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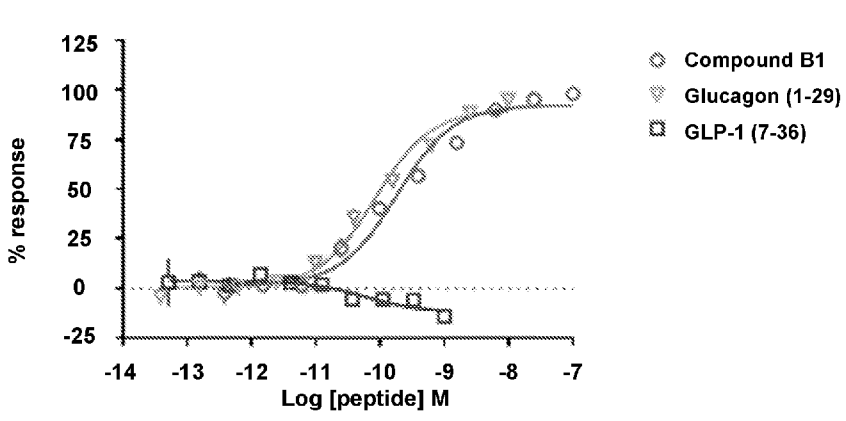
(54) Title: GLUCAGON RECEPTOR/GLP-1 RECEPTOR SELECTIVE ANALOG POLYPEPTIDES AND METHODS OF USE THEREOF

FIG. 1A




(57) Abstract: This invention relates to isolated polypeptides that are dual glucagon receptor analogs and glucagon-like peptide-1 (GLP-1) receptor analogs, as well as peptide derivatives thereof. This invention also relates to methods of using such polypeptides in a variety of therapeutic and diagnostic indications, as well as methods of producing such polypeptides.

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# GLUCAGON RECEPTOR/GLP-1 RECEPTOR SELECTIVE ANALOG POLYPEPTIDES AND METHODS OF USE THEREOF

## Related Application

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/337,007, filed May 16, 2016, the contents of which are incorporated herein by reference in their entirety.

## Field

**[0002]** This invention relates to isolated polypeptides that are dual glucagon receptor analogs and glucagon-like peptide-1 (GLP-1) receptor analogs, as well as peptide derivatives thereof. These analogs exhibit dual action at the human glucagon receptor and human GLP-1 receptor with improved solubility, thermal stability and physicochemical properties compared to native endogenous glucagon. This invention also relates to methods of using such polypeptides in a variety of therapeutic and diagnostic indications, as well as methods of producing such polypeptides. These analogs are useful in methods of treating obesity, diabetes, metabolic disorders, and other diseases or disorders.

## Background

**[0003]** Glucagon, a peptide hormone that is produced by the alpha cells of the pancreas, 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 include, for example, glucagon, GLP-1, glucagon-like peptide-2 (GLP-2), and oxyntomodulin (OXM), which are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake.

**[0004]** Accordingly, there exists a need for therapeutics and therapies that mimic GLP-1 and/or glucagon activity.

## Summary

**[0005]** This invention relates to isolated polypeptides that are dual glucagon receptor analogs and glucagon-like peptide-1 (GLP-1) receptor analogs, as well as peptide derivatives thereof. Glucagon is a 29 amino acid peptide hormone that is produced by alpha

cells in the pancreas and that interacts with the glucagon receptor ("GCGR," also abbreviated as "GlucR"). GLP-1 is produced by the L-cell located mainly in the ileum and colon, and to a lesser extent by L-cells in the duodenum and jejunum. GLP-1 is a regulatory peptide to a G-coupled protein receptor on β cell and via adenyl cyclase activity and production of cAMP stimulates the insulin response to the nutrients that are absorbed from the gut (Baggio 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).

**[0006]** The isolated polypeptides of the disclosure are potent, stable, and soluble glucagon analogs that exhibit dual action on the human glucagon receptor and GLP-1 receptor. Recently published data has shown that balanced co-agonism at both the glucagon and GLP-1 receptors resulted in significant weight-loss, with improved lipid and body composition profiles. (*See e.g.*, Ranabir Sinha Roy at. al., "Glucagon-Like Peptide 1/Glucagon Receptor Dual Agonism Reverses Obesity in Mice." Diabetes. vol. 58(10): 2258–2266 (2009).

**[0007]** The isolated polypeptides of the disclosure are derived from a genus that imparts selectivity, solubility, and improved clearance of the molecule from the kidney.

**[0008]** In some embodiments, an isolated polypeptide of the disclosure is a dual glucagon receptor/GLP-1 receptor agonist. In some embodiments, an isolated polypeptide of the disclosure binds to a glucagon receptor (GCGR) and binds to a GLP-1 receptor.

**[0009]** In some embodiments, an isolated polypeptide of the disclosure comprises a modified amino acid sequence based on the amino acid sequence of human glucagon: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21), where the modified amino acid sequence includes at least one amino acid substitution, at least two amino acid substitutions, at least three amino acid substitutions, at least four amino acid substitutions, at least five amino acid substitutions, at least six amino acid substitutions, at least seven amino acid substitutions, at least eight amino acid substitutions, at least nine amino acid substitutions, at least 10 amino acid substitutions, at least 11 amino acid substitutions, at least 12 amino acid substitutions, at least 13 amino acid substitutions, at least 14 amino acid substitutions, at least 15 amino acid substitutions, at least 16 amino acid substitutions, at least 17 amino acid substitutions, at least 18 amino acid substitutions, at least 19 amino acid substitutions, at least 20 amino acid substitutions, at least 21 amino acid substitutions, at least 22 amino acid substitutions, at least 23 amino acid substitutions, at

least 24 amino acid substitutions, at least 25 amino acid substitutions, at least 26 amino acid substitutions, at least 27 amino acid substitutions, at least 28 amino acid substitutions, and/or at least 29 amino acid substitutions, provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0010]** In some embodiments, an isolated polypeptide of the disclosure comprises a modified amino acid sequence based on the amino acid sequence of human glucagon: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21), where the modified amino acid sequence includes at least one amino acid substitution, at least two amino acid substitutions, at least three amino acid substitutions, at least four amino acid substitutions, at least five amino acid substitutions, at least six amino acid substitutions, at least seven amino acid substitutions, at least eight amino acid substitutions, at least nine amino acid substitutions, at least 10 amino acid substitutions, at least 11 amino acid substitutions, at least 12 amino acid substitutions, or at least 13 amino acid substitutions, wherein the amino acid substitution(s) is selected from the group consisting of:

(i) an amino acid substitution at position 1 selected from the group consisting of H, W, pyroglutamate (pGlu), Q, P, Y, and N;

(ii) an amino acid substitution at position 2 selected from the group consisting of S, D-Ser, and 2-Aminoisobutyric acid (Aib);

(iii) an amino acid substitution at position 16 selected from the group consisting of S and K*, where K* at position 16 is connected with E* at position 20 via lactam bridge;

(iv) an amino acid substitution at position 17 selected from the group consisting of R, A, K and K*, where K* at position 17 is connected with E* at position 21 via lactam bridge;

(v) an amino acid substitution at position 18 selected from the group consisting of R and A;

(vi) an amino acid substitution at position 20 selected from the group consisting of Q and E*, where E* at position 20 is connected with K* at position 16 via lactam bridge;

(vii) an amino acid substitution at position 21 selected from the group consisting of D and E*, where E* at position 21 is connected with K* at position 17 via lactam bridge;

(viii) an amino acid substitution at position 23 selected from the group consisting of V and D-Val

(ix) an amino acid substitution at position 24 to K**, where K** at position 24 is connected with E** at position 28 via lactam bridge;

(x) an amino acid substitution at position 25 selected from the group consisting of W and H;

(xi) an amino acid substitution at position 26 selected from the group consisting of L and K;

(xii) an amino acid substitution at position 27 selected from the group consisting of M and Q;

