CH}_2)\text{CO}-]_r$. In some embodiments, the spacer is $-\text{NH}(\text{CH}_2)_3\text{CO}-[\text{N}(\text{R}_3)((\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O})_q(\text{CH}_2)\text{CO}-]_r$.

In some embodiments, the spacer comprises a bivalent group of Formula XIV:



Formula XIV

wherein

Y is selected from the group consisting of γ Glu, Asp, Lys and Gly;

n is from 1 to 10.

In some embodiments, Y is selected from the group consisting of γ Glu and Gly. In some embodiments, Y is γ Glu. In some embodiments, Y is Gly.

In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a $\text{CO}-$ group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

In some embodiments, the spacer comprises a bivalent group of Formula XV:



Formula XV

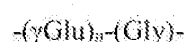
wherein

n is from 1 to 10.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

5 In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

In some embodiments, the spacer comprises a bivalent group of Formula XVI:



10 Formula XVI

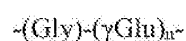
wherein

n is from 1 to 10.

In some embodiments, $(\gamma\text{Glu})_n$ is selected from the group consisting of γGlu ; $2(\gamma\text{Glu})$; $3(\gamma\text{Glu})$; $4(\gamma\text{Glu})$; and $5(\gamma\text{Glu})$. In some embodiments, $-(\gamma\text{Glu})_n-(\text{Gly})-$ is selected from the group
15 consisting of $2(\gamma\text{Glu}), \text{Gly}$; and $3(\gamma\text{Glu}), \text{Gly}$.

In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

20 In some embodiments, the spacer comprises a bivalent group of Formula XVII:



Formula XVII

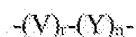
wherein

n is from 1 to 10.

25 In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and
30 10. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

In some embodiments, the spacer comprises a bivalent group of Formula XVIII:



Formula XVIII

wherein

5 Y is selected from the group consisting of γ Glu, Asp, and Gly;

V is $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_r\text{NH]}-$;

r is from 1 to 6;

n is from 1 to 10; and

t is from 1 to 6.

10 In some embodiments, Y is γ Glu. In some embodiments, Y is Asp. In some embodiments, Y is Gly.

In some embodiments, n is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n is from 1 to 5. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

15 In some embodiments, r is from 1 to 3. In some embodiments, r is 1. In some embodiments, r is 2. In some embodiments, r is 3. In some embodiments, r is 4. In some embodiments, r is 5.

In some embodiments, t is from 1 to 3. In some embodiments, t is selected from the group consisting of 1, 2, 3, 4, 5 and 6.

In an embodiment, $-(V)_r-(Y)_n-$ is $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_r\text{NH]}_r-\gamma\text{Glu}-$.

20 In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

In some embodiments, the spacer comprises a bivalent group of Formula XIX:



Formula XIX

wherein

Y1 is selected from the group consisting of γ Glu, Asp, and Gly;

Y2 is selected from the group consisting of γ Glu, Asp, and Gly;

30 dpeg is $-\text{[CO(CH}_2\text{)O(CH}_2\text{)}_2\text{O(CH}_2\text{)NH]}-$;

r is from 1 to 8;

n1 is from 0 to 10; and

n2 is from 0 to 10.

In some embodiments, Y1 is γ Glu. In some embodiments, Y1 is Asp. In some embodiments, Y1 is Gly.

5 In some embodiments, Y2 is γ Glu. In some embodiments, Y2 is Asp. In some embodiments, Y2 is Gly.

In some embodiments, n1 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n1 is from 0 to 3. In some embodiments, n1 is 0. In some embodiments, n1 is 1. In some embodiments, n1 is 2. In some embodiments, n1 is 3. In some
10 embodiments, n1 is 4. In some embodiments, n1 is 5.

In some embodiments, n2 is selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, n2 is from 0 to 3. In some embodiments, n2 is 0. In some embodiments, n2 is 1. In some embodiments, n2 is 2. In some embodiments, n2 is 3. In some embodiments, n2 is 4. In some embodiments, n2 is 5.

15 In some embodiments, r is from 1 to 3. In some embodiments, r is 1. In some embodiments, r is 2. In some embodiments, r is 3. In some embodiments, r is 4. In some embodiments, r is 5. In some embodiments, r is 6. In some embodiments, r is 7. In some embodiments, r is 8.

In some embodiments, r is 1, n1 is 2, and n2 is 0.

In some embodiments, r is 1, n1 is 2, and n2 is 2.

20 In some embodiments, Y1 is γ Glu and Y2 is γ Glu.

In some embodiments, Y1 is γ Glu and n2 is 0.

In some embodiments, Y1 is γ Glu, r is 1, n1 is 2, and n2 is 0.

In some embodiments, $-(Y1)_{n1}-(dpeg)_r-(Y2)_{n2}-$ is selected from the group consisting of - γ Glu- γ Glu-dpeg-, - γ Glu- γ Glu-dpeg- γ Glu- γ Glu-, - γ Glu- γ Glu-dpeg- γ Glu-, - γ Glu- γ Glu-dpeg-dpeg-,
25 - γ Glu- γ Glu-dpeg-dpeg- γ Glu-, -dpeg-dpeg- γ Glu-, - γ Glu- γ Glu- γ Glu-dpeg-, and - γ Glu-dpeg-.

In some embodiments, the spacer forms a bridge between an amino group of the disclosed polypeptide and a CO— group of a lipophilic substituent. In some embodiments, one end of the spacer forms a covalent bond with an amino group of the disclosed polypeptide and the other end of the spacer forms a covalent bond with a hydrogen atom or a protecting group.

30 Accordingly, in some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group

consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent, and optionally comprises a spacer. In some embodiments, an isolated polypeptide provided herein comprises an amino acid
5 sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent, and optionally comprises a spacer.

10 In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent of Formula I. In some embodiments, an isolated
15 polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent of Formula I

20 In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent of Formula I and a spacer selected from the group
25 consisting of those described by Formula XII, XIII, XIV, XV, XVI, XVII, XVIII, and XIX. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID
30 NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent of

Formula I and a spacer selected from the group consisting of those described by Formula XII, XIII, XIV, XV, XVI, XVII, XVIII, and XIX.

5 In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula I, II, III, IV, V, VI, VII, VIII, IX, X, and XI. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by
10 a consensus sequence selected from the group consisting of 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula I, II, III, IV, V, VI, VII, VIII, IX, X, and XI.

15 In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula I. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises
20 a lipophilic substituent and a spacer selected from the group consisting of those described by Formula I.

In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of
30 SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting

of those described by Formula II. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula II.

In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula III. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula III.

In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula IV. In some embodiments, an isolated polypeptide provided herein comprises an amino acid sequence, or a pharmaceutically acceptable salt thereof, selected from the group consisting of amino acid sequences represented by a consensus sequence selected from the group consisting of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255-258, wherein the isolated peptide further comprises a lipophilic substituent and a spacer selected from the group consisting of those described by Formula IV.

As used herein, $(\gamma\text{Glu})_2$ and $2(\gamma\text{Glu})$ both mean $-(\gamma\text{Glu})-(\gamma\text{Glu})-$ or $-\text{CO}(\text{CH}_2)_2\text{CH}(\text{CO}_2\text{H})\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{CO}_2\text{H})\text{NH}-$; $(\gamma\text{Glu})_3$ and $3(\gamma\text{Glu})$ both mean $-(\gamma\text{Glu})-(\gamma\text{Glu})-(\gamma\text{Glu})-$ or $-\text{CO}(\text{CH}_2)_2\text{CH}(\text{CO}_2\text{H})\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{CO}_2\text{H})\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{CO}_2\text{H})\text{NH}-$; etc.; where a variable is present more than once in a given formula, each occurrence of that variable is independently determined. For example, for group $-(Y)_3-$, where Y may be γGlu , Asp, Lys, or Gly, each Y is independently selected to be one of the four amino acids. Accordingly, by non-limiting example, $-(Y)_3-$ may be $-(\gamma\text{Glu})-(\gamma\text{Glu})-(\gamma\text{Glu})-$, $-(\gamma\text{Glu})-(\text{Asp})-(\gamma\text{Glu})-$, $-(\text{Gly})-(\text{Asp})-(\gamma\text{Glu})-$, or $-(\text{Gly})-(\gamma\text{Glu})-(\gamma\text{Glu})-$.

Bridging Moiety

In some embodiments, any of the disclosed polypeptides is optionally substituted with one or more bridging moieties. As used herein, the term “bridging moiety” means a covalent bond or any bivalent linker or moiety that joins two sidechains of two separate amino acid residues. In some embodiments, any of the disclosed polypeptides is optionally substituted with one or more lactam bridging moieties. As used herein, the term “lactam bridging moiety” means a lactam bridge or lactam bond that joins amino-containing and carboxy-containing sidechains of two separate amino acid residues. In some embodiments, the lactam bridging moiety is formed between a lysine residue and an aspartic acid residue, and the amino-containing sidechain of lysine and the carboxy-containing sidechain of aspartic acid are covalently joined, with loss of water, to form a lactam bridging moiety. In some embodiments, the lactam bridging moiety is formed between a lysine residue and a glutamic acid residue, and the amino-containing sidechain of lysine and the carboxy-containing sidechain of glutamic acid are covalently joined, with loss of water, to form a lactam bridging moiety. In some embodiments, the lactam bridging moiety is formed between two amino acids that are spaced three, four, or five residues apart on the peptide. In some embodiments, the lactam bridging moiety is formed between two amino acids that are spaced four residues apart on the peptide.

Polypeptide intermediates & general methods of providing the present compounds

The compounds of this invention may be prepared or isolated in general by synthetic and/or semi-synthetic methods known to those skilled in the art for analogous compounds and by methods described in detail in the Examples, herein.

In the Schemes below, where a particular protecting group ("PG"), leaving group ("LG"), or transformation condition is depicted, one of ordinary skill in the art will appreciate that other protecting groups, leaving groups, and transformation conditions are also suitable and are contemplated. Such groups and transformations are described in detail in *March's Advanced*
5 *Organic Chemistry: Reactions, Mechanisms, and Structure*, M. B. Smith and J. March, 5th Edition, John Wiley & Sons, 2001, *Comprehensive Organic Transformations*, R. C. Larock, 2nd Edition, John Wiley & Sons, 1999, and *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of each of which is hereby incorporated herein by reference.

10 As used herein, the phrase "leaving group" (LG) includes, but is not limited to, halogens (e.g. fluoride, chloride, bromide, iodide), sulfonates (e.g. mesylate, tosylate, benzenesulfonate, brosylate, nosylate, triflate), diazonium, and the like.

As used herein, the phrase "oxygen protecting group" includes, for example, carbonyl protecting groups, hydroxyl protecting groups, etc. Hydroxyl protecting groups are well known
15 in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Examples of suitable hydroxyl protecting groups include, but are not limited to, esters (e.g., acetyl, benzyl), allyl ethers, ethers, silyl ethers, alkyl ethers, arylalkyl ethers, benzyl ethers and alkoxyalkyl ethers. Examples of such esters include formates, acetates,
20 carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate (trimethylacetyl), crotonate, 4-methoxy-crotonate, benzoate, p-benylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-
25 (phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl. Examples of such silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-
30 (trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of arylalkyl ethers include

benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, and 2- and 4-picoyl.

Amino protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Suitable amino protecting groups include, but are not limited to, aralkylamines, carbamates, cyclic imides, allyl amines, amides, and the like. Examples of such groups include t-butyloxycarbonyl (Boc), ethyloxycarbonyl, methyloxycarbonyl, trichloroethyloxycarbonyl, allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), allyl, phthalimide, benzyl (Bn), dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dmb), 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde), fluorenylmethylcarbonyl (Fmoc), formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, phenylacetyl, trifluoroacetyl, benzoyl, and the like.

In certain embodiments, the present invention also relates to synthetic peptide intermediates of disclosed glucagon analogs. In some embodiments, a polypeptide intermediate of the disclosure is an isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein at least one amino acid is covalently bound to a protecting group. In some embodiments, a polypeptide intermediate of the disclosure is an isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255 to 258, wherein at least one amino acid is covalently bound to a protecting group.