(xiii) an amino acid substitution at position 28 to E**, where E** at position 28 is connected with K** at position 24 via lactam bridge;

(xiv) an amino acid substitution at position 30 selected from the group consisting of OH and $NH_2$; and

(xv) combinations thereof,

provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0011]** In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of amino acid sequences represented by the consensus sequence of SEQ ID NO: 1:

$X_1X_2QGTFTSDYSKYLDX_{16}X_{17}X_{18}AX_{20}X_{21}FVX_{24}WX_{26}X_{27}X_{28}TX_{30}$ (SEQ ID NO: 1), wherein:

$X_1$ is H, W, pyroglutamate (pGlu), Q, P, Y, or N;

$X_2$ is S, D-Ser, or 2-Aminoisobutyric acid (Aib);

$X_{16}$ is S or K*, where K* at position 16 is connected with E* at position 20 via lactam bridge;

$X_{17}$ is R, A, K, or K*, where K* at position 17 is connected with E* at position 21 via lactam bridge;

$X_{18}$ is R or A;

$X_{20}$ is Q or E*, where E* at position 20 is connected with K* at position 16 via lactam bridge;

$X_{21}$ is D or E*, where E* at position 21 is connected with K* at position 17 via lactam bridge;

$X_{23}$ is V or D-Val;

$X_{24}$ is K**, where K** at position 24 is connected with E** at position 28 via lactam bridge;

$X_{25}$ is W or H;

$X_{26}$ is L or K;

$X_{27}$ is M or Q;

$X_{28}$ is E**, where E** at position 28 is connected with K** at position 24 via lactam bridge; and

$X_{30}$ is OH or $NH_2$; and combinations thereof,

provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0012]** In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-$NH_2$ (SEQ ID NO: 2), which is also referred to herein as Compound C1;
HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 3), which is also referred to herein as Compound C2;
HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-OH (SEQ ID NO: 4), which is also referred to herein as Compound C3;
NSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 5), which is also referred to herein as Compound C4;
QSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 6), which is also referred to herein as Compound C5;
WSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 7), which is also referred to herein as Compound C6;
PSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 8), which is also referred to herein as Compound C7;
(pGlu)SQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 9), which is also referred to herein as Compound C8;

HSQGTFTSDYSKYLDSK*RAQE*FVK**HLQE**T-$NH_2$ (SEQ ID NO: 10), which is also referred to herein as Compound C9; HSQGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 11), which is also referred to herein as Compound C10; H(D-Ser)QGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 12), which is also referred to herein as Compound C11; HSQGTFTSDYSKYLDSKAAQDFVK**WLME**T- $NH_2$ (SEQ ID NO: 13), which is also referred to herein as Compound C12; HSQGTFTSDYSKYLDSRRAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 14), which is also referred to herein as Compound C13; HSQGTFTSDYSKYLDSRRAQDFVK**WKME**T-$NH_2$ (SEQ ID NO: 15), which is also referred to herein as Compound C14; HSQGTFTSDYSKYLDSRRAQDFVK**HLME**T-$NH_2$ (SEQ ID NO: 16), which is also referred to herein as Compound C15; HSQGTFTSDYSKYLDSARAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 17), which is also referred to herein as Compound C16; Y(Aib)QGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 18), which is also referred to herein as Compound C17; YSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 19), which is also referred to herein as Compound C18; and HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 20), which is also referred to herein as Compound C19. In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-20.

**[0013]** The isolated polypeptides of the disclosure contain amino acid motifs that allow for maintaining solubility, stability, e.g., metabolic stability, and/or potency of the dual-action analogs.

**[0014]** The isolated polypeptides of SEQ ID NOs: 2-20 are derived from a genus that is responsible for selectivity, solubility, and improved clearance of the molecule from the kidney. This genus is based on (a) determination of the residue(s) required for binding to both glucagon receptor and GLP-1 receptor, (b) identification of amino acids and secondary structure motifs that provide for solubility while enhancing or maintaining the potency of the parent peptide, and (c) identification of amino acid substitutions for imparting chemical stability.

**[0015]** In some embodiments, the carboxyl group consisting of the C-terminal amino acid residue of an isolated polypeptide of the disclosure is amidated. In some embodiments, the carboxyl group consisting of the C-terminal amino acid residue of an isolated polypeptide of the disclosure unmodified.

**[0016]** In some embodiments, an isolated polypeptide provided herein is an agonist of glucagon activity. In some embodiments, an isolated polypeptide provided herein can bind to a glucagon receptor. In some embodiments, the glucagon receptor is a human glucagon receptor. In some embodiments, the isolated polypeptide of the disclosure binds to a human glucagon receptor with a pEC50 in the cAMP assay (as described herein) greater than or equal to about 9.0.

**[0017]** In some embodiments, an isolated polypeptide of the disclosure binds to a human glucagon receptor and a human GLP-1 receptor, but does not substantially bind any other human receptor. As used herein, the term "does not substantially bind" and variations thereof refer to polypeptides that exhibit low affinity to no affinity for a human receptor other than the human glucagon receptor and the human GLP-1 receptor. In some embodiments, an isolated polypeptide of the disclosure binds to a human glucagon receptor and/or a human GLP-1 receptor at an affinity that is at least 100-fold greater than the affinity of the same isolated polypeptide for any other human receptor. In preferred embodiments, an isolated polypeptide of the disclosure binds to a human glucagon receptor and/or a human GLP-1 receptor at an affinity that is at least 1,000-fold greater than the affinity of the same isolated polypeptide for any other human receptor. In some embodiments, the isolated polypeptide of the disclosure binds to a human glucagon receptor with a pEC50 in the cAMP assay greater than or equal to about 9.0, and the isolated polypeptide of the disclosure binds to human GLP-1 receptor with a pEC50 in the cAMP assay that is greater than or equal to about 7.0.

**[0018]** In some embodiments, an isolated polypeptide as provided herein can further comprise a heterologous moiety associated with the polypeptide. In some embodiments, the heterologous moiety is a protein, a peptide, a protein domain, a linker, an organic polymer, an inorganic polymer, a polyethylene glycol (PEG), biotin, an albumin, a human serum albumin (HSA), a HSA FcRn binding portion, an antibody, a domain of an antibody, an antibody fragment, a single chain antibody, a domain antibody, an albumin binding domain, an enzyme, a ligand, a receptor, a binding peptide, a non-FnIII scaffold, an epitope tag, a

recombinant polypeptide polymer, a cytokine, or any combination of two or more of such moieties.

**[0019]** The isolated polypeptides provided herein exhibit dual glucagon receptor/GLP-1 receptor agonistic activity, for example by binding a glucagon receptor and a GLP-1 receptor. The isolated polypeptides provided herein completely or partially agonize or otherwise stimulate glucagon activity upon binding to or otherwise interacting with a glucagon receptor and a GLP-1 receptor. The stimulation or modulation of a biological function of glucagon is complete or partial upon interaction between the dual glucagon receptor/GLP-1 receptor agonist and the glucagon receptor and the GLP-1 receptor.

**[0020]** The isolated polypeptides of the disclosure, which are dual-action agonists, are useful alone or in combination with at least a second agent. In preferred embodiments, the second agent is a polypeptide. In preferred embodiments, the second polypeptide is an insulinotrophic peptide. For example, the insulinotrophic polypeptide is selected from the group consisting of exenatide, a derivative of exenatide, an analogue of exenatide, glucagon-like peptide-1 (GLP-1), a derivative of GLP-1, and an analogue of GLP-1.

**[0021]** In preferred embodiments, the insulinotrophic polypeptide is exenatide, a derivative of exenatide, or an analog of exenatide. In preferred embodiments, the exenatide is synthetic exenatide. In preferred embodiments, the synthetic exenatide comprises the amino acid sequence H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-$NH_2$ (SEQ ID NO: 23).

**[0022]** In combination therapies, the use of a dual-action analog allows for the titration of proper therapeutic doses of glucagon and any GLP-1 receptor agonist. This allows for the desired effects of glucagon/GLP-1 agonism (*i.e.*, weight-loss, increase in energy expenditure), without a deleterious spike in blood glucose.

**[0023]** In some embodiments, an isolated polypeptide as provided herein and the additional agent are formulated into a single therapeutic composition, and the isolated polypeptide and additional agent are administered simultaneously. In some embodiments, the isolated polypeptide and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the isolated polypeptide and the additional agent are administered simultaneously, or the isolated polypeptide and the additional agent are administered at different times during a treatment regimen. For example, the isolated polypeptide is administered prior to the administration of the

additional agent, the isolated polypeptide is administered subsequent to the administration of the additional agent, or the isolated polypeptide and the additional agent are administered in an alternating fashion. As described herein, the isolated polypeptide and additional agent are administered in single doses or in multiple doses.

**[0024]** Also provided herein are methods for treating, delaying the onset of, delaying the progression of, or otherwise ameliorating a symptom of a disease or condition caused, characterized by, or otherwise associated with aberrant glucagon activity. In some embodiments, the disease or condition is type 2 diabetes mellitus.

**[0025]** Also provided herein are methods of treating a metabolic disorder by administering an isolated polypeptide of the disclosure or any pharmaceutical composition described herein to a subject in need thereof.

**[0026]** Also provided herein are methods of treating obesity by administering an isolated polypeptide of the disclosure or any pharmaceutical composition described herein to a subject in need thereof.

**[0027]** Also provided herein are methods of treating, preventing, delaying the onset of, delaying the progression of, and/or otherwise ameliorating a symptom of a metabolic disease or disorder associated with elevated blood glucose in a patient by administering an isolated polypeptide of the disclosure or any pharmaceutical composition described herein to the patient in need thereof.

**[0028]** Also provided herein are methods of treating, preventing, delaying the onset of, delaying the progression of, and/or otherwise ameliorating a symptom of a disease or disorder in which co-agonism at both the glucagon and GLP-1 receptors is desired, such as, by way of non-limiting example, chronic pain, hemophilia and other blood disorders, endocrine disorders, metabolic disorders, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Alzheimer's disease, cardiovascular diseases, hypoglycemia unawareness, restrictive lung disease, chronic obstructive pulmonary disease, lipoatrophy, metabolic syndrome, leukemia, hepatitis, renal failure, autoimmune diseases (e.g., Grave's disease, systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis), shock and/or wasting disorders.

**[0029]** Also provided herein are methods of treating, preventing, delaying the onset of, delaying the progression of, and/or otherwise ameliorating a symptom of an infectious disease requiring chronic treatment(s).

**[0030]** In the methods disclosed herein, the isolated polypeptides of the disclosure and/or pharmaceutical compositions described herein are administered alone or in combination with pharmaceutically acceptable carriers and/or excipients and/or polymers and/or organic solvent, in either single or multiple doses.

**[0031]** Pharmaceutical compositions according to the invention can include a polypeptide of the disclosure, along with a suitable carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

## Brief Description of the Drawings

**[0032]** Figures 1A and 1B are a series of graphs depicting peptide-receptor activation profiles at the human glucagon receptor (GCGR) and the human GLP-1 receptor (GLP-1R) by human glucagon, GLP-1 (7-37), and GCGR-GLP-1R balanced co-agonist peptide referred to herein as Compound C1. These figures demonstrate that glucagon and Compound C1 activate GCGR (Figure 1A) and GLP-1R (Figure 1B) at nearly equal potencies.

**[0033]** Figure 2 is a graph and a table depicting mean plasma concentration vs. time plot of glucagon and the dual agonist Compound C1 in male rats after intravenous bolus administration at a dose of 0.1 mg/kg. For the graph, n=3, and the error bars represent standard error. The data presented in the table is shown as mean ± standard error (n=3).

**[0034]** Figure 3 is a graph depicting the efficacy of Compound C1 singly or in combination with exendin-4 in DIO LE rats. $p<0.05$ compared to vehicle control.

**[0035]** Figure 4 is a schematic representation of the dual analog referred to herein as Compound C1.

## Detailed Description

**[0036]** This invention relates to isolated polypeptides that are dual glucagon receptor analogs and glucagon-like peptide-1 (GLP-1) receptor analogs, as well as peptide derivatives thereof. Glucagon is produced by the pancreas and interacts with the glucagon receptor ("GCGR," also abbreviated as "GlucR"). In some embodiments, an isolated polypeptide of the disclosure is a dual glucagon receptor/GLP-1 receptor agonist. In some embodiments, an isolated polypeptide of the disclosure binds to a glucagon receptor (GCGR) and to a GLP-1 receptor.

*Definitions*

**[0037]** 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".

**[0038]** 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."

**[0039]** 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.

**[0040]** 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.

**[0041]** In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

**[0042]** 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.

**[0043]** 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 mimetic). 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 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-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 $C_1$-$C_4$ 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-molecule drug.

**[0044]** 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 terminus of the peptide.

**[0045]** 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.

**[0046]** The term "insulinotrophic" 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 insulinotrophic hormone). Such compounds typically stimulate or otherwise affect the secretion or biosynthesis of insulin in a subject. Thus, an "insulinotrophic peptide" is an amino acid-containing molecule capable of stimulating or otherwise affecting secretion or biosynthesis of insulin.

**[0047]** The term "insulinotrophic peptide" as used herein includes, but is not limited to, glucagon-like peptide 1 (GLP-1), as well as derivatives and analogues thereof, exenatide, exenatide having the amino acid sequence of SEQ ID NO; 1, as well as derivatives and analogues thereof.

**[0048]** The phrase "incretin mimetics" as used herein includes, but is not limited to GLP-1 peptide, peptide derivatives of GLP-1, peptide analogs of GLP-1; exenatide, exenatide having the amino acid sequence of SEQ ID NO: 1, exenatide peptide, peptide derivatives of exenatide, and peptide analogs of exenatide. Examples of preferred incretin mimetics include exenatide, exenatide having the amino acid sequence of exendin-4 (the naturally-occurring form of exenatide, and has the amino acid sequence of SEQ ID NO: 1), exenatide-LAR, lixisenatide, GLP-l (7-36), liraglutide, dulaglutide, albiglutide, and taspoglutide. Incretin mimetics are also referred to herein as "insulinotrophic peptides." Incretin mimetics which target the GLP-1 receptor are also known in the literature as"GLP-1 receptor agonists."

**[0049]** The term "an exenatide" as used herein includes, but is not limited to exenatide, exenatide having the amino acid sequence of SEQ ID NO: 1, native exendin-4, exenatide peptides, exenatide peptide analogs, and exenatide peptide derivatives.

**[0050]** The term "GLP-1" refers to a polypeptide that is produced by the L-cell located mainly in the ileum and colon, and to a lesser extent by L-cells in the duodenum and jejunum. GLP-1 is a regulatory peptides to a G-coupled protein receptor on β cell and via adenyl cyclase activity and production of cAMP stimulates the insulin response to the nutrients that are absorbed from the gut (Baggio 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 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 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.

**[0051]** The term "glucagon" refers to a 29 amino acid peptide hormone that is produced by alpha cells in the pancreas and that interacts with the glucagon receptor ("GCGR," also abbreviated as "GlucR"). The amino acid sequence of glucagon is HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21).

**[0052]** Glucagon originates from the 158 amino acid pre-proglucagon peptide which also acts as a precursor for the peptide hormones GLP-1, GLP-2, oxyntomodulin, glicentin, and glicentin-related pancreatic polypeptide via tissue-specific processing. Glucagon corresponds to the amino acid residues 33 to 61 of the precursor peptide and acts via interaction with a class B seven transmembrane G protein-coupled receptor located primarily in the liver. Immunostaining has also indicated the presence of glucagon receptors in the kidneys, gastrointestinal tract, heart, spleen, brain, adipocytes, and lymphoblasts. In response to low blood glucose levels, glucagon is released and stimulates hepatic glucose output via glycogenolysis and gluconeogenesis. Glucagon acts as a counter to the glucose-lowering effect of insulin and tight regulation via a feed-back system between the two hormones allows for effective glucose homeostasis.