In some embodiments, a polypeptide intermediate of the disclosure is an isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 101 to 112, 115 to 139, 211, 212, 221, 225 to 235, 238, and 239, wherein at least one amino acid is covalently bound to a spacer as defined herein, wherein the spacer is further covalently bound to a protecting group or a hydrogen atom. In some embodiments, a polypeptide intermediate of the disclosure is an isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 101 to 112, 115 to 140, 142 to 147, 149 to 160, 211, 212, 221, 225 to 235, 238 to 245, 251, 252, and 255 to 258, wherein at least one amino acid is covalently bound to a spacer as defined herein, wherein the spacer is further covalently bound to a protecting group or a hydrogen atom. In some embodiments, the polypeptide intermediate comprises a lysine residue bound to a protecting group via the amino group of its sidechain. In some embodiments, the lysine residue is covalently bound to a Alloc or

ivDde. In some embodiments, the polypeptide intermediate comprises an aspartic acid residue bound to a protecting group via the carboxyl group of its sidechain. In some embodiments, the aspartic acid residue is covalently bound to an allyl group. In some embodiments, the polypeptide intermediate comprises a glutamic acid residue bound to a protecting group via the carboxyl group of its sidechain. In some embodiments, the glutamic acid residue is covalently bound to an allyl group.

Methods of Use

According to another embodiment, the invention relates to a method of treating metabolic disease or disorder in a subject in need of treatment, comprising providing the subject with an effective amount of a glucagon analog polypeptide of the disclosure or a pharmaceutical composition thereof. Metabolic diseases or disorders include type 1 diabetes, type 2 diabetes, and obesity. Additionally, the invention relates to a method of effecting weight loss in a subject, including a diabetic subject, comprising providing the subject with an effective amount of a glucagon analog polypeptide of the disclosure. In certain embodiments, the invention also relates to methods of treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH).

In some embodiments, provided is a method of treating obesity in a human subject, providing weight loss to the human subject, or suppressing appetite in the human subject, comprising administering to the subject any of the polypeptides disclosed herein, a pharmaceutical composition comprising any of the disclosed polypeptides, or an osmotic delivery device comprising any of the disclosed polypeptides.

In some embodiments, provided is a method of treating diabetes in a human subject, comprising administering to the subject any of the polypeptides disclosed herein, a pharmaceutical composition comprising any of the disclosed polypeptides, or an osmotic delivery device comprising any of the disclosed polypeptides. In some embodiments, the diabetes is type 1 diabetes. In some embodiments, the diabetes is type 2 diabetes.

In some embodiments, provided is a method of treating nonalcoholic fatty liver disease (NAFLD) in a human subject, comprising administering to the subject any of the polypeptides

disclosed herein, a pharmaceutical composition comprising any of the disclosed polypeptides, or an osmotic delivery device comprising any of the disclosed polypeptides.

5 In some embodiments, provided is a method of treating nonalcoholic steatohepatitis (NASH) in a human subject, comprising administering to the subject any of the polypeptides disclosed herein, a pharmaceutical composition comprising any of the disclosed polypeptides, or an osmotic delivery device comprising any of the disclosed polypeptides.

10 Glucagon analog polypeptides of the disclosure are particularly useful for the treatment of diabetes, the method comprising providing a diabetic subject with an effective amount of a glucagon analog polypeptide. In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of a subject with type 1 or type 2 diabetes to control, or reduce, concentrations of blood sugar in the subject, where blood sugar levels can be monitored or approximated based on measured blood concentrations of glycated hemoglobin (hemoglobin A1c, HbA1c).

- 15 (i) In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of a subject with type 1 diabetes;
- (ii) In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of a subject with type 2 diabetes;
- (iii) In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of obesity; and
- 20 (iv) In some embodiments, a glucagon analog polypeptide of the disclosure is used to provide weight loss to a subject, such as a diabetic subject,
- (v) In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of nonalcoholic fatty liver disease (NAFLD),
- 25 (vi) In some embodiments, a glucagon analog polypeptide of the disclosure is used for the treatment of nonalcoholic steatohepatitis (NASH),

wherein the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises any isolated polypeptide of this disclosure including those represented by any of the consensus sequences of SEQ ID NO: 1 through SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof.

30 In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises any isolated polypeptide of this disclosure including those represented by any of

the consensus sequences of SEQ ID NO: 1 through SEQ ID NO: 60, or a pharmaceutically acceptable salt thereof.

5 In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises any isolated polypeptide of this disclosure including those selected from the group consisting of SEQ ID NOs: 3, 6, 21, and 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises the isolated polypeptide of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises the isolated polypeptide of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises the isolated polypeptide of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide of usage (i), (ii), (iii), (iv), (v) or (vi) comprises the isolated polypeptide of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

15 In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 1 diabetes wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 1 diabetes wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 1 diabetes wherein the isolated polypeptide is of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 1 diabetes wherein the isolated polypeptide is of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

25 In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 2 diabetes wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 2 diabetes wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 2 diabetes wherein the isolated polypeptide is of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some

embodiments, the glucagon analog polypeptide is used for the treatment of a subject with type 2 diabetes wherein the isolated polypeptide is of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

5 In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with obesity wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with obesity wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with obesity wherein the isolated polypeptide is of SEQ ID
10 NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with obesity wherein the isolated polypeptide is of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucagon analog polypeptide is used to provide weight loss to a subject wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used to provide weight loss to a subject wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used to provide weight loss to a subject wherein the isolated polypeptide is of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used to provide
15 weight loss to a subject wherein the isolated polypeptide is of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NAFLD wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NAFLD wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NAFLD wherein the isolated polypeptide is of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NAFLD wherein the isolated polypeptide is
20 of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NASH wherein the isolated polypeptide is of SEQ ID NO: 3 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NASH wherein the isolated polypeptide is of SEQ ID NO: 6 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NASH wherein the isolated polypeptide is of SEQ ID NO: 21 or a pharmaceutically acceptable salt thereof. In some embodiments, the glucagon analog polypeptide is used for the treatment of a subject with NASH wherein the isolated polypeptide is of SEQ ID NO: 24 or a pharmaceutically acceptable salt thereof.

The terms "patient" or "subject" as used herein, refer to a rodent or an animal, preferably a mammal, and most preferably a human.

Combinations

In some embodiments, a glucagon analog polypeptide of the disclosure is co-formulated in combination with a second agent. In some embodiments, a glucagon analog polypeptide of the disclosure is co-formulated in combination with a second agent, wherein the second agent is an incretin mimetic. In some embodiments, a glucagon analog polypeptide of the disclosure is co-formulated in combination with a second agent, wherein the second agent is an insulinotropic compound.

The phrase "incretin mimetics" as used herein includes, but is not limited to GLP-1 peptide; GLP-1 (7-36); GLP-1 receptor agonists; peptide derivatives of GLP-1; peptide analogs of GLP-1; exenatide; exenatide having the amino acid sequence of exendin-4 (the naturally-occurring form of exenatide; exenatide-LAR; lixisenatide; liraglutide; semaglutide; dulaglutide; albiglutide; taspoglutide; tirzepatide (Eli Lilly's LY3298176 or Y-(Aib)-EGTFTSDYSH-(Aib)-LDKIAQ-[diacid- γ -Glu-(AEEA)₂-Lys]-AFVQWLIAGGPSSGAPPPS-NH₂) SEQ ID NO: 303); glucagon as well as peptide analogs and peptide derivatives thereof; glucagon like polypeptide-2 (GLP-2); PYY as well as peptide analogs and peptide derivatives thereof; PYY(3-36); oxyntomodulin as well as peptide analogs and peptide derivatives thereof; amylin as well as peptide analogs and peptide derivatives thereof; and gastric inhibitory peptide (GIP). Incretin mimetics are also referred to herein as "insulinotropic peptides."

Incretin mimetics which target the GLP-1 receptor are also known in the literature as “GLP-1 receptor agonists” or “GLP-1 agonists,” with both terms being used interchangeably herein.

Some embodiments of the present invention comprise use of a disclosed glucagon analog polypeptide of the present invention in combination with a second therapeutic agent, such as a second polypeptide, such as, by way of, non-limiting example, insulinotropic peptides. In some
5 embodiments, a pharmaceutical composition comprising a glucagon analog polypeptide in combination with a second agent is used to treat type 2 diabetes.

In some embodiments, provided is a pharmaceutical composition comprising any of the isolated polypeptides as disclosed herein. In some embodiments, provided is a pharmaceutical
10 composition comprising any of the isolated polypeptides as disclosed herein and further comprising a second polypeptide. In some embodiments, the second polypeptide is a PYY analog. In some embodiments, the second polypeptide is an amylin analog. In a preferred embodiment, the second polypeptide is a GLP-1 receptor agonist.

The term “GLP-1” refers to a polypeptide, glucagon-like peptide-1(7-36)amide, a 30-
15 residue peptide hormone released from intestinal L cells following nutrient consumption. GLP-1 has the amino acid sequence of (HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH₂), SEQ ID NO: 301. GLP-1 is a regulatory peptide that binds to the extracellular region of the GLP-1 receptor (GLP-1R), a G-coupled protein receptor on β cell and via adenylyl cyclase activity and production of cAMP stimulates the insulin response to the nutrients that are absorbed from the gut [Baggio
20 2007, “Biology of incretins: GLP-1 and GIP,” Gastroenterology, vol. 132(6):2131-57; Holst 2008, “The incretin system and its role in type 2 diabetes mellitus,” Mol Cell Endocrinology, vol. 297(1-2):127-36]. The effects of GLP-1R agonism are multiple. GLP-1 maintains glucose homeostasis by enhancing endogenous glucose dependent insulin secretion, rendering the β cells glucose competent and sensitive to GLP-1, suppressing glucagon release, restoring first and second phase
25 insulin secretion, slowing gastric emptying, decreasing food intake, and increasing satiety [Holst 2008 Mol. Cell Endocrinology; Kjems 2003 “The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects,” Diabetes, vol. 52(2): 380-86; Holst 2013 “Incretin hormones and the satiation signal,” Int J Obes (Lond), vol. 37(9):1161-69; Seufert 2014, “The extra-pancreatic effects of GLP-1 receptor agonists: a focus on
30 the cardiovascular, gastrointestinal and central nervous systems,” Diabetes Obes Metab, vol. 16(8): 673-88]. The risk of hypoglycemia is minimal given the mode of action of GLP-1.

Glucagon-like peptide-1(7-36)amide (GLP-1) is a 30-residue peptide hormone released from intestinal L cells following nutrient consumption. It potentiates the glucose-induced secretion of insulin from pancreatic beta cells, increases insulin expression, inhibits beta-cell apoptosis, promotes beta-cell neogenesis, reduces glucagon secretion, delays gastric emptying, promotes satiety and increases peripheral glucose disposal. These multiple effects have generated a great deal of interest in the discovery of long-lasting agonists of the GLP-1 receptor (GLP-1R) in order to treat type 2 diabetes. The term "exenatide" as used herein includes, but is not limited to exenatide, exenatide having the amino acid sequence of (HGE~~G~~TFTSDLSKQME~~E~~EA~~V~~RLFI~~E~~WLKNGGPSSGAPPPS-NH₂), SEQ ID NO: 302, native extendin-4, exenatide peptides, exenatide peptide analogs, and exenatide peptide derivatives.

Endogenous GLP-1 is released from the gut in response to nutrient ingestion. Following food intake and digestion, carbohydrates and fats appear in the lumen of the gut, which stimulate a so-called incretin effect, the release of incretins such as GLP-1 from intestinal L-cells. GLP-1, once released, targets the pancreas where it enhances secretion of insulin in a "glucose dependent manner." In other words, this GLP-1-mediated effect upon insulin persists when glucose levels are high yet safely dissipates as glucose levels fall. GLP-1 activity thus self-regulates to reduce the risk of hypoglycemia (the condition by which glucose levels drop dangerously low). Since GLP-1 has a short elimination half-life ($t_{1/2}$) of less than five minutes, this endogenous peptide is unsuitably short-lived for use as a therapeutic.

Synthetic analogs of GLP-1 have been designed to have longer half-lives and similarly enhance secretion of insulin in a glucose dependent manner like endogenous GLP-1, for use in the treatment of type 2 diabetes and for providing weight loss.

Numerous GLP-1 receptor agonists (e.g., GLP-1 peptide derivatives and peptide analogs) demonstrating insulintropic action are known in the art (see, e.g., U.S. Pat. Nos. 5,118,666; 5,120,712; 5,512,549; 5,545,618; 5,574,008; 5,574,008; 5,614,492; 5,958,909; 6,191,102; 6,268,343; 6,329,336; 6,451,974; 6,458,924; 6,514,500; 6,593,295; 6,703,359; 6,706,689; 6,720,407; 6,821,949; 6,849,708; 6,849,714; 6,887,470; 6,887,849; 6,903,186; 7,022,674; 7,041,646; 7,084,243; 7,101,843; 7,138,486; 7,141,547; 7,144,863; and 7,199,217), as well as in clinical trials (e.g., taspoglutide and albiglutide).

Certain GLP-1 receptor agonists, including Bydureon® (exenatide), marketed by AstraZeneca of Cambridge, U.K.; Trulicity® (dulaglutide), marketed by Eli Lilly and Co., of

Indianapolis, IN, U.S.A.; and Victoza® (liraglutide), Ozempic® (injectable semaglutide) & Rybelsus® (orally administered semaglutide), marketed by Novo Nordisk A/S of Bagsværd, Denmark, have each been approved by numerous regulatory authorities, including the United States Food and Drug Administration (U.S. FDA) and European Medicines Agency (EMA) for the treatment of patients suffering from type 2 diabetes. These marketed GLP-1 receptor agonists were developed and formulated for injectable and/or oral administration to patients. However, patient adherence to injectable and orally administered therapies for type 2 diabetes is notoriously poor which prohibits many patients from realizing a full and lasting therapeutic potential of GLP-1 receptor agonists. Many patients skip or cease periodic self-administrations of prescribed injectable and orally administered GLP-1 receptor agonists and thus fail to adequately treat and control their own type 2 diabetic condition.

In some embodiments, provided herein is a pharmaceutical combination comprising a glucagon analog polypeptide of the disclosure and a GLP-1 receptor agonist, or pharmaceutically acceptable salts thereof. In some embodiments, provided herein is a pharmaceutical combination comprising an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 60 and a GLP-1 receptor agonist, or pharmaceutically acceptable salts thereof. In some embodiments, provided herein is a pharmaceutical combination comprising an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 39 and a GLP-1 receptor agonist, or pharmaceutically acceptable salts thereof.

In some embodiments, the pharmaceutical combination comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 21, and 24 together with a GLP-1 receptor agonist, or pharmaceutically acceptable salts thereof. In some embodiments, the pharmaceutical combination comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 21, and 24 together with A303, or pharmaceutically acceptable salts thereof. In some embodiments, the pharmaceutical combination comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 6, 21, and 24 together with A304, or pharmaceutically acceptable salts thereof.

In some embodiments, the pharmaceutical combination comprises an amino acid sequence of SEQ ID NO: 3 and a GLP-1 receptor agonist, or a pharmaceutically acceptable salts thereof. In some embodiments, the pharmaceutical combination comprises an amino acid sequence of SEQ ID NO: 6 and a GLP-1 receptor agonist, or a pharmaceutically acceptable salts

thereof. In some embodiments, the pharmaceutical combination comprises an amino acid sequence of SEQ ID NO: 21 and a GLP-1 receptor agonist, or a pharmaceutically acceptable salts thereof. In some embodiments, the pharmaceutical combination comprises an amino acid sequence of SEQ ID NO: 24 and a GLP-1 receptor agonist, or a pharmaceutically acceptable salts thereof.

The glucagon analog polypeptides of the disclosure in combination with a GLP-1 receptor agonist have been found to deliver bariatric surgery-like efficacy for weight loss. The observation that improved glycemic control following Roux-en-Y gastric bypass surgery (RYGB) in obese or obese T2D patients precedes the weight loss seen following RYGB, suggests that surgical rearrangement of the gut leads to physiological adaptations beyond those driven by weight loss alone. Indeed, RYGB leads to enhanced post-prandial secretion of GLP-1 and glucagon. The gut peptide hormones GLP-1 and glucagon each play a role in whole body energy balance through several overlapping biological responses to energy input. These responses principally include potentiation of glucose-induced insulin secretion, inhibition of gastric emptying, inducing satiety, and inhibition of food intake.

Accordingly, combinations of a glucagon analog polypeptide of the disclosure together with a GLP-1 receptor agonist are suitable for the treatment of the diseases and disorders disclosed herein. In some embodiments, the GLP-1 receptor agonist is a long acting GLP-1 receptor agonist.

Oxyntomodulin is a naturally occurring 37 amino acid peptide hormone found in the colon that has been found to suppress appetite and facilitate weight loss (Wynne K, et al., *Int J Obes (Lond)* 30(12):1729-36(2006)). The sequence of oxyntomodulin, as well as peptide analogs and peptide derivatives thereof, are known in the art (e.g., Bataille D, et al., *Peptides* 2(Suppl 2):41-44 (1981); and U.S. Patent Publication Nos. 2005/0070469 and 2006/0094652).

Gastric Inhibitory Peptide (GIP) is an insulinotropic peptide hormone (Efendic, S., et al., *Horm Metab Res.* 36:742-6 (2004)) and is secreted by the mucosa of the duodenum and jejunum in response to absorbed fat and carbohydrate that stimulate the pancreas to secrete insulin. GIP circulates as a biologically active 42-amino acid peptide. GIP is also known as glucose-dependent insulinotropic protein. GIP is a 42-amino acid gastrointestinal regulatory peptide that stimulates insulin secretion from pancreatic beta cells in the presence of glucose (Tseng, C., et al., *PNAS* 90:1992-1996 (1993)). The sequence of GIP, as well as peptide analogs and peptide derivatives

thereof, are known in the art (e.g., Meier J. J., Diabetes Metab Res Rev. 21(2):91-117 (2005) and Efendic S., Horm Metab Res. 36(11-12):742-6 (2004)).

Glucagon is a peptide hormone, produced by alpha cells of the pancreas, which raises the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers the glucose concentration. The pancreas releases glucagon when the concentration of glucose in the bloodstream falls too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.

Human amylin, or islet amyloid polypeptide (IAPP), is a 37-residue polypeptide hormone. Amylin is co-secreted with insulin from pancreatic β -cells in the ratio of approximately 100:1 (insulin:amylin). Pro-islet amyloid polypeptide (i.e., pro-IAPP) is produced in the pancreatic β -cells as a 67 amino acid, 7404 Dalton pro-peptide that undergoes post-translational modifications including protease cleavage to produce the 37-residue amylin. Loss of β -cell function that occurs early in type 1 diabetes and can occur late in type 2 diabetes leads to deficiencies in the secretion of insulin and amylin.

Amylin functions as part of the endocrine pancreas, those cells within the pancreas that synthesize and secrete hormones. Amylin contributes to glycemic control; it is secreted from the pancreatic islets into the blood circulation and is cleared by peptidases in the kidney. Amylin's metabolic function is well-characterized as an inhibitor of the appearance of nutrients, such as glucose, in the plasma. It thus functions as a synergistic partner to insulin, a peptide that regulates blood glucose levels and coordinates the body's distribution and uptake of glucose. Insulin's role in the body is, among other things, to prevent blood glucose levels from rising too high, particularly after a meal.

Amylin is believed to play a role in glycemic regulation by slowing gastric emptying and promoting satiety (i.e., feeling of fullness), thereby preventing post-prandial (i.e., after-meal) spikes in blood glucose levels. The overall effect is to slow the rate of appearance of glucose in the blood after eating. Amylin also lowers the secretion of glucagon by the pancreas. Glucagon's role in the body is, among other things, to prevent blood glucose levels dropping too low. This is significant because certain type 1 diabetes, for example, are prone to secrete excess amounts of the blood glucose-raising glucagon just after meals.

For numerous reasons, human amylin, having a half-life in serum of about 13 minutes, is not amenable for use as a therapeutic agent. Rather, pramlintide (Symlin®, developed by Amylin Pharmaceuticals, Inc., San Diego, CA, USA and marketed by AstraZeneca plc, Cambridge, UK) was developed as a synthetic analogue of human amylin for the treatment of patients with types 1 or 2 diabetes, who use meal-time insulin but cannot achieve desired glycemic control despite optimal insulin therapy. Pramlintide differs from human amylin in 3 of its 37 amino acids. These modifications provide pramlintide a longer half-life of approximately 48 minutes in humans and reduce its propensity to aggregate, a characteristic found of human amylin. Further analogues of human amylin have been disclosed such as those in U.S. Patent Application No. 16/598,915 (corresponding to PCT International Application No. PCT/US2019/055696), both filed October 10, 2019.

Implantable Delivery

In some embodiments, provided is an osmotic delivery device, as described herein, comprising any of the long acting glucagon analog polypeptides, as disclosed herein, or a pharmaceutical composition comprising any of the long acting glucagon analog polypeptides.

In some embodiments, the osmotic delivery device comprises an impermeable reservoir comprising interior and exterior surfaces and first and second open ends; a semi-permeable membrane in sealing relationship with the first open end of the reservoir; an osmotic engine within the reservoir and adjacent the semi-permeable membrane; a piston adjacent the osmotic engine, wherein the piston forms a movable seal with the interior surface of the reservoir, the piston divides the reservoir into a first chamber and a second chamber, the first chamber comprising the osmotic engine; a suspension formulation, wherein the second chamber comprises the suspension formulation and the suspension formulation is flowable and comprises the isolated polypeptide; and a diffusion moderator inserted in the second open end of the reservoir, the diffusion moderator adjacent the suspension formulation.

An implantable, osmotic delivery device typically includes a reservoir having at least one orifice through which the suspension formulation is delivered. The suspension formulation may be stored within the reservoir. In a preferred embodiment, the implantable, drug delivery device is an osmotic delivery device, wherein delivery of the drug is osmotically driven. Some osmotic

delivery devices and their component parts have been described, for example, the DUROS® delivery device or similar devices (see, e.g., U.S. Pat. Nos. 5,609,885; 5,728,396; 5,985,305; 5,997,527; 6,113,938; 6,132,420; 6,156,331; 6,217,906; 6,261,584; 6,270,787; 6,287,295; 6,375,978; 6,395,292; 6,508,808; 6,544,252; 6,635,268; 6,682,522; 6,923,800; 6,939,556; 5 6,976,981; 6,997,922; 7,014,636; 7,207,982; and 7,112,335; 7,163,688; U.S. Patent Publication Nos. 2005/0175701, 2007/0281024, 2008/0091176, and 2009/0202608).

The osmotic delivery device typically consists of a cylindrical reservoir which contains the osmotic engine, piston, and drug formulation. The reservoir is capped at one end by a controlled-rate, semi-permeable membrane and capped at the other end by a diffusion moderator through
10 which suspension formulation, comprising the drug, is released from the drug reservoir. The piston separates the drug formulation from the osmotic engine and utilizes a seal to prevent the water in the osmotic engine compartment from entering the drug reservoir. The diffusion moderator is designed, in conjunction with the drug formulation, to prevent body fluid from entering the drug reservoir through the orifice.

15 The osmotic device releases a drug at a predetermined rate based on the principle of osmosis. Extracellular fluid enters the osmotic delivery device through a semi-permeable membrane directly into a salt engine that expands to drive the piston at a slow and even delivery rate. Movement of the piston forces the drug formulation to be released through the orifice or exit port at a predetermined shear rate. In one embodiment of the present invention, the reservoir of the
20 osmotic device is loaded with a suspension formulation wherein the device is capable of delivering the suspension formulation to a subject over an extended period of time (e.g., about 1, about 3, about 6, about 9, about 10, and about 12 months) at a pre-determined, therapeutically effective delivery rate.

The release rate of the drug from the osmotic delivery device typically provides a subject
25 with a predetermined target dose of a drug, for example, a therapeutically effective daily dose delivered over the course of a day; that is, the release rate of the drug from the device, provides substantial steady-state delivery of the drug at a therapeutic concentration to the subject.

Typically, for an osmotic delivery device, the volume of a beneficial agent chamber comprising the beneficial agent formulation is between about 100 μ l to about 1000 μ l, more
30 preferably between about 120 μ l and about 500 μ l, more preferably between about 150 μ l and about 200 μ l.