**[0053]** In addition to its effect on blood glucose levels, glucagon is also known to increase energy expenditure and thermogenesis. Increased signaling has a direct effect on the regulation of triglycerides, free fatty acids, apolipoprotein, and bile acid metabolism. Current therapeutic uses for glucagon have primarily focused on its use as a rescue agent for hypoglycemic episodes. However, recent work has taken advantage of the hormone's ability to affect energy balance and lipid metabolism resulting in potential treatments for various metabolic disorders.

**[0054]** The poor solubility of glucagon (<1.0 mg/ml in aqueous or saline buffer) has prevented the opportunity for chronic study via continuous infusion. A summary of the solubility and the pEC50 value of glucagon with respect to glucagon receptor (GLUR) and GLP1, as determined in cAMP assays, is shown in Table 1 below:

Table 1.

| | Sequence | GLUR pEC50 (cAMP) | GLP1 pEC50 (cAMP) | Solubility in Aqueous |
|---|---|---|---|---|
| Glucagon | HSQGTFTSDYSKYLDSRRAQDFVQW LMNT-OH (SEQ ID NO: 21) | 10 | 9.9 | 0.03-10 µg/ml |

**[0055]** 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.

**[0056]** 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.

**[0057]** The phrase "single-phase" as used herein refers to a solid, semisolid, or liquid homogeneous system that is physically and chemically uniform throughout.

**[0058]** 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.

**[0059]** 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.

**[0060]** 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.

**[0061]** 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:

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

where F/A=shear stress (force per unit area),
μ=a proportionality constant (viscosity), and
V/L=the velocity per layer thickness (shear rate).

**[0062]** 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 for the determination of viscosity include, measurement of a kinematic viscosity using viscometers, for example, a Cannon-Fenske viscometer, a Ubbelohde viscometer for the Cannon-Fenske opaque solution, or a 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 delivery device.

**[0063]** 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.

**[0064]** 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.

**[0065]** The term "osmotic delivery device" as used herein typically refers to a device used for delivery of a drug (*e.g.*, an isolated dual glucagon receptor/GLP-1 receptor agonist 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.*, an isolated dual glucagon receptor/GLP-1 receptor agonist 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.

**[0066]** 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.

**[0067]** 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.*, an isolated dual glucagon receptor/GLP-1 receptor analog) 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).

**[0068]** 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).

**[0069]** The terms "µg" and "mcg" and "ug" are understood to mean "micrograms". Similarly, each of the terms "µl" or "uL" is understood to mean "microliter".

**[0070]** 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".

**[0071]** 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).

## 2.0.0 General Overview of the Invention

**[0072]** Before describing the present invention in detail, it is to be understood that this invention is not limited to particular types of drug delivery devices, particular sources of drugs, particular solvents, particular polymers, and the like, as use of such particulars may be selected in view of the teachings of the present specification. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

**[0073]** In a first aspect, the present invention relates to isolated polypeptides that are glucagon analogs and peptide derivatives thereof. In some embodiments, an isolated polypeptide of the disclosure is a dual glucagon receptor/GLP-1 receptor agonist. In some embodiments, an isolated polypeptide of the disclosure binds to a glucagon receptor (GCGR) and a GLP-1 receptor.

**[0074]** In some embodiments, an isolated polypeptide of the disclosure comprises a modified amino acid sequence based on the amino acid sequence of human glucagon: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21), where the modified amino acid sequence includes at least one amino acid substitution, at least two amino acid substitutions, at least three amino acid substitutions, at least four amino acid substitutions, at least five amino acid substitutions, at least six amino acid substitutions, at least seven amino acid substitutions, at least eight amino acid substitutions, at least nine amino acid substitutions, at least 10 amino acid substitutions, at least 11 amino acid substitutions, at least 12 amino acid substitutions, at least 13 amino acid substitutions, at least 14 amino acid substitutions, at least 15 amino acid substitutions, at least 16 amino acid substitutions, at least 17 amino acid substitutions, at least 18 amino acid substitutions, at least 19 amino acid substitutions, at least 20 amino acid substitutions, at least 21 amino acid

substitutions, at least 22 amino acid substitutions, at least 23 amino acid substitutions, at least 24 amino acid substitutions, at least 25 amino acid substitutions, at least 26 amino acid substitutions, at least 27 amino acid substitutions, at least 28 amino acid substitutions, and/or at least 29 amino acid substitutions, provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0075]** In some embodiments, an isolated polypeptide of the disclosure comprises a modified amino acid sequence based on the amino acid sequence of human glucagon: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21), where the modified amino acid sequence includes at least one amino acid substitution, at least two amino acid substitutions, at least three amino acid substitutions, at least four amino acid substitutions, at least five amino acid substitutions, at least six amino acid substitutions, at least seven amino acid substitutions, at least eight amino acid substitutions, at least nine amino acid substitutions, at least 10 amino acid substitutions, at least 11 amino acid substitutions, at least 12 amino acid substitutions, or at least 13 amino acid substitutions, wherein the amino acid substitution(s) is selected from the group consisting of:

(i) an amino acid substitution at position 1 selected from the group consisting of H, W, pyroglutamate (pGlu), Q, P, Y, and N;

(ii) an amino acid substitution at position 2 selected from the group consisting of S, D-Ser, and 2-Aminoisobutyric acid (Aib);

(iii) an amino acid substitution at position 16 selected from the group consisting of S and K*, where K* at position 16 is connected with E* at position 20 via lactam bridge;

(iv) an amino acid substitution at position 17 selected from the group consisting of R, A, K and K*, where K* at position 17 is connected with E* at position 21 via lactam bridge;

(v) an amino acid substitution at position 18 selected from the group consisting of R and A;

(vi) an amino acid substitution at position 20 selected from the group consisting of Q and E*, where E* at position 20 is connected with K* at position 16 via lactam bridge;

(vii) an amino acid substitution at position 21 selected from the group consisting of D and E*, where E* at position 21 is connected with K* at position 17 via lactam bridge;

(viii) an amino acid substitution at position 23 selected from the group consisting of V and D-Val

(ix) an amino acid substitution at position 24 to K**, where K** at position 24 is connected with E** at position 28 via lactam bridge;

(x) an amino acid substitution at position 25 selected from the group consisting of W and H;

(xi) an amino acid substitution at position 26 selected from the group consisting of L and K;

(xii) an amino acid substitution at position 27 selected from the group consisting of M and Q;

(xiii) an amino acid substitution at position 28 to E**, where E** at position 28 is connected with K** at position 24 via lactam bridge;

(xiv) an amino acid substitution at position 30 selected from the group consisting of OH and $NH_2$; and

(xv) combinations thereof,

provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0076]** In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of amino acid sequences represented by the consensus sequence of SEQ ID NO: 1:

$X_1X_2QGTFTSDYSKYLDX_{16}X_{17}X_{18}AX_{20}X_{21}FVX_{24}WX_{26}X_{27}X_{28}TX_{30}$ (SEQ ID NO: 1), wherein:

$X_1$ is H, W, pyroglutamate (pGlu), Q, P, Y, or N;

$X_2$ is S, D-Ser, or 2-Aminoisobutyric acid (Aib);

$X_{16}$ is S or K*, where K* at position 16 is connected with E* at position 20 via lactam bridge;

$X_{17}$ is R, A, K, or K*, where K* at position 17 is connected with E* at position 21 via lactam bridge;

$X_{18}$ is R or A;

$X_{20}$ is Q or E*, where E* at position 20 is connected with K* at position 16 via lactam bridge;

$X_{21}$ is D or E*, where E* at position 21 is connected with K* at position 17 via lactam bridge;

$X_{23}$ is V or D-Val;

$X_{24}$ is K**, where K** at position 24 is connected with E** at position 28 via lactam bridge;

$X_{25}$ is W or H;

$X_{26}$ is L or K;

$X_{27}$ is M or Q;

$X_{28}$ is E**, where E** at position 28 is connected with K** at position 24 via lactam bridge;

$X_{30}$ is OH or $NH_2$; and combinations thereof,

provided that the isolated polypeptide having a modified amino acid sequence retains the ability to function as a dual glucagon receptor/GLP-1 receptor agonist.