Typically, the osmotic delivery device is implanted within the subject, for example, subdermally or subcutaneously to provide subcutaneous drug delivery. The device(s) can be implanted subdermally or subcutaneously into either or both arms (e.g., in the inside, outside, or back of the upper arm) or the abdomen. Preferred locations in the abdominal area are under the abdominal skin in the area extending below the ribs and above the belt line. To provide a number of locations for implantation of one or more osmotic delivery device within the abdomen, the abdominal wall can be divided into 4 quadrants as follows: the upper right quadrant extending at least 2-3 centimeters below the right ribs, e.g., at least about 5-8 centimeters below the right ribs, and at least 2-3 centimeters to the right of the midline, e.g., at least about 5-8 centimeters to the right of the midline; the lower right quadrant extending at least 2-3 centimeters above the belt line, e.g., at least about 5-8 centimeters above the belt line, and at least 2-3 centimeters to the right of the midline, e.g., at least about 5-8 centimeters to the right of the midline; the upper left quadrant extending at least 2-3 centimeters below the left ribs, e.g., at least about 5-8 centimeters below the left ribs, and at least 2-3 centimeters to the left of the midline, e.g., at least about 5-8 centimeters to the left of the midline; and the lower left quadrant extending at least 2-3 centimeters above the belt line, e.g., at least about 5-8 centimeters above the belt line, and at least 2-3 centimeters to the left of the midline, e.g., at least about 5-8 centimeters to the left of the midline. This provides multiple available locations for implantation of one or more devices on one or more occasions. Implantation and removal of osmotic delivery devices are generally carried out by medical professionals using local anesthesia (e.g., lidocaine).

Termination of treatment by removal of an osmotic delivery device from a subject is straightforward, and provides the important advantage of immediate cessation of delivery of the drug to the subject.

Preferably, the osmotic delivery device has a fail-safe mechanism to prevent an inadvertent excess or bolus delivery of drug in a theoretical situation like the plugging or clogging of the outlet (diffusion moderator) through which the drug formulation is delivered. To prevent an inadvertent excess or bolus delivery of drug the osmotic delivery device is designed and constructed such that the pressure needed to partially or wholly dislodge or expel the diffusion moderator from the reservoir exceeds the pressure needed to partially or wholly dislodge or expel the semi-permeable membrane to the extent necessary to de-pressurize the reservoir. In such a scenario, pressure would build within the device until it would push the semi-permeable membrane at the other end outward,

thereby releasing the osmotic pressure. The osmotic delivery device would then become static and no longer deliver the drug formulation provided that the piston is in a sealing relationship with the reservoir.

A dose and delivery rate can be selected to achieve a desired blood concentration of a drug generally within less than about 6 half-lives of the drug within the subject after implantation of the device. The blood concentration of the drug is selected to give the optimal therapeutic effects of the drug while avoiding undesirable side effects that may be induced by excess concentration of the drug, while at the same time avoiding peaks and troughs that may induce side effects associated with peak or trough plasma concentrations of the drug.

The suspension formulations may also be used in infusion pumps, for example, the ALZET® (DURECT Corporation, Cupertino, Calif.) osmotic pumps which are miniature, infusion pumps for the continuous dosing of laboratory animals (e.g., mice and rats).

Modes of administration

In some embodiments, the method comprises providing a glucagon analog polypeptide of the disclosure or a pharmaceutical composition thereof, to a subject in need of treatment, via injection. In some embodiments, the method comprises providing a glucagon analog polypeptide of the disclosure or a pharmaceutical composition thereof, formulated for oral administration, to a subject in need of treatment.

In some embodiments, the method comprises providing a glucagon analog polypeptide of the disclosure or a pharmaceutical composition thereof, to a subject in need of treatment, via implantation. In some embodiments, the method comprises providing continuous delivery of a glucagon analog polypeptide, to a subject in need of treatment, from an osmotic delivery device. The delivery device, such as an osmotic delivery device, comprises sufficient glucagon analog polypeptide of the disclosure for continuous administration for up to 3 months, 6 months, 9 months, 12 months, 18 months or 24 months. As such, continuous administration of a glucagon analog polypeptide of the disclosure via osmotic delivery device eliminates daily, or multiple daily dosing of marketed glucagon analog polypeptides.

The substantial steady-state delivery of the glucagon analog polypeptide from the osmotic delivery device is continuous over an administration period. In some embodiments, the subject or patient is a human subject or human patient.

5 In some embodiments of the present invention, the administration period is, for example, at least about 3 months, at least about 3 months to about a year, at least about 4 months to about a year, at least about 5 months to about a year, at least about 6 months to about a year, at least about 8 months to about a year, at least about 9 months to about a year, at least about 10 months to about a year, at least about one year to about two years, at least about two years to about three years.

10 In further embodiments, the treatment methods of the present invention provide significant decrease in the subject's fasting plasma glucose concentration after implantation of the osmotic delivery device in the subject (relative to the subject's fasting plasma glucose concentration before implantation of the osmotic delivery device) that is achieved within about 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, 1 day or less after implantation of the osmotic delivery device in the subject. The significant decrease in fasting plasma glucose is typically statistically significant as demonstrated by application of an appropriate statistical test or is considered significant for the
15 subject by a medical practitioner. A significant decrease in fasting plasma glucose relative to the baseline before implantation is typically maintained over the administration period.

In some embodiments, the present invention relates to a method of treating a disease or condition in a subject in need of treatment. The method comprises providing continuous delivery
20 of a drug from an osmotic delivery device, wherein substantial steady-state delivery of the drug at therapeutic concentrations is achieved in the subject. The substantial steady-state delivery of the drug from the osmotic delivery device is continuous over an administration period of at least about 3 months. The drug has a known or determined half-life in a typical subject. Humans are preferred subjects for the practice of the present invention. The present invention includes a drug effective
25 for treatment of the disease or condition, as well as an osmotic delivery device comprising the drug for use in the present methods of treating the disease or condition in a subject in need of treatment. Advantages of the present invention include mitigation of peak-associated drug toxicities and attenuation of sub-optimal drug therapy associated with troughs.

In some embodiments, the substantial steady-state delivery of a drug at therapeutic
30 concentrations is achieved within a period of about 1 month, 7 days, 5 days, 3 days or 1 day after implantation of the osmotic delivery device in the subject.

The invention also provides a method for promoting weight loss in a subject in need thereof, a method for treating excess weight or obesity in a subject in need thereof, and/or a method for suppressing appetite in a subject in need thereof. The method comprises providing delivery of an isolated glucagon analog polypeptide. In some embodiments, the isolated glucagon analog polypeptide is continuously delivered from an implantable osmotic delivery device. In some
5 embodiments, substantial steady-state delivery of the glucagon analog polypeptide from the osmotic delivery device is achieved and is substantially continuous over an administration period. In some embodiments, the subject is human.

The present invention includes an osmotic delivery device comprising a glucagon analog polypeptide for use in the present methods in a subject in need of treatment. The subject may have
10 type 2 diabetes. The subject in need thereof may have a baseline HbA1c % of greater than 10.0%, i.e., a high baseline (HBL) subject. The subject may not have previously received a drug for treating type 2 diabetes mellitus.

In further embodiments, the treatment methods of the present invention provide significant
15 decrease in the subject's fasting plasma glucose concentration after implantation of the osmotic delivery device in the subject (relative to the subject's fasting plasma glucose concentration before implantation of the osmotic delivery device) that is achieved within about 7 days or less after implantation of the osmotic delivery device in the subject, within about 6 days or less after implantation of the osmotic delivery device in the subject, within about 5 days or less after
20 implantation of the osmotic delivery device in the subject, within about 4 days or less after implantation of the osmotic delivery device in the subject, within about 3 days or less after implantation of the osmotic delivery device in the subject, within about 2 days or less after implantation of the osmotic delivery device in the subject, or within about 1 day or less after implantation of the osmotic delivery device in the subject. In preferred embodiments of the present
25 invention, the significant decrease in the subject's fasting plasma glucose concentration after implantation of the osmotic delivery device, relative to the subject's fasting plasma glucose concentration before implantation, is achieved within about 2 days or less, preferably within about 1 day or less after implantation of the osmotic delivery device in the subject, or more preferably within about 1 day after implantation of the osmotic delivery device in the subject. The significant
30 decrease in fasting plasma glucose is typically statistically significant as demonstrated by application of an appropriate statistical test or is considered significant for the subject by a medical

practitioner. A significant decrease in fasting plasma glucose relative to the baseline before implantation is typically maintained over the administration period.

In embodiments of all aspects of the present invention relating to methods of treating a disease or condition in a subject, an exemplary osmotic delivery device comprises the following:

5 an impermeable reservoir comprising interior and exterior surfaces and first and second open ends; a semi-permeable membrane in sealing relationship with the first open end of the reservoir; an osmotic engine within the reservoir and adjacent the semi-permeable membrane; a piston adjacent the osmotic engine, wherein the piston forms a movable seal with the interior surface of the reservoir, the piston divides the reservoir into a first chamber and a second chamber, the first

10 chamber comprising the osmotic engine; a drug formulation or suspension formulation comprising the drug, wherein the second chamber comprises the drug formulation or suspension formulation and the drug formulation or suspension formulation is flowable; and a diffusion moderator inserted in the second open end of the reservoir, the diffusion moderator adjacent the suspension formulation. In preferred embodiments, the reservoir comprises titanium or a titanium alloy.

15 In embodiments of all aspects of the present invention relating to methods of treating a disease or condition in a subject, the drug formulation can comprise the drug and a vehicle formulation. Alternatively, suspension formulations are used in the methods and can, for example, comprise a particle formulation comprising the drug and a vehicle formulation. Vehicle formulations for use in forming the suspension formulations of the present invention can, for

20 example, comprise a solvent and a polymer.

The reservoir of the osmotic delivery devices may, for example, comprise titanium or a titanium alloy.

In embodiments of all aspects of the present invention the implanted osmotic delivery device can be used to provide subcutaneous delivery.

25 In embodiments of all aspects of the present invention the continuous delivery can, for example, be zero-order, controlled continuous delivery.

Pharmaceutical compositions

According to another embodiment, the invention provides a pharmaceutical composition comprising a compound, *i.e.*, isolated polypeptide, of this invention or a pharmaceutically acceptable derivative thereof and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

5 In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed polypeptides formulated as pharmaceutically acceptable salts thereof, such as a trifluoroacetate salt, acetate salt or hydrochloride salt. In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed polypeptides formulated as a trifluoroacetate salt. In some embodiments, provided is a pharmaceutical composition comprising
10 any of the disclosed polypeptides formulated as an acetate salt. In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed polypeptides formulated as a hydrochloride salt.

The term "pharmaceutically acceptable carrier, adjuvant, or vehicle" refers to a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound
15 with which it is formulated.

As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, polymers, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples
20 of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can
25 also be incorporated into the compositions.

Representative pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such
30 as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen

phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

5 In some embodiments, provided is a pharmaceutical composition comprising a pharmaceutically acceptable derivative of any of the disclosed polypeptides formulated as pharmaceutically acceptable salts thereof, such as a trifluoroacetate salt, acetate salt or hydrochloride salt. A "pharmaceutically acceptable derivative" means any non-toxic salt, ester, salt of an ester or other derivative of a compound of this invention that, upon administration to a
10 recipient, is capable of providing, either directly or indirectly, a compound of this invention or an active metabolite or residue thereof.

The pharmaceutical composition comprises a drug and may be formulated as a "particle formulation" as described in greater detail below. The pharmaceutical composition and/or particle formulation may include stabilizing components (also referred to herein as "excipients").
15 Examples of stabilizing components include, but are not limited to, carbohydrates, antioxidants, amino acids, buffers, inorganic compounds, and surfactants.

The amount of compound in compositions of this invention is such that is effective to measurably activate one or more glucagon receptors (*e.g.*, human, rat, monkey *etc.*), in a biological sample or in a patient. In certain embodiments, the amount of compound in compositions of this
20 invention is such that is effective to measurably agonize the human glucagon receptor in the absence or presence of human serum albumin, in a biological sample or in a patient. In certain embodiments, a composition of this invention is formulated for administration to a patient in need of such composition. In some embodiments, a composition of this invention is formulated for injectable administration to a patient. In some embodiments, a composition of this invention is
25 formulated for administration to a patient via an implantable delivery device such as an osmotic deliver device.

The isolated polypeptides of the disclosure (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the isolated
30 polypeptide, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, (e.g., intravenous, intradermal, subdermal, subcutaneous), oral (e.g., inhalation), transdermal (i.e., topical), transmucosal, rectal, or combinations thereof. In some embodiments, a pharmaceutical composition or an isolated polypeptide of the disclosure is formulated for administration by topical administration. In some embodiments, a pharmaceutical composition or an isolated polypeptide of the disclosure is formulated for administration by inhalation administration. In some embodiments, the pharmaceutical composition is formulated for administration by a device or other suitable delivery mechanism that is suitable for subdermal or subcutaneous implantation and delivers the pharmaceutical composition subcutaneously. In some embodiments, the pharmaceutical composition is formulated for administration by an implant device that is suitable for subdermal or subcutaneous implantation and delivers the pharmaceutical composition subcutaneously. In some embodiments, the pharmaceutical composition is formulated for administration by an osmotic delivery device, e.g., an implantable osmotic delivery device, that is suitable for subdermal or subcutaneous placement or other implantation and delivers the pharmaceutical composition subcutaneously. Solutions or suspensions used for parenteral application, intradermal application, subdermal application, subcutaneous application, or combinations thereof can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage

and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a
5 coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition.
10 Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by
15 incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

20 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and
25 expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose
30 or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For
5 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels,
10 or creams as generally known in the art.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen,
15 polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit
20 form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the
25 dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser
30 together with instructions for administration.