**[0077]** In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-$NH_2$ (SEQ ID NO: 2), which is also referred to herein as Compound C1;
HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 3), which is also referred to herein as Compound C2;
HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-OH (SEQ ID NO: 4), which is also referred to herein as Compound C3;
NSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 5), which is also referred to herein as Compound C4;
QSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 6), which is also referred to herein as Compound C5;
WSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 7), which is also referred to herein as Compound C6;
PSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 8), which is also referred to herein as Compound C7;

(pGlu)SQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 9), which is also referred to herein as Compound C8; HSQGTFTSDYSKYLDSK*RAQE*FVK**HLQE**T-$NH_2$ (SEQ ID NO: 10), which is also referred to herein as Compound C9; HSQGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 11), which is also referred to herein as Compound C10; H(D-Ser)QGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 12), which is also referred to herein as Compound C11; HSQGTFTSDYSKYLDSKAAQDFVK**WLME**T- $NH_2$ (SEQ ID NO: 13), which is also referred to herein as Compound C12; HSQGTFTSDYSKYLDSRRAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 14), which is also referred to herein as Compound C13; HSQGTFTSDYSKYLDSRRAQDFVK**WKME**T-$NH_2$ (SEQ ID NO: 15), which is also referred to herein as Compound C14; HSQGTFTSDYSKYLDSRRAQDFVK**HLME**T-$NH_2$ (SEQ ID NO: 16), which is also referred to herein as Compound C15; HSQGTFTSDYSKYLDSARAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 17), which is also referred to herein as Compound C16; Y(Aib)QGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 18), which is also referred to herein as Compound C17; YSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 19), which is also referred to herein as Compound C18; and HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 20), which is also referred to herein as Compound C19. In some embodiments, an isolated polypeptide of the disclosure comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-20.

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

**[0078]** In some embodiments, the disclosed peptide is optionally substituted with one or more lipophilic substituents each optionally via a spacer.

**[0079]** Conjugation of one or more lipophilic substituents, each optionally via spacer, to a disclosed peptide is intended to prolong the action of the peptide by facilitating binding to serum albumin and delayed renal clearance of the conjugated peptide. As used herein, a "lipophilic substituent" comprises a substituent comprising 4 to 40 carbon atoms, 8 to 25 carbon atoms, or 12 to 22 carbon atoms. The lipophilic substituent may be attached to

an amino group of the peptide (*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 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 peptide comprises three, two, or preferably one lipophilic substituent each with or without an optional spacer.

**[0080]** 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 the acyl group of the formula $CH_3(CH_2)_nCO$—, wherein n is an integer from 4 to 38, an integer from 4 to 24, such as $CH_3(CH_2)_6CO$—, $CH_3(CH_2)_8CO$—, $CH_3(CH_2)_{10}CO$—, $CH_3(CH_2)_{12}CO$—, $CH_3(CH_2)_{14}CO$—, $CH_3(CH_2)_{16}CO$—, $CH_3(CH_2)_{18}CO$—, $CH_3(CH_2)_{20}CO$— or $CH_3(CH_2)_{22}CO$—. In some embodiments, n is 6, 8, 10, 12, 14, 16, 18, 20 or 22. In some embodiments, the lipophilic substituent is an acyl group of the formula $CH_3(CH_2)_{14}CO$—.

**[0081]** 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 lipophilic substituent is an acyl group of the formula $HOOC(CH_2)_mCO$—, wherein m is an integer from 4 to 38, an integer from 4 to 24, such as $HOOC(CH_2)_{14}CO$—, $HOOC(CH_2)_{16}CO$—, $HOOC(CH_2)_{18}CO$—, $HOOC(CH_2)_{20}CO$— or $HOOC(CH_2)_{22}CO$—. In some embodiments, the lipophilic substituent is $HOOC(CH_2)_{16}CO$—. In some embodiments, m is 6, 8, 10, 12, 14, 16, 18, 20 or 22.

**[0082]** In some embodiments, the lipophilic substituent is attached to the parent peptide by means of a "spacer." In some embodiments, the spacer comprises a bivalent group of Formula I:

$$-N(R_1)(CHR_2)_pCO—[N(R_3)((CH_2)_2O(CH_2)_2O)_q(CH_2)CO—]_r$$

(Formula I)

wherein

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.

which spacer forms a bridge between an amino group of the disclosed peptide and a CO— group of the lipophilic substituent.

**[0083]** 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.

**[0084]** In some embodiments, at least one $R_2$ is $CO_2H$. In some embodiments, one $R_2$ is $CO_2H$.

**[0085]** 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.

**[0086]** In some embodiments, q is 1. In some embodiments, q is 2. In some embodiments, q is 3.

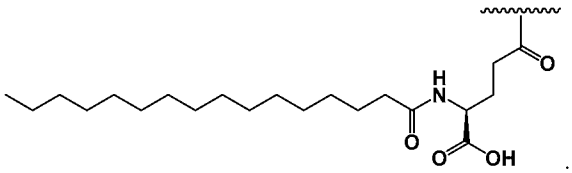
**[0087]** In some embodiments, r is 0. In some embodiments, r is 1.

**[0088]** In some embodiments, the spacer is γ-glutamyl, *i.e.*, $–NH(CHCO_2H)(CH_2)_2CO--$. In some embodiments, the spacer is γ-aminobutanoyl, *i.e.*, $–NH(CH_2)_3CO--$. In some embodiments, the spacer is β-asparagyl, *i.e.*, $–NH(CHCO_2H)(CH_2)CO--$. In some embodiments, the spacer is $–NH(CH_2)_2CO--$. In some embodiments, the spacer is glycyl. In some embodiments, the spacer is β-alanyl. In some embodiments, provided is the peptide of SEQ ID NO: 151, described below, wherein the lipophilic substituent is linked to the ε-amino group of a lysine via a spacer.

**[0089]** In some embodiments, the spacer is $–NHCH(CO_2H)(CH_2)_2CO--[N(R_3)((CH_2)_2O(CH_2)_2O)_q(CH_2)CO—]_r$. In some embodiments, the spacer is $–NH(CH_2)_3CO--[N(R_3)((CH_2)_2O(CH_2)_2O)_q(CH_2)CO—]_r$. In some embodiments, the spacer is $–NHCH(CO_2H)(CH_2)_2CO-NH((CH_2)_2O(CH_2)_2O)_2(CH_2)CO-$. In some embodiments, the spacer is $–NH(CH_2)_3CO-NH((CH_2)_2O(CH_2)_2O)_2(CH_2)CO-$. In some embodiments, the spacer is $–NHCH(CO_2H)CH_2CO--[N(R_3)((CH_2)_2O(CH_2)_2O)_q(CH_2)CO—]_r$. In some embodiments, the spacer is $–NH(CH_2)_2CO--[N(R_3)((CH_2)_2O(CH_2)_2O)_q(CH_2)CO—]_r$.