Drug Particle Formulations

Compounds, i.e., isolated polypeptides or pharmaceutically acceptable salts thereof, for use in the practice of the present invention are typically added to particle formulations, which are used to make polypeptide-containing particles that are uniformly suspended, dissolved or dispersed in a suspension vehicle to form a suspension formulation. In some embodiments, the glucagon analog polypeptide is formulated in a particle formulation and converted (e.g., spray dried) to particles. In some embodiments, the particles comprising the glucagon analog polypeptide are suspended in a vehicle formulation, resulting in a suspension formulation of vehicle and suspended particles comprising the glucagon analog polypeptide.

Preferably, particle formulations are formable into particles using processes such as spray drying, lyophilization, desiccation, freeze-drying, milling, granulation, ultrasonic drop creation, crystallization, precipitation, or other techniques available in the art for forming particles from a mixture of components. In one embodiment of the invention the particles are spray dried. The particles are preferably substantially uniform in shape and size.

In some embodiments, the present invention provides drug particle formulations for pharmaceutical use. The particle formulation typically comprises a drug and includes one or more stabilizing component (also referred to herein as "excipients"). Examples of stabilizing components include, but are not limited to, carbohydrates, antioxidants, amino acids, buffers, inorganic compounds, and surfactants. The amounts of stabilizers in the particle formulation can be determined experimentally based on the activities of the stabilizers and the desired characteristics of the formulation, in view of the teachings of the present specification.

In any of the embodiments, the particle formulation may comprise about 50 wt % to about 90 wt % drug, about 50 wt % to about 85 wt % drug, about 55 wt % to about 90 wt % drug, about 60 wt % to about 90 wt % drug, about 65 wt % to about 85 wt % drug, about 65 wt % to about 90 wt % drug, about 70 wt % to about 90 wt % drug, about 70 wt % to about 85 wt % drug, about 70 wt % to about 80 wt % drug, or about 70 wt % to about 75 wt % drug.

Typically, the amount of carbohydrate in the particle formulation is determined by aggregation concerns. In general, the carbohydrate amount should not be too high so as to avoid promoting crystal growth in the presence of water due to excess carbohydrate unbound to drug.

Typically, the amount of antioxidant in the particle formulation is determined by oxidation concerns, while the amount of amino acid in the formulation is determined by oxidation concerns and/or formability of particles during spray drying.

Typically, the amount of buffer in the particle formulation is determined by pre-processing concerns, stability concerns, and formability of particles during spray drying. Buffer may be required to stabilize drug during processing, e.g., solution preparation and spray drying, when all stabilizers are solubilized.

Examples of carbohydrates that may be included in the particle formulation include, but are not limited to, monosaccharides (e.g., fructose, maltose, galactose, glucose, D-mannose, and sorbose), disaccharides (e.g., lactose, sucrose, trehalose, and cellobiose), polysaccharides (e.g., raffinose, melezitose, maltodextrins, dextrans, and starches), and alditols (acyclic polyols; e.g., mannitol, xylitol, maltitol, lactitol, xylitol sorbitol, pyranosyl sorbitol, and myoinositol). Suitable carbohydrates include disaccharides and/or non-reducing sugars, such as sucrose, trehalose, and raffinose.

Examples of antioxidants that may be included in the particle formulation include, but are not limited to, methionine, ascorbic acid, sodium thiosulfate, catalase, platinum, ethylenediaminetetraacetic acid (EDTA), citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate. Further, amino acids that readily oxidize can be used as antioxidants, for example, cysteine, methionine, and tryptophan.

Examples of amino acids that may be included in the particle formulation include, but are not limited to, arginine, methionine, glycine, histidine, alanine, leucine, glutamic acid, iso-leucine, L-threonine, 2-phenylamine, valine, norvaline, proline, phenylalanine, tryptophan, serine, asparagines, cysteine, tyrosine, lysine, and norleucine. Suitable amino acids include those that readily oxidize, e.g., cysteine, methionine, and tryptophan.

Examples of buffers that may be included in the particle formulation include, but are not limited to, citrate, histidine, succinate, phosphate, maleate, tris, acetate, carbohydrate, and gly-gly. Suitable buffers include citrate, histidine, succinate, and tris.

Examples of inorganic compounds that may be included in the particle formulation include, but are not limited to, NaCl, Na₂SO₄, NaHCO₃, KCl, KH₂PO₄, CaCl₂, and MgCl₂.

In addition, the particle formulation may include other stabilizers/excipients, such as surfactants and salts. Examples of surfactants include, but are not limited to, Polysorbate 20, Polysorbate 80, PLURONIC® (BASF Corporation, Mount Olive, N.J.) F68, and sodium dodecyl sulfate (SDS). Examples of salts include, but are not limited to, sodium chloride, calcium chloride, and magnesium chloride.

The particles are typically sized such that they can be delivered via an implantable osmotic delivery device. Uniform shape and size of the particles typically helps to provide a consistent and uniform rate of release from such a delivery device; however, a particle preparation having a non-normal particle size distribution profile may also be used. For example, in a typical implantable osmotic delivery device having a delivery orifice, the size of the particles is less than about 30%, more preferably is less than about 20%, more preferably is less than about 10%, of the diameter of the delivery orifice. In an embodiment of the particle formulation for use with an osmotic delivery system, wherein the delivery orifice diameter of the implant is about 0.5 mm, particle sizes may be, for example, less than about 150 microns to about 50 microns. In an embodiment of the particle formulation for use with an osmotic delivery system, wherein the delivery orifice diameter of the implant is about 0.1 mm, particle sizes may be, for example, less than about 30 microns to about 10 microns. In one embodiment, the orifice is about 0.25 mm (250 microns) and the particle size is about 2 microns to about 5 microns.

Those of ordinary skill in the art will appreciate that a population of particles follow principles of particle size distribution. Widely used, art-recognized methods of describing particle size distributions include, for example, average diameters and D values, such as the D50 value, which is commonly used to represent the mean diameter of the range of the particle sizes of a given sample.

Particles of a particle formulation have diameters of between about 2 microns to about 150 micron, e.g., less than 150 microns in diameter, less than 100 microns in diameter, less than 50 microns in diameter, less than 30 microns in diameter, less than 10 microns in diameter, less than 5 microns in diameter, and about 2 microns in diameter. Preferably, particles have diameters of between about 2 microns and about 50 microns.

Particles of a particle formulation comprising an isolated glucagon analog polypeptide have average diameters of between about 0.3 microns to about 150 microns. Particles of a particle formulation comprising an isolated glucagon analog polypeptide have average diameters of

between about 2 microns to about 150 microns, e.g., less than 150 microns in average diameter, less than 100 microns in average diameter, less than 50 microns in average diameter, less than 30 microns in average diameter, less than 10 microns in average diameter, less than 5 microns in average diameter, and about 2 microns in average diameter. In some embodiments, particles have
5 average diameters of between about 0.3 microns and 50 microns, for example, between about 2 microns and about 50 microns. In some embodiments, the particles have an average diameter between 0.3 microns and 50 microns, for example, between about 2 microns and about 50 microns, where each particle is less than about 50 microns in diameter.

Typically, the particles of the particle formulations, when incorporated in a suspension
10 vehicle, do not settle in less than about 3 months, preferably do not settle in less than about 6 months, more preferably do not settle in less than about 12 months, more preferably do not settle in less than about 24 months at delivery temperature, and most preferably do not settle in less than about 36 months at delivery temperature. The suspension vehicles typically have a viscosity of between about 5,000 to about 30,000 poise, preferably between about 8,000 to about 25,000 poise,
15 more preferably between about 10,000 to about 20,000 poise. In one embodiment, the suspension vehicle has a viscosity of about 15,000 poise, plus or minus about 3,000 poise. Generally speaking, smaller particles tend to have a lower settling rate in viscous suspension vehicles than larger particles. Accordingly, micron- to nano-sized particles are typically desirable. In viscous suspension formulation, particles of about 2 microns to about 7 microns of the present invention
20 will not settle for at least 20 years at room temperature based on simulation modeling studies. In an embodiment of the particle formulation of the present invention, for use in an implantable osmotic delivery device, comprises particles of sizes less than about 50 microns, more preferably less than about 10 microns, more preferably in a range from about 2 microns to about 7 microns.

In summary, disclosed polypeptides, or pharmaceutically acceptable salts thereof, are
25 formulated into dried powders in solid state particles, which preserve maximum chemical and biological stability of the drug. Particles offers long-term storage stability at high temperature, and therefore, allows delivery to a subject of stable and biologically effective drug for extended periods of time. Particles are suspended in suspension vehicles for administration to patients.

Particle suspensions in vehicles

In one aspect, the suspension vehicle provides a stable environment in which the drug particle formulation is dispersed. The drug particle formulations are chemically and physically stable (as described above) in the suspension vehicle. The suspension vehicle typically comprises one or more polymer and one or more solvent that form a solution of sufficient viscosity to uniformly suspend the particles comprising the drug. The suspension vehicle may comprise further components, including, but not limited to, surfactants, antioxidants, and/or other compounds soluble in the vehicle.

The viscosity of the suspension vehicle is typically sufficient to prevent the drug particle formulation from settling during storage and use in a method of delivery, for example, in an implantable, osmotic delivery device. The suspension vehicle is biodegradable in that the suspension vehicle disintegrates or breaks down over a period of time in response to a biological environment, while the drug particle is dissolved in the biological environment and the active pharmaceutical ingredient (i.e., the drug) in the particle is absorbed.

In embodiments, the suspension vehicle is a “single-phase” suspension vehicle, which is a solid, semisolid, or liquid homogeneous system that is physically and chemically uniform throughout.

The solvent in which the polymer is dissolved may affect characteristics of the suspension formulation, such as the behavior of drug particle formulation during storage. A solvent may be selected in combination with a polymer so that the resulting suspension vehicle exhibits phase separation upon contact with the aqueous environment. In some embodiments of the invention, the solvent may be selected in combination with the polymer so that the resulting suspension vehicle exhibits phase separation upon contact with the aqueous environment having less than approximately about 10% water.

The solvent may be an acceptable solvent that is not miscible with water. The solvent may also be selected so that the polymer is soluble in the solvent at high concentrations, such as at a polymer concentration of greater than about 30%. Examples of solvents useful in the practice of the present invention include, but are not limited to, lauryl alcohol, benzyl benzoate, benzyl alcohol, lauryl lactate, decanol (also called decyl alcohol), ethyl hexyl lactate, and long chain (C8 to C24) aliphatic alcohols, esters, or mixtures thereof. The solvent used in the suspension vehicle

may be “dry,” in that it has a low moisture content. Preferred solvents for use in formulation of the suspension vehicle include lauryl lactate, lauryl alcohol, benzyl benzoate, and mixtures thereof.

Examples of polymers for formulation of the suspension vehicles of the present invention include, but are not limited to, a polyester (e.g., polylactic acid and polylacticpolyglycolic acid), a
5 polymer comprising pyrrolidones (e.g., polyvinylpyrrolidone having a molecular weight ranging from approximately 2,000 to approximately 1,000,000), ester or ether of an unsaturated alcohol (e.g., vinyl acetate), polyoxyethylenepolyoxypropylene block copolymer, or mixtures thereof. Polyvinylpyrrolidone can be characterized by its K-value (e.g., K-17), which is a viscosity index. In one embodiment, the polymer is polyvinylpyrrolidone having a molecular weight of 2,000 to
10 1,000,000. In a preferred embodiment, the polymer is polyvinylpyrrolidone K-17 (typically having an approximate average molecular weight range of 7,900-10,800). The polymer used in the suspension vehicle may include one or more different polymers or may include different grades of a single polymer. The polymer used in the suspension vehicle may also be dry or have a low moisture content.