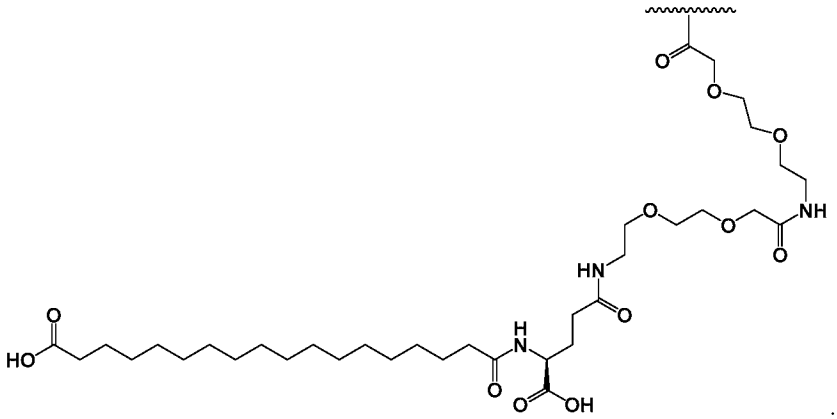
**[0090]** In some embodiments, the spacer is an amino acid, for example, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. In some embodiments, when the spacer is Lys, Glu or Asp, one carboxyl group of the spacer may form an amide bond with an amino group of the disclosed peptide, and an amino group of the spacer may form an amide bond with a carboxyl group of the lipophilic substituent.

**[0091]** In some embodiments, the lipophilic substituent and spacer combine to form the structure of Formula II:



Formula II

**[0092]** In some embodiments, the lipophilic substituent and spacer combine to form the structure of Formula III:



Formula III

**[0093]** In some embodiments, each lipophilic substituent is attached, optionally via a spacer, to the ε-amino group of a lysine residue contained in the parent peptide.

**[0094]** In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed peptides formulated as a trifluoroacetate salt, acetate salt or hydrochloride salt. In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed peptides formulated as a trifluoroacetate salt. In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed peptides formulated as an acetate salt. In some embodiments, provided is a pharmaceutical composition comprising any of the disclosed peptides formulated as a hydrochloride salt.

**[0095]** The invention also provides a method of treating type 2 diabetes mellitus in a subject in need of treatment. The method comprises providing one or more of the isolated dual glucagon receptor/GLP-1 receptor agonist polypeptides of the disclosure. In some embodiments, the method comprises providing continuous delivery of an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide from an osmotic delivery device,

wherein substantial steady-state delivery of the isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide at a therapeutic concentration is achieved within a time period of about 7 days or less after implantation of the osmotic delivery device in the subject. The substantial steady-state delivery of the isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide from the osmotic delivery device is continuous over an administration period. Humans are preferred subjects for the practice of the present invention.

**[0096]** 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, or at least about 10 months to about a year.

**[0097]** In some embodiments of the present invention, the substantial steady-state delivery of an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide at therapeutic concentrations is achieved within about 5 days or less after 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 invention, the substantial steady-state delivery of the isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide at therapeutic concentrations is achieved within about 2 days or less, more preferably within about 1 day or less after implantation of the osmotic delivery device in the subject.

**[0098]** 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 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 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 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 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.

**[0099]** In yet further embodiments of the first aspect of the present invention, the treatment methods further comprise the capability to terminate the continuous delivery of the dual glucagon receptor/GLP-1 receptor agonist polypeptide such that the concentration of the dual glucagon receptor/GLP-1 receptor agonist polypeptide is substantially undetectable in a blood sample from the subject within about 6 half-lives or less of the dual glucagon receptor/GLP-1 receptor agonist polypeptide after termination of continuous delivery, within about 5 half-lives or less of the dual glucagon receptor/GLP-1 receptor agonist polypeptide after termination of continuous delivery, within about 4 half-lives or less of the dual glucagon receptor/GLP-1 agonist receptor polypeptide after termination of continuous delivery, or within about 3 half-lives or less of the dual glucagon receptor/ GLP-1 receptor agonist polypeptide after termination of continuous delivery. The dual glucagon receptor/GLP-1 receptor agonist polypeptide may be detected, for example, by an RIA, a chromatographic method, an ECL assay, an ELISA, or an IEMA. Termination of the continuous delivery can be accomplished, for example, by removal of the osmotic delivery device from the subject.

**[00100]** In related embodiments of the present invention, the treatment methods further comprise the capability to terminate the continuous delivery of the dual glucagon receptor/GLP-1 receptor agonist polypeptide such that the concentration of the polypeptide is substantially undetectable in a blood sample from the subject in less than about 72 hours after termination of continuous delivery, in less than about 48 hours after termination of continuous delivery, in less than about 24 hours after termination of continuous delivery, in less than about 18 hours after termination of continuous delivery, in less than about 14

hours after termination of continuous delivery, in less than about 12 hours after termination of continuous delivery, in less than about 6 hours after termination of continuous delivery, or in less than about 4 hours after termination of continuous delivery. In preferred embodiments, the dual glucagon receptor/GLP-1 receptor agonist polypeptide is substantially undetectable in a blood sample from the subject in less than about 24 hours after termination of continuous delivery, in less than about 18 hours after termination of continuous delivery, or more preferably in less than about 14 hours after termination of continuous delivery.

**[00101]** In some embodiments, the dual glucagon receptor/GLP-1 receptor agonist polypeptide is formulated in a suspension formulation. In some embodiments, the suspension formulation comprises a particle formulation comprising the dual glucagon receptor/GLP-1 receptor agonist polypeptide, and a vehicle formulation. In some embodiments, the dual glucagon receptor/GLP-1 receptor agonist polypeptide comprises an isolated polypeptide of the disclosure, a peptide analog thereof, or a peptide derivative thereof. In some embodiments, the dual glucagon receptor/GLP-1 receptor agonist polypeptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20.

**[00102]** In embodiments of all aspects of the present invention relating to methods of treating type 2 diabetes mellitus, suspension formulations for use in the methods can, for example, comprise a particle formulation comprising an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide, and a vehicle formulation.

**[00103]** The reservoir of the osmotic delivery devices may, for example, comprise titanium or a titanium alloy.

**[00104]** In a fifth aspect, 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 of a drug from an osmotic delivery device, wherein substantial steady-state delivery of the drug at therapeutic concentrations is achieved within a time period of about 7 days or less after implantation of the osmotic delivery device 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 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.

**[00105]** 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, or at least about 10 months to about a year.

**[00106]** In some embodiments of this aspect of the present invention, the substantial steady-state delivery of a drug at therapeutic concentrations is achieved within a period of about 7 days or less after implantation of the osmotic delivery device in the subject, about 5 days or less after implantation of the osmotic delivery device in the subject, about 4 days or less after implantation of the osmotic delivery device in the subject, about 3 days or less after implantation of the osmotic delivery device in the subject, about 2 days or less after implantation of the osmotic delivery device in the subject, or about 1 day or less after implantation of the osmotic delivery device in the subject.

**[00107]** In some embodiments of this aspect of the present invention, establishment of the substantial steady-state delivery of the drug at therapeutic concentrations, after implantation of the osmotic delivery device in the subject, may take a longer period of time, for example, a period of about 2 weeks or less, or within less than about 6 half-lives of the drug within the subject after implantation of the device.

**[00108]** 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 dual glucagon receptor/GLP-1 receptor agonist polypeptide. In some embodiments, the isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide is continuously delivered from an osmotic delivery device, wherein substantial steady-state delivery of the dual glucagon receptor/GLP-1 receptor agonist polypeptide at a therapeutic concentration is achieved within a time period of about 7 days or less after implantation of the osmotic delivery device in the subject. The substantial steady-state delivery of the dual glucagon receptor/GLP-1 receptor agonist polypeptide from the osmotic delivery device is continuous over an administration period. Humans are preferred subjects for the practice of the present invention. The present invention includes

an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide, as well as an osmotic delivery device comprising an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide for use in the present methods in a subject in need of treatment. They subject may have 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.

**[00109]** 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, or at least about 10 months to about a year.

**[00110]** 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 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 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 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 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.

**[00111]** 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: 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 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.

**[00112]** 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 example, comprise a solvent and a polymer.

**[00113]** The reservoir of the osmotic delivery devices may, for example, comprise titanium or a titanium alloy.

**[00114]** In embodiments of all aspects of the present invention the implanted osmotic delivery device can be used to provide subcutaneous delivery.

**[00115]** In embodiments of all aspects of the present invention the continuous delivery can, for example, be zero-order, controlled continuous delivery.

**[00116]** Any of the above aspects and embodiments can be combined with any other aspect or embodiment as disclosed herein.

### 3.0.0 Formulations and Compositions

**[00117]** Drugs for use in the practice of the present invention are typically uniformly suspended, dissolved or dispersed in a suspension vehicle to form a suspension formulation.

**[00118]** 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 fusion protein and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, 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 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 also be incorporated into the compositions.

**[00119]** 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.

**[00120]** 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 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. 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.

**[00121]** 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 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.

**[00122]** 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 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 or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

**[00123]** 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.

**[00124]** Systemic administration can also be by transmucosal or transdermal means. For 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, or creams as generally known in the art.

**[00125]** The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

**[00126]** 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, 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.

**[00127]** It is especially advantageous to formulate oral or parenteral compositions in dosage unit 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 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.

**[00128]** The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### 3.1.0 Drug Particle Formulations

**[00129]** In one aspect, 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.

**[00130]** 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, about or about 70 wt % to about 75 wt % drug.

**[00131]** In any of the embodiments, a particle formulation comprises a drug, as described above, and one or more stabilizer. The stabilizers may be, for example, carbohydrate, antioxidant, amino acid, buffer, inorganic compound, or surfactant. 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.

**[00132]** Typically, the amount of carbohydrate in the 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.

**[00133]** Typically, the amount of antioxidant in the 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.

**[00134]** Typically, the amount of buffer in the 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.

**[00135]** 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 myoinsitol). Suitable carbohydrates include disaccharides and/or non-reducing sugars, such as sucrose, trehalose, and raffinose.

**[00136]** 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 hydroxanisol, butylated hydroxyltoluene, and propyl gallate. Further, amino acids that readily oxidize can be used as antioxidants, for example, cysteine, methionine, and tryptophan.

**[00137]** Examples of amino acids that may be included in the particle formulation include, but are not limited to, arginine, methionine, glycine, histidine, alanine, L-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.

**[00138]** 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.

**[00139]** Examples of inorganic compounds that may be included in the particle formulation include, but are not limited to, NaCl, $Na_2SO_4$, $NaHCO_3$, KCl, $KH_2PO_4$, $CaCl_2$, and $MgCl_2$.

**[00140]** 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.

### 3.1.1 Exemplary Drugs

**[00141]** The drug particle formulations comprise a drug. The drug may be any physiologically or pharmacologically active substance, particularly those known to be delivered to the body of a human or an animal.

**[00142]** Suitable drugs include, but are not limited to: peptides, proteins, polypeptides or synthetic analogs of these species, as well as mixtures thereof.

**[00143]** In one embodiment, preferred drugs include macromolecules. Such macromolecules include, but are not limited to, pharmacologically active peptides, proteins, or polypeptides. Numerous peptides, proteins, or polypeptides that are useful in the practice of the present invention are described herein. In addition to the peptides, proteins, or polypeptides described, modifications of these peptides, proteins, or polypeptides are also known to one of skill in the art and can be used in the practice of the present invention following the guidance presented herein. Such modifications include, but are not limited to, amino acid analogs, amino acid mimetics, analog polypeptides, or derivative polypeptides. Further, the drugs disclosed herein may be formulated or administered singly or in combination (*e.g.*, using mixtures of drugs or multiple devices; U.S. Patent Publication No. 2009/0202608).

**[00144]** Some embodiments of the present invention comprise use of polypeptides of SEQ ID NOs: 2-20.

**[00145]** Some embodiments of the present invention comprise use of a dual glucagon receptor/GLP-1 receptor agonist polypeptide in combination with a second polypeptide,

such as, by way of, non-limiting example, peptide hormones, for example, glucagon and incretin mimetics (*e.g.,* GLP-1 and exenatide), as well as peptide analogs and peptide derivatives thereof; PYY (also known as peptide YY, peptide tyrosine tyrosine), as well as peptide analogs and peptide derivatives thereof, for example, PYY(3-36); oxyntomodulin, as well as peptide analogs and peptide derivatives thereof); and gastric inhibitory peptide (GIP), as well as peptide analogs and peptide derivatives thereof.

**[00146]** GLP-1, including three forms of the peptide, GLP-1(1-37), GLP-1(7-37) and GLP-1(7-36) amide, as well as peptide analogs of GLP-1 have been shown to stimulate insulin secretion (*i.e.,* is insulinotropic), which induces glucose uptake by cells and results in decreases in serum glucose concentrations (*see, e g.*, Mojsov, S., *Int. J. Peptide Protein Research*, 40:333-343 (1992)).

**[00147]** Numerous GLP-1 peptide derivatives and peptide analogs demonstrating insulinotropic 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). One example of a GLP-1 peptide derivative useful in the practice of the present invention is Victoza® (Novo Nordisk A/S, Bagsvaerd D K) (liraglutide; U.S. Pat. Nos. 6,268,343, 6,458,924, and 7,235,627). Once-daily injectable Victoza® (liraglutide) is commercially available in the United States, Europe, and Japan. For ease of reference herein, the family of GLP-1 peptides, GLP-1 peptide derivatives and GLP-1 peptide analogs having insulinotropic activity is referred to collectively as "GLP-1."

**[00148]** The molecule exenatide has the amino acid sequence of exendin-4 (Kolterman O. G., *et al.*, *J. Clin. Endocrinol. Metab.* 88(7):3082-9 (2003)) and is produced by chemical synthesis or recombinant expression. Twice-daily injectable exenatide is commercially available in the United States and Europe, and sold under the trade name of Byetta® (Amylin Pharmaceuticals, Inc., San Diego, Calif.). Exendin-3 and exendin-4 are known in the art and were originally isolated from *Heloderma spp.* (Eng, J., *et al.*, *J. Biol. Chem.*, 265:20259-62 (1990); Eng., J., *et al.*, J. Biol. Chem., 267:7402-05 (1992)). Use of exendin-3 and exendin-4 for the treatment of type 2 diabetes mellitus and the prevention of hyperglycemia has been proposed (*see, e.g.,* U.S. Pat. No. 5,424,286). Numerous exenatide

peptide derivatives and peptide analogs (including, *e.g.,* exendin-4 agonists) are known in the art (*see, e.g.,* U.S. Pat. Nos. 5,424,286; 6,268,343; 6,329,336; 6,506,724; 6,514,500; 6,528,486; 6,593,295; 6,703,359; 6,706,689; 6,767,887; 6,821,949; 6,849,714; 6,858,576; 6,872,700; 6,887,470; 6,887,849; 6,924,264; 6,956,026; 6,989,366; 7,022,674; 7,041,646; 7,115,569; 7,138,375; 7,141,547; 7,153,825; and 7,157,555). One example of an exenatide derivative useful in the practice of the present invention is lixisenatide (also known as ZP10, AVE0010) (*see, e.g.,* U.S. Pat. No. 6,528,486), which is in clinical trials. For ease of reference herein, the family of exenatide peptides (*e.g.,* including exendin-3, exendin-4, and exendin-4-amide), exenatide peptide derivatives, and exenatide peptide analogs is referred to collectively as "exenatide."

**[00149]** Peptide YY (PYY) is a 36 amino acid residue peptide amide. PYY inhibits gut motility and blood flow (Laburthe, M., *Trends Endocrinol Metab.* 1(3):168-74 (1990), mediates intestinal secretion (Cox, H. M., *et al.*, *Br J Pharmacol* 101(2):247-52 (1990); Playford, R. J., *et al.*, *Lancet* 335(8705):1555-7 (1990)), and stimulate net absorption (MacFayden, R. J., *et al.*, *Neuropeptides* 7(3):219-27 (1986)). Two major in vivo variants, PYY(1-36) and PYY(3-36), have been identified (*e.g.,* Eberlein, G. A., *et al.*, *Peptides* 10(4), 797-803 (1989)). The sequence of PYY, as well as peptide analogs and peptide derivatives thereof, are known in the art (*e.g.,* U.S. Pat. Nos. 5,574,010 and 5,552,520).