15 Generally speaking, a suspension vehicle for use in the present invention may vary in composition based on the desired performance characteristics. In one embodiment, the suspension vehicle may comprise about 40 wt % to about 80 wt % polymer(s) and about 20 wt % to about 60 wt % solvent(s). Preferred embodiments of a suspension vehicle include vehicles formed of polymer(s) and solvent(s) combined at the following ratios: about 25 wt % solvent and about 75
20 wt % polymer; about 50 wt % solvent and about 50 wt % polymer; about 75 wt % solvent and about 25 wt % polymer. Accordingly, in some embodiments, the suspension vehicle may comprise selected components and in other embodiments consist essentially of selected components.

The suspension vehicle may exhibit Newtonian behavior. The suspension vehicle is typically formulated to provide a viscosity that maintains a uniform dispersion of the particle
25 formulation for a predetermined period of time. This helps facilitate making a suspension formulation tailored to provide controlled delivery of the drug contained in the drug particle formulation. The viscosity of the suspension vehicle may vary depending on the desired application, the size and type of the particle formulation, and the loading of the particle formulation in the suspension vehicle. The viscosity of the suspension vehicle may be varied by altering the
30 type or relative amount of the solvent or polymer used.

The suspension vehicle may have a viscosity ranging from about 100 poise to about 1,000,000 poise, preferably from about 1,000 poise to about 100,000 poise. In preferred embodiments, the suspension vehicles typically have a viscosity, at 33° C., of between about 5,000 to about 30,000 poise, preferably between about 8,000 to about 25,000 poise, more preferably
5 between about 10,000 to about 20,000 poise. In one embodiment, the suspension vehicle has a viscosity of about 15,000 poise, plus or minus about 3,000 poise, at 33° C. The viscosity may be measured at 33° C., at a shear rate of 10-4/sec, using a parallel plate rheometer.

The suspension vehicle may exhibit phase separation when contacted with the aqueous environment; however, typically the suspension vehicle exhibits substantially no phase separation
10 as a function of temperature. For example, at a temperature ranging from approximately 0° C. to approximately 70° C. and upon temperature cycling, such as cycling from 4° C. to 37° C. to 4° C., the suspension vehicle typically exhibits no phase separation.

The suspension vehicle may be prepared by combining the polymer and the solvent under dry conditions, such as in a dry box. The polymer and solvent may be combined at an elevated
15 temperature, such as from approximately 40° C. to approximately 70° C., and allowed to liquefy and form the single phase. The ingredients may be blended under vacuum to remove air bubbles produced from the dry ingredients. The ingredients may be combined using a conventional mixer, such as a dual helix blade or similar mixer, set at a speed of approximately 40 rpm. However, higher speeds may also be used to mix the ingredients. Once a liquid solution of the ingredients is
20 achieved, the suspension vehicle may be cooled to room temperature. Differential scanning calorimetry (DSC) may be used to verify that the suspension vehicle is a single phase. Further, the components of the vehicle (e.g., the solvent and/or the polymer) may be treated to substantially reduce or substantially remove peroxides (e.g., by treatment with methionine; see, e.g., U.S., Patent Application Publication No. 2007-0027105).

The drug particle formulation is added to the suspension vehicle to form a suspension
25 formulation. In some embodiments, the suspension formulation may comprise a drug particle formulation and a suspension vehicle and in other embodiments consist essentially of a drug particle formulation and a suspension vehicle.

The suspension formulation may be prepared by dispersing the particle formulation in the
30 suspension vehicle. The suspension vehicle may be heated and the particle formulation added to the suspension vehicle under dry conditions. The ingredients may be mixed under vacuum at an

elevated temperature, such as from about 40° C. to about 70° C. The ingredients may be mixed at a sufficient speed, such as from about 40 rpm to about 120 rpm, and for a sufficient amount of time, such as about 15 minutes, to achieve a uniform dispersion of the particle formulation in the suspension vehicle. The mixer may be a dual helix blade or other suitable mixer. The resulting
5 mixture may be removed from the mixer, sealed in a dry container to prevent water from contaminating the suspension formulation, and allowed to cool to room temperature before further use, for example, loading into an implantable, drug delivery device, unit dose container, or multiple-dose container.

The suspension formulation typically has an overall moisture content of less than about 10
10 wt %, preferably less than about 5 wt %, and more preferably less than about 4 wt %.

In preferred embodiments, the suspension formulations of the present invention are substantially homogeneous and flowable to provide delivery of the drug particle formulation from the osmotic delivery device to the subject.

In summary, the components of the suspension vehicle provide biocompatibility.
15 Components of the suspension vehicle offer suitable chemico-physical properties to form stable suspensions of drug particle formulations. These properties include, but are not limited to, the following: viscosity of the suspension; purity of the vehicle; residual moisture of the vehicle; density of the vehicle; compatibility with the dry powders; compatibility with implantable devices; molecular weight of the polymer; stability of the vehicle; and hydrophobicity and hydrophilicity
20 of the vehicle. These properties can be manipulated and controlled, for example, by variation of the vehicle composition and manipulation of the ratio of components used in the suspension vehicle.

The suspension formulations described herein may be used in an implantable, osmotic delivery device to provide zero-order, continuous, controlled, and sustained delivery of a
25 compound over an extended period of time, such as over weeks, months, or up to about one year or more. Such an implantable osmotic delivery device is typically capable of delivering the suspension formulation, comprising the drug, at a desired flow rate over a desired period of time. The suspension formulation may be loaded into the implantable, osmotic delivery device by conventional techniques.

30

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to practice the present invention, and are not intended to limit the scope of what the inventors regard as the invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, concentrations, and percent changes) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, temperature is in degrees Centígrade and pressure is at or near atmospheric.

Example 1: Generation of a long acting Glucagon analog polypeptides

Long acting glucagon analog polypeptides of the invention, as provided in Table 3, were synthesized on a Prelude peptide synthesizer (Protein Technologies Inc., Tucson, AZ)) by solid-phase methods using Fmoc strategy with N-[(dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU) or 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) activation (5-fold molar excess to amino acid) in N,N-dimethylformamide (DMF), and N,N-diisopropylethylamine (DIEA) was used as base. A 20% piperidine/DMF solution was used for Fmoc deprotection. The resin used was Rink Amide MBHA LL (Novabiochem) with loading of (0.30 – 0.40) mmol/g on a (20-400) μ mol scale.

Upon completion of solid phase synthesis of the linear polypeptide, the resin was washed with dichloromethane (DCM) and dried under vacuum for 30 minutes. For analogs containing the allyloxycarbonyl (Alloc) protecting group, removal was accomplished via a solution of Pd (PPh₃)₃ in (chloroform/acetic acid/n-methyl-morpholine, 37:2:1). For analogs containing the *tert*-butyloxycarbonyl (BOC)-Lys-fluorenylmethyloxycarbonyl (Fmoc)-OH, the Fmoc protecting group was removed using 20% piperidine/DMF. The resulting Fmoc-deprotected resin was washed with DMF (6 x 30 secs). Next, elongation of the spacer region was carried out in step-wise manner with the manual addition of each building block under pre-activation conditions. Addition of the lipophilic substituent (also referred to as “acyl chain”) was carried out under solid-phase peptide synthesis (SPPS) conditions with no pre-activation step. Final deprotection and cleavage of the peptide from the solid support were performed by treatment of the resin with (95% TFA, 2% water, 2% thioanisole, and 1% triisopropylsilane) for 2-3 hours. The cleaved peptide was

precipitated using cold diethyl ether. The diethyl ether layer was decanted, and the solids triturated again with cold diethyl ether and pelleted by centrifugation.

For analogs containing a lactam bridge, the appropriate allyl-protected amino acid building blocks were installed under normal solid-phase conditions as described above. Also, Fmoc-Lys- ϵ -1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl(ivDde)-OH was installed as a handle to later incorporate the acyl spacer and side-chain. Upon completion of the linear peptide the allyl-protecting groups were removed as described above. Lactam-bridge formation was afforded via solid-phase protocol using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, .5M) activation and DIEA as the base. Deprotection of the Fmoc & ivDde groups was afforded via 4% solution of hydrazine in DMF. The resulting de-protected resin was washed with DMF (6 x 30 secs). Elongation of the spacer region and addition of a lipophilic substituent was carried out as described in the preceding paragraphs. Final deprotection and cleavage of the peptide from the solid support was performed by treatment of the resin with (95% TFA, 2% water, 2% thioanisole, and 1% triisopropylsilane) for 2-3 hours. The cleaved peptide was precipitated using cold diethyl ether. The diethyl ether was decanted, and the solids triturated again with cold diethyl ether and pelleted by centrifugation.

The crude product was next dissolved in a solution of acetonitrile (ACN)/H₂O, 0.1% TFA. A 10% solution of acetic acid was added to each solution of crude peptide product and allowed to stir until analysis via LC/MS indicated removal of any CO₂ adducts. The solution was frozen and lyophilized. Purification was afforded via the methods described in Example 2.

Example 2: Purification and characterization of long acting glucagon receptor agonist polypeptides, i.e., linear polypeptide, without any lipophilic substituent and optional spacer

The product of Example 1 was lyophilized and analyzed by electrospray ionization- liquid chromatography/mass spectrometry (ESI-LC/MS) and analytical high-pressure liquid chromatography (HPLC) and was demonstrated to be pure (>98%). Mass results were consistent with calculated values.

Characterizations of peptide analogs were performed via C18 HPLC and LC/MS analysis (Acquity SQD Waters Corp, Milford, MA) and UV detection provided by dual absorbance signals at 215 nm and 280 nm, using one of Method A, Method B, Method C or Method D.

Method A, LC/MS conditions: performed using a Phenomenex HPLC Acris™ Peptide XB C18 35 column, 1.7 μ m, 2.1 X 100 mm or Acquity BEH300 or BEH130 CT8 column, 1.77 μ m.

2.1 X 100 mm using 5 - 65% acetonitrile/water with 0.05% TFA over 30 minutes with a flow rate 0.5 mL/min, λ - 215 nm, 280 nm.

Method B, C18 HPLC conditions: HPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 μ m, 100 X 2.10 mm column at 25 °C, 5 - 65% acetonitrile/water with 0.05% TFA over 30 minutes, flow rate 0.5 mL/min, λ - 215 nm, 280 nm.

Method C, HPLC conditions: HPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 μ m, 100 X 2.10 mm column at 25°C, 5 - 65% acetonitrile/water with 0.05% TFA over 20 minutes, flow rate 0.5 mL/min, λ - 215 nm, 280 nm.

Method D, HPLC conditions: HPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 μ m, 100 X 2.10 mm column at 25°C, 5 - 65% acetonitrile/water with 0.05% TFA over 10 minutes, flow rate 0.5 mL/min, λ - 215 nm, 280 nm. 5.0 μ L of sample was injected using a PLNO (partial loop w/ needle over-fill) injection mode.

Table 9 provides exemplary long acting glucagon receptor agonist polypeptides of the disclosure.

Polypeptide analogs without a lipophilic substituent and optional spacer are sometimes referred to herein as "linear polypeptides." Polypeptide analogs having at least one covalently bound lipophilic substituent and optional spacer are sometimes referred to herein as "conjugated polypeptides."

Table 9: Exemplary compounds: long acting glucagon analog polypeptides

Compound No.	SEQ ID NO:	Parent MW	Calculated (M+3) Mass	Observed (M+3) Mass
A1	SEQ ID NO: 1	5011.57	1671.52	1673
A2	SEQ ID NO: 2	5041.55	1681.52	1682.9
A3	SEQ ID NO: 3	4998.49	1667.16	1668.5
A4	SEQ ID NO: 4	4963.48	1655.49	1656.8
A5	SEQ ID NO: 5	4998.53	1667.18	TBD

Compound No.	SEQ ID NO:	Parent MW	Calculated (M+3) Mass	Observed (M+3) Mass
A6	SEQ ID NO: 6	4997.55	1666.85	1668
A7	SEQ ID NO: 7	5070.55	1692	1692
A8	SEQ ID NO: 8	5071.54	1691.9	1691.9
A9	SEQ ID NO: 9	5070.6	1692	1692
A10	SEQ ID NO: 10	5029.5	1678.6	1678.6
A11	SEQ ID NO: 11	5067.59	1691.3	1691.3
A12	SEQ ID NO: 12	5067.59	1691.2	1691.2
A13	SEQ ID NO: 13	4896.4	1633.13	1633.9
A14	SEQ ID NO: 14	4852.39	1618.46	1619.6
A15	SEQ ID NO: 15	5055.58	1687.6	1687.6
A16	SEQ ID NO: 16	5040.57	1682.4	1682.4
A17	SEQ ID NO: 17	5054.6	1687.2	1687.2
A18	SEQ ID NO: 18	5069.61	1692	1692
A19	SEQ ID NO: 19	5013.54	1673.5	1673.5
A20	SEQ ID NO: 20	5054.64	1687.1	1687.1
A21	SEQ ID NO: 21	5066.65	1689.88	1691.3
A22	SEQ ID NO: 22	5013.59	1672.2	1673.9

Compound No.	SEQ ID NO:	Parent MW	Calculated (M+3) Mass	Observed (M+3) Mass
A23	SEQ ID NO: 23	5014.53	1672.51	1673.6
A24	SEQ ID NO: 24	4972.49	1658.5	1659.8
A25	SEQ ID NO: 25	5080.68	1695.4	1695.4
A26	SEQ ID NO: 26	5079.74	1695.2	1695.2
A27	SEQ ID NO: 27	5065.67	1691.1	1691.1
A28	SEQ ID NO: 28	5038.64	1681.7	1681.7
A29	SEQ ID NO: 29	5036.63	1681	1681
A30	SEQ ID NO: 30	5022.6	1680.9	1680.9
A31	SEQ ID NO: 31	5124.69	1709.23	1710
A32	SEQ ID NO: 32	5123.75	1708.92	1710
A33	SEQ ID NO: 33	5082.65	1695.22	1696.1
A34	SEQ ID NO: 34	5065.67	1689.56	1691
A35	SEQ ID NO: 35	4287.84	1430.28	1431.3
A36	SEQ ID NO: 36	5108.69	1705.9	1705.9
A37	SEQ ID NO: 37	5037.61	1680.8	1680.8
A38	SEQ ID NO: 38	5036.67	1680.8	1680.8
A39	SEQ ID NO: 39	4995.57	1667.6	1667.6

Example 3: Functional assays of long acting glucagon receptor agonist polypeptides: human and rat receptors

Activation of the human, cyno and rat Glucagon receptor (GCGR) and human, cyno and rat GLP-1 receptors (GLP-1R), leads to increases in cellular cyclic adenosine monophosphate (cAMP). In the presence of the non-specific cAMP/cGMP phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), accumulating cAMP can be measured *in vitro* using common detection methods. Thus, it is possible to estimate an *in vitro* potency (pEC50) for peptides activating each of these receptors using fit dose-response curves for cAMP accumulation.

Cell handling and cAMP accumulation assays

CHO-K1 cells stably expressing human GLP-1R (Genebank accession #NM_002062) were grown in 90% F12-K media supplemented with 10% FBS and 250 µg/ml G418. For rat GLP-1R expression, a cDNA fragment encoding the full-length open reading frame of the rat GLP-1R (Genebank accession #NM_012728) was sub-cloned into the expression vector pcDNA3.1+ to enable transient receptor expression in CHO-K1 cells.

A cDNA fragment encoding the full-length open reading frame of the cynomolgus monkey GLP-1R (Genebank accession # XM_028847603) was cloned into pcDNA3.1+ for expression in CHO-K1 cells. A cDNA fragment encoding the full-length open reading frame of the human GCGR (Genebank accession #NM_000160) was cloned into pcDNA3.1+ for expression in CHO-K1 cells. A cDNA fragment encoding the full-length open reading frame of the rat GCGR (Genebank accession # NM_172091) was cloned into pcDNA3.1+ for expression in CHO-K1 cells. A cDNA fragment encoding the full-length open reading frame of the cynomolgus monkey GCGR (Genebank accession # XM_005585255) was cloned into pcDNA3.1+ for expression in CHO-K1 cells.

On day 1, CHO-K1 cells were plated at 1.5 million cells per T75 flask in 90% F12-K media supplemented with 10% FBS. On day 3 cells were transfected with 40 mcg of each expression plasmid using Lipofectamine 2000 transfection reagent. On day 5, cells were dispensed at 1000 cells per well in white 384-well OptiPlates in 5 mL of assay buffer consisting of 1X HBSS, 5 mM HEPES, 0.5 mM IBMX, and 0.1% casein.

Peptides were serially diluted 4-fold in assay buffer to final concentrations ranging from 1×10^{-9} M to 9.5×10^{-16} M. Two assay controls consisting of 50 mM forskolin (cAMP system

maximum) or assay buffer only (cAMP system minimum) were also prepared. Five microliters of each peptide-concentration, or assay control, was added to triplicate wells and incubated for thirty minutes at room temperature. During this incubation step a 4x europium labelled cAMP tracer solution and a 4x Ulight®-anti-cAMP solution (consisting of an anti-cAMP monoclonal antibody labelled with Ulight™ dye) was prepared according to the manufacturer's protocol (PerkinElmer LANCE Ultra cAMP kit). Following this incubation, 5 mL europium labelled cAMP and 5 mL Ulight anti-cAMP antibody was added to each well. The plate was covered to prevent evaporation and incubated for 60 minutes at room temperature in the dark. Plates were read on an Envision fluorescent plate reader (PerkinElmer).

10 Data analysis

Test values were first normalized to the system maximum and system minimum averaged values in Excel using the formula: (test value – system min average) / (system max average – system min average) * 100. Normalized test values represent a baseline corrected percentage of the system maximum cAMP response induced by forskolin. Data from up to 3 replicate tests were analyzed for each peptide. Potency values were estimated using GraphPad Prism software (v7.04) by fitting data to a 4-parameter logistic curve model: $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC50} - X)})$. The Hill slope was constrained to 1.0. EC50 values were converted to pEC50 values using the formula: $\text{pEC50} = -\text{Log}(\text{EC50})$. Mean pEC50 values for glucagon analogs of the disclosure are reported in Table 10. Comparative activity data of glucagon, exenatide, and compound A6 (SEQ ID NO: 6) against the human, cyno, and rat glucagon and GLP-1 receptors is provided in Table 11 and in Figure 2.

Table 10: Activity of glucagon analog polypeptides against the glucagon receptor and the GLP-1 receptor

Compound No.	glucagon receptor pEC50	GLP-1 receptor pEC50
A1	11.5	ND
A2	12.5	ND
A3	12.5	ND
A4	11.7	ND
A5	11.6	ND
A6	12.2	< 7
A7	10.7	< 9
A8	9.9	< 9
A9	10.6	< 9
A10	10.4	< 9

Compound No.	glucagon receptor pEC ₅₀	GLP-1 receptor pEC ₅₀
A11	11.7	<9
A12	11.8	<9
A13	12	<9
A14	11.2	<9
A15	12.1	<9
A16	12.2	<9
A17	12.1	<9
A18	10.4	<9
A19	12.1	<9
A20	12.1	<9
A21	12.1	<9
A22	11.7	<9
A23	11.5	<9
A24	12.5	<9
A25	11.9	<9
A26	11.1	<9
A27	12	<9
A28	11.7	<9
A29	12.4	<9
A30	12.5	<9
A31	11.9	<9
A32	11.7	<9
A33	11.9	<9
A34	11.9	<9
A35	11.9	<9
A36	10.8	<9
A37	11.5	<9
A38	11.4	<9
A39	11.7	<9

ND = not determined

Table 11: Activity (as EC₅₀ values) of compound A6 against human, cyno, and rat glucagon and GLP-1 receptors

	hGCGR	cGCGR	rGCGR	hGLP1R	cGLP1R	rGLP1R	hGIPR
Glucagon	12.1	10.3	10.2	9.24	<7	<7	<9
Exenatide	8.61	<7	<7	12.1	11.4	11.8	<9
Compound A6	12.2	9.77	10.3	<7	ND	ND	<9
hGIP	ND	ND	ND	ND	ND	ND	11.8

5 ND = not determined

Example 4: In vitro metabolic stability studies of glucagon analogs

Rat and Human Kidney Brush Border Membranes

In vitro incubations in subcutaneous (SC) tissue homogenates were used to characterize the ability of peptides to resist pre-systemic degradation by proteases and peptidases after SC

administration. In vivo nonclinical studies have shown that peptidase activity in the SC space can limit the bioavailability of a peptide after SC administration. Peptides with high SC bioavailability are stable in this assay, while peptides with low SC bioavailability are unstable in this assay.

Brush border membranes from rat and human kidney tissue were prepared via centrifugation and stored at -70°C. Thawed stocks of rat or human kBBM were diluted to the appropriate concentration in 25 mM HEPES buffer (pH 7.4) containing 1% casein and aliquoted into a 96-well plate. The kBBM solutions were pre-warmed for 10 minutes at 37°C. Reactions were initiated by the addition of test peptide (1 mM final concentration) also dissolved in 25 mM HEPES buffer (pH 7.4) containing 1% casein. The final concentration of kBBM in each incubation was 50 mcg protein/mL. Reactions were maintained at 37°C in a shaking water bath. At 0, 0.25, 0.5, 1.0, 2.0, and 4.0 h post-initiation, 30 mL of the reaction mixture was removed and placed into a 96-well plate containing 120 mL of ice-cold methanol containing 2.5% formic acid. Quenched samples were centrifuged at $2178 \times g$ for 10 min and then a portion of the supernatants were transferred to a clean 96-well plate and diluted 1:1 with water. Samples were analyzed by UPLC-MS/MS. The results of this analysis for compound A6 (SEQ ID NO: 6) are shown in Table 12.

Human Subcutaneous Tissue Homogenates

In vitro incubations in subcutaneous (SC) tissue homogenates were used to characterize the ability of peptides to resist pre-systemic degradation by proteases and peptidases after SC administration. In vivo nonclinical studies have shown that peptidase activity in the SC space can limit the bioavailability of a peptide after SC administration. Peptides with high SC bioavailability are stable in this assay, while peptides with low SC bioavailability are unstable in this assay.

Human SC tissue was homogenized in cold 25 mM HEPES buffer (pH 7.4, 10-fold volume based on sample weight), and then filtered through a double layer of cheesecloth. The filtrates were aliquoted, flash frozen on a methanol/dry ice bath and stored at -80°C. The protein concentration of each pooled batch was determined using the BCA protein assay. Thawed stocks of human SC tissue homogenates were diluted to 1.0 mg protein/mL in 25 mM HEPES buffer (pH 7.4) and aliquoted into a 96-well plate. The diluted SC homogenates were pre-warmed for 10 minutes at 37°C. Reactions were initiated by the addition of test peptide (10 mM final concentration) also dissolved in 25 mM HEPES buffer (pH 7.4). Reactions were maintained at

37°C in a shaking water bath. At 0, 0.25, 0.5, 1.0, 2.0, and 4.0 h post-initiation, 50 µL of the reaction mixture was removed and placed into a 96-well plate containing 150 µL of ice-cold methanol containing 2.5% formic acid. Quenched samples were centrifuged at $2178 \times g$ for 10 min and then a portion of the supernatants were transferred to a clean 96-well plate and diluted 1:10 with water. Samples were analyzed by UPLC-MS/MS. The results of this analysis for compound A6 (SEQ ID NO: 6) are shown in Table 12.

Fraction unbound (f_u) in plasma

Conventional methods of measuring plasma protein binding, such as equilibrium dialysis, ultrafiltration and ultracentrifugation, are not reliable with peptides because of their tendency to adsorb to the surface of plastic tubing, dialysis membranes and molecular weight cut-off filters. As a result, the extent to which acylated peptides bind to serum albumin was evaluated using surface plasmon resonance (SPR). The utility of this technique to provide a reasonable estimation of the fraction of a drug bound to plasma protein has been demonstrated in the literature. The results of this analysis for compound A6 (SEQ ID NO: 6) are shown in Table 12.

Table 12: Metabolic stability pharmacokinetics and fraction unbound of glucagon analog compound A6

	kBBM t _{1/2} (hr)	(H)SC tissue stability t _{1/2} (hr)	% f_u
Rat	6.6	9.0	0.83%
Monkey	>12	5.0	0.48%
Human	>72	10.6	0.72%

Example 5: Pharmacokinetic analysis of glucagon analog polypeptides

Intravenous infusion of long-acting glucagon analog polypeptides to assess intravenous pharmacokinetics of polypeptides

Peptides were dissolved in 0.05% Tween-20 in phosphate buffered saline and administered as a 1-hour intravenous infusion to non-fasted male Sprague-Dawley rats (n=3 per group) via femoral vein cannula at a final dose of 0.033 mg/kg. Formulations were administered at a rate of 1.67 mL/kg/h. Blood samples (approximately 250 µL) were collected for pharmacokinetic analysis via a jugular vein cannula at 0.25, 0.5, 0.75, 1, 1.17, 1.33, 1.5, 2, 4, 8, 24, 48, 72, and 96 hours post-start of infusion into microtainer tubes containing K₂EDTA as anticoagulant and 25 µL

of a protease inhibitor cocktail. Plasma was prepared by centrifugation and stored at -80°C until analysis.

Subcutaneous bolus injection of long-acting glucagon analog polypeptides to assess bioavailability (F) of polypeptides

5 Peptides were dissolved in sterile saline and administered to non-fasted male Sprague-Dawley rats (n=3 per group) at a dose of 0.1 mg/kg via a single bolus injection into the subcutaneous space between the scapulae. Blood samples (approximately 250 µL) were collected for pharmacokinetic analysis via a jugular vein cannula at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, and 120 hr post-dose into microtainer tubes containing K₂EDTA as anticoagulant and 25 µL
10 of a protease inhibitor cocktail. Plasma was prepared by centrifugation and stored at -80°C until analysis.

Plasma sample preparation for pharmacokinetic studies

A 70 µL aliquot of each plasma sample was placed into to a 96-well plate. To each well was added 210 µL of 0.1% TFA in 2:1 ethanol:acetonitrile containing an appropriate internal
15 standard. Plates were vortex mixed for 10 min at 1300 rpm, and then centrifuged for 10 min at 500 x g. Supernatants (210 µL) were placed into a clean 96-well plate and evaporated under a nitrogen stream at 45°C. Residues were reconstituted in 80 µL of 20% acetonitrile (aq) containing 0.1% formic acid.

LC/MS quantification of glucagon analog polypeptides in plasma

20 All calibration standards were prepared in control rat plasma containing K₂EDTA and protease inhibitor cocktail.

Samples and standards were analyzed by electrospray ionization (ESI) UPLC-MS/MS using a system consisting of a CTC HTS PAL auto-injector (Leap, Carrboro, NC), an Agilent Infinity 1290 system with column oven (Palo Alto, CA), a Valco switching valve (Houston, TX),
25 and a Sciex TripleTOF® 5600 mass spectrometer (Framingham, MA). Samples were injected onto a 2.1 × 50 mm reverse phase C18 analytical column, typically a Waters CORTECS UPLC C18+, 1.6 µm (Waters Corporation, Milford, MA) or similar. Chromatographic separation was

achieved with a gradient method using water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) as mobile phase. Initial conditions consisted of 95% A and 5% B. The organic component was increased to 95% B over a period of 3-4 minutes, depending on the peptide. Typical flow rates were 550 $\mu\text{L}/\text{min}$. The column temperature was held constant at 45°C. Peptides were quantified by monitoring one or more product ions produced from a multiply charged parent ion. The results of this analysis for compound A6 (SEQ ID NO: 6) are shown in Table 13. The change in plasma concentration of A6 following bolus injection and intravenous infusion are presented in Figure 3.

Table 13: Pharmacokinetic and ADME profile of compound A6

Route	Dose (mg/kg)	CL (mL/min/kg)	V_{ss} (mL/kg)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	F (%)
IV infusion	0.033	0.0829	86.7	13.0	ND	ND	ND
SC bolus	0.11	ND	ND	15.8	8-24	462	94.9

ND = not determined

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

What is claimed is:

1. An isolated polypeptide, comprising the amino acid sequence of SEQ ID NO: 80: X_1 SHGTFTSX₉YSKYLYX₁₅X₁₆X₁₇X₁₈AX₂₀X₂₁FX₂₃X₂₄WX₂₆EX₂₈E-Z-tail-(OH/NH₂) (SEQ ID NO: 80), or a pharmaceutically acceptable salt thereof, wherein:
 - X₁ is Y or acetyl-Y;
 - X₉ is D or E;
 - X₁₅ is D, E, or K;
 - X₁₆ is Aib or K;
 - X₁₇ is K;
 - X₁₈ is K, R, or Y;
 - X₂₀ is E, K, Q, or S;
 - X₂₁ is E or K;
 - X₂₃ is Aib or V;
 - X₂₄ is Aib, E, K, Q, or S;
 - X₂₆ is E, L, or Q;
 - X₂₈ is D, E, K, N, Q, S, or N-methylglutamate;
 - Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);
 - wherein when X₁₅, X₁₆, X₁₇, X₁₈, X₂₀, or X₂₁ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,
 - provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and
 - wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₂₀ and X₂₄ or at positions X₂₄ and X₂₈.

2. An isolated polypeptide, comprising the amino acid sequence of SEQ ID NO: 81: X_1 SHGTFTSDYSKYL X_{15} X_{16} X_{17} X_{18} A X_{20} X_{21} F X_{23} X_{24} WLEX X_{28} E-Z-tail-(OH/NH₂) (SEQ ID NO: 81), or a pharmaceutically acceptable salt thereof, wherein:

X_1 is Y or acetyl-Y;

X_{15} is D or K;

X_{16} is Aib or K;

X_{17} is K;

X_{18} is K or Y;

X_{20} is E, K, Q, or S;

X_{21} is E or K;

X_{23} is Aib or V;

X_{24} is Aib, E, K, Q, or S;

X_{28} is D, E, K, N, Q, S, or N-methylglutamate;

Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);

wherein when X_{15} , X_{16} , X_{17} , X_{18} , X_{20} , or X_{21} are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X_{20} and X_{24} or at positions X_{24} and X_{28} .

3. An isolated polypeptide, comprising the amino acid sequence of SEQ ID NO: 82: X_1 SHGTFTSDYSKYLD X_{16} X_{17} YAX X_{20} X_{21} F X_{23} X_{24} WLEX X_{28} E-Z-tail-(OH/NH₂) (SEQ ID NO: 82), or a pharmaceutically acceptable salt thereof, wherein:

X_1 is Y or acetyl-Y;

X_{16} is Aib or K;

X_{17} is K;

X₂₀ is E, K, Q, or S;

X₂₁ is E or K;

X₂₃ is Aib or V;

X₂₄ is Aib, E, K, Q, or S;

X₂₈ is D, E, K, N, Q, S, or N-methylglutamate;

Z-tail is absent or selected from the group consisting of PSSGAPPPS (SEQ ID NO: 90), PSSGEPPPS (SEQ ID NO: 91), PSSGKPPPS (SEQ ID NO: 92), or PSSGSPPPS (SEQ ID NO: 93);

wherein when X₁₆, X₁₇, or X₂₁ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to a lipophilic substituent, optionally via a spacer; and

wherein the isolated polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₂₀ and X₂₄ or at positions X₂₄ and X₂₈.

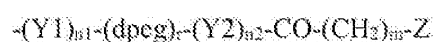
4. The isolated polypeptide of claim 1 or claim 2, wherein one of X₁₆, X₁₇, or X₂₁ is lysine bound to a lipophilic substituent, optionally via a spacer.
5. The isolated polypeptide of any one of claims 1-3, wherein when X₂₀ is Q, X₁₆ is K, wherein the lysine at residue X₁₆ is covalently bound to a lipophilic substituent, optionally via a spacer; or X₂₁ is K, wherein the lysine residue at X₂₁ is optionally covalently bound to a lipophilic substituent, optionally via a spacer.
6. The isolated polypeptide of any one of claims 1-3, wherein when X₂₀ is Q, X₂₁ is K, wherein the lysine residue at X₂₁ is optionally covalently bound to a lipophilic substituent, optionally via a spacer.

7. The isolated polypeptide of any one of claims 1-3, wherein when X_{21} is E, X_{20} is E, K, or S.
8. The isolated polypeptide of any one of claims 1-3, wherein X_{16} is Aib or K covalently bound to a lipophilic substituent, optionally via a spacer.
9. The isolated polypeptide of any one of claims 1-3, wherein X_{24} is Aib or K.
10. The isolated polypeptide of any one of claims 1-3, wherein X_{28} is D or S.
11. The isolated polypeptide of any one of claims 1-3, wherein the lipophilic substituent forms an amide bond between an amino group of the isolated polypeptide and the CO-group of the lipophilic substituent.
12. The isolated polypeptide of claim 11, wherein the lipophilic substituent is of Formula I:
$$-\text{CO}-(\text{CH}_2)_m-\text{Z}$$

Formula I

wherein
Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$; and
m is from 4 to 24.
13. The isolated polypeptide of claim 12, wherein Z is $-\text{CO}_2\text{H}$.
14. The isolated polypeptide of claim 12, wherein m is from 14 to 20.
15. The isolated polypeptide of claim 12, wherein m is 16, 17, or 18.
16. The isolated polypeptide of any one of claims 1-3, wherein the lipophilic substituent is covalently bound to the isolated polypeptide via a spacer.

17. The isolated polypeptide of claim 16, wherein the lipophilic substituent, $-\text{CO}-(\text{CH}_2)_m-\text{Z}$, is linked to an amino group of the isolated polypeptide via the spacer, wherein the spacer forms a bridge between the amino group of the isolated polypeptide and the $-\text{CO}-$ group of the lipophilic substituent.
18. The isolated polypeptide of claim 16, wherein the lipophilic substituent and spacer are of Formula IV:



Formula IV

wherein

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24;

Y1 is selected from the group consisting of γGlu , Asp, and Gly;

Y2 is selected from the group consisting of γGlu , Asp, and Gly;

dpeg is $-\text{[CO(CH}_2\text{)O(CH}_2\text{)}_2\text{O(CH}_2\text{)NH]}-$;

r is from 1 to 8;

n1 is from 0 to 10; and

n2 is from 0 to 10.

19. The isolated polypeptide of claim 18, wherein Z is $-\text{CO}_2\text{H}$.
20. The isolated polypeptide of claim 18, wherein m is from 14 to 20.
21. The isolated polypeptide of claim 18, wherein m is 16, 17, or 18.
22. The isolated polypeptide of claim 18, wherein Y1 is γGlu .
23. The isolated polypeptide of claim 18, wherein n1 is 2.
24. The isolated polypeptide of claim 18, wherein n2 is 0.

25. The isolated polypeptide of claim 18, wherein r is 1.
26. An isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 1 to 60, or a pharmaceutically acceptable salt thereof.
27. The isolated polypeptide of claim 26, comprising the amino acid sequence of any of SEQ ID NOs: 1 to 39, or a pharmaceutically acceptable salt thereof.
28. The isolated polypeptide of claim 27 comprising the amino acid sequence of SEQ ID NO: 3, or a pharmaceutically acceptable salt thereof.
29. The isolated polypeptide of claim 27 comprising the amino acid sequence of SEQ ID NO: 6, or a pharmaceutically acceptable salt thereof.
30. The isolated polypeptide of claim 27 comprising the amino acid sequence of SEQ ID NO: 21, or a pharmaceutically acceptable salt thereof.
31. The isolated polypeptide of claim 27 comprising the amino acid sequence of SEQ ID NO: 24, or a pharmaceutically acceptable salt thereof.
32. A pharmaceutical composition comprising the isolated polypeptide of any one of claims 1-3 or 26-31, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant, carrier, or vehicle.
33. A pharmaceutical combination comprising the isolated polypeptide of any one of claims 1-3 or 26-31, or a pharmaceutically acceptable salt thereof, and a GLP-1 receptor agonist.
34. An osmotic delivery device, comprising the isolated polypeptide of any one of claims 1-3 or 26-31.

35. The osmotic delivery device of claim 34, comprising
- an impermeable reservoir comprising interior and exterior surfaces and first and second open ends;
 - a semi-permeable membrane in sealing relationship with the first open end of the reservoir;
 - an osmotic engine within the reservoir and adjacent the semi-permeable membrane;
 - a piston adjacent the osmotic engine, wherein the piston forms a movable seal with the interior surface of the reservoir, the piston divides the reservoir into a first chamber and a second chamber, the first chamber comprising the osmotic engine;
 - a suspension formulation, wherein the second chamber comprises the suspension formulation and the suspension formulation is flowable and comprises the isolated polypeptide; and
 - a diffusion moderator inserted in the second open end of the reservoir, the diffusion moderator adjacent the suspension formulation.
36. A method of treating obesity in a human subject, providing weight loss to the human subject, or suppressing appetite in the human subject, comprising administering to the subject a pharmaceutical composition comprising the isolated polypeptide of any one of claims 1-3 or 26-31.
37. A method of treating diabetes in a human subject, comprising administering to the subject a pharmaceutical composition comprising the isolated polypeptide of any one of claims 1-3 or 26-31.
38. The method of claim 37, wherein the diabetes is type 1 diabetes.
39. The method of claim 37 wherein the diabetes is type 2 diabetes.
40. A method of treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH) in a human subject, comprising administering to the subject a

pharmaceutical composition comprising the isolated polypeptide of any one of claims 1-3 or 26-31.