**[00150]** 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).

**[00151]** 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)).

**[00153]** 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.

**[00154]** Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide with the sequence HADGSFSDEMNTILDNLAARDFINWLIQTKITD (SEQ ID NO: 22) in humans. GLP-2 is created by specific post-translational proteolytic cleavage of proglucagon in a process that also liberates the related glucagon-like peptide-1 (GLP-1). GLP-2 is produced by the intestinal endocrine L cell and by various neurons in the central nervous system. Intestinal GLP-2 is co-secreted along with GLP-1 upon nutrient ingestion. When externally administered, GLP-2 produces a number of effects in humans and rodents, including intestinal growth, enhancement of intestinal function, reduction in bone breakdown and neuroprotection. GLP-2 may act in an endocrine fashion to link intestinal growth and metabolism with nutrient intake.

**[00155]** Examples of half-lives of some of the peptides are as follows: exenatide, approximately 2.5 hours; GLP-1, approximately 2 minutes; GIP, approximately 5 minutes; PYY, approximately 8 minutes; glucagon, approximately 6 minutes; oxyntomodulin, approximately 6 minutes; and GLP-2, approximately 6 minutes.

**[00156]** The drugs can also be in various forms including, but not limited to, the following: uncharged molecules; components of molecular complexes; and pharmacologically acceptable salts such as hydrochloride, hydrobromide, sulfate, laurates, palmatates, phosphate, nitrate, borate, acetate, maleate, tartrate, oleates, or salicylates. For acidic drugs, salts of metals, amines or organic cations, for example, quaternary ammonium, can be employed. Furthermore, simple derivatives of the drug such as esters, ethers, amides and the like that have solubility characteristics suitable for the purpose of the invention can also be used herein.

**[00156]** The above drugs and other drugs known to those of skill in the art are useful in methods of treatment for a variety of conditions including but not limited to the following: chronic pain, hemophilia and other blood disorders, endocrine disorders, metabolic disorders, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Alzheimer's disease, cardiovascular diseases (*e.g.*, heart failure, atherosclerosis, and acute coronary syndrome), rheumatologic disorders, diabetes (including type 1, type 2 diabetes mellitus, human immunodeficiency virus treatment-induced, latent autoimmune diabetes in adults, and steroid-induced), hypoglycemia unawareness, restrictive lung disease, chronic obstructive pulmonary disease, lipoatrophy, metabolic syndrome, leukemia, hepatitis, renal failure, infectious diseases (including bacterial infection, viral infection (*e.g.*, infection by human immunodeficiency virus, hepatitis C virus, hepatitis B virus, yellow fever virus, West Nile virus, Dengue virus, Marburg virus, and Ebola virus), and parasitic infection), hereditary diseases (such as cerbrosidase deficiency and adenosine deaminase deficiency), hypertension, septic shock, autoimmune diseases (*e.g.*, Grave's disease, systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis), shock and wasting disorders, cystic fibrosis, lactose intolerance, Crohn's diseases, inflammatory bowel disease, gastrointestinal cancers (including colon cancer and rectal cancer), breast cancer, leukemia, lung cancer, bladder cancer, kidney cancer, non-Hodgkin lymphoma, pancreatic cancer, thyroid cancer, endometrial cancer, and other cancers. Further, some of the above agents are useful for the treatment of infectious diseases requiring chronic treatments including, but not limited to, tuberculosis, malaria, leishmaniasis, trypanosomiasis (sleeping sickness and Chagas disease), and parasitic worms.

**[00157]** The amount of drug in drug particle formulations is that amount necessary to deliver a therapeutically effective amount of the agent to achieve the desired therapeutic result in the subject to which the drug is being delivered. In practice, this will vary depending upon such variables, for example, as the particular agent, the severity of the condition, and the desired therapeutic effect. Beneficial agents and their dosage unit amounts are known to the prior art in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Ed., (2005), McGraw Hill; Remington's Pharmaceutical Sciences, 18th Ed., (1995), Mack Publishing Co.; and Martin's Physical Pharmacy and Pharmaceutical Sciences, 1.00 edition (2005), Lippincott Williams & Wilkins. Further, highly concentrated drug particles are described in U.S. Patent Publication No. 2010/0092566. Typically, for an osmotic delivery system, the volume of the chamber comprising the drug formulation is

between about 100 µl to about 1000 µl, more preferably between about 140 µl and about 200 µl. In one embodiment, the volume of the chamber comprising the drug formulation is about 150 µl.

**[00158]** Drug particle formulations of the invention are preferably chemically and physically stable for at least 1 month, preferably at least 3 months, more preferably at least 6 months, more preferably at least 12 months at delivery temperature. The delivery temperature is typically normal human body temperature, for example, about 37° C., or slightly higher, for example, about 40° C. Further, drug particle formulations of the present invention are preferably chemically and physically stable for at least 3 months, preferably at least 6 months, more preferably at least 12 months, at storage temperature. Examples of storage temperatures include refrigeration temperature, for example, about 5° C.; or room temperature, for example, about 25° C.

**[00159]** A drug particle formulation may be considered chemically stable if less than about 25%; preferably less than about 20%, more preferably less than about 15%, more preferably less than about 10%, and more preferably less than about 5% breakdown products of the drug particles are formed after about 3 months, preferably after about 6 months, preferably after about 12 months at delivery temperature and after about 6 months, after about 12 months, and preferably after about 24 months at storage temperature.

**[00160]** A drug particle formulation may be considered physically stable if less than about 10%, preferably less than about 5%, more preferably less than about 3%, more preferably less than 1% aggregates of the drug are formed after about 3 months, preferably after about 6 months, at delivery temperature and about 6 months, preferably about 12 months, at storage temperature.

**[00161]** When the drug in the drug particle formulation is a protein, the protein solution is kept in a frozen condition and lyophilized or spray dried to a solid state. Tg (glass transition temperature) may be one factor to consider in achieving stable compositions of protein. While not intending to be bound by any particular theory, the theory of formation of a high Tg amorphous solid to stabilize peptides, polypeptides, or proteins has been utilized in pharmaceutical industry. Generally, if an amorphous solid has a higher Tg, such as 100° C., peptide products will not have mobility when stored at room temp or even at 40° C. because the storage temperature is below the Tg. Calculations using molecular information have shown that if a glass transition temperature is above a storage temperature of 50° C. that there is zero mobility for molecules. Zero mobility of molecules

correlates with better stability. Tg is also dependent on the moisture concentration in the product formulation. Generally, the more moisture, the lower the Tg of the composition.

**[00162]** Accordingly, in some aspects of the present invention, excipients with higher Tg may be included in the protein formulation to improve stability, for example, sucrose (Tg=75° C.) and trehalose (Tg=110° C.). 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.

**[00163]** 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 than 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.

**[00164]** 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 $D_{50}$ value, which is commonly used to represent the mean diameter of the range of the particle sizes of a given sample.

**[00165]** 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.

**[00166]** Particles of a particle formulation comprising an isolated dual glucagon receptor/GLP-1 receptor agonist polypeptide have average diameters of between about 0.3 microns to about 150 microns. Particles of a particle formulation comprising an isolated dual glucagon receptor/GLP-1 receptor agonist 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 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.

**[00167]** Typically, the particles of the particle formulations, when incorporated in a suspension 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, 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 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.

**[00168]** In one embodiment, a drug particle formulation comprises a drug, as described above, one or more stabilizers, and optionally a buffer. The stabilizers may be, for example, carbohydrate, antioxidant, amino acid, buffer, inorganic compound, or surfactant. The amounts of stabilizers and buffer in the particle formulation can be determined experimentally based on the activities of the stabilizers and buffers and the desired characteristics of the formulation, in view of the teachings of the present specification. Typically, the amount of carbohydrate in the 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 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 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 excipients are solubilized.

**[00169]** 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 myoinsitol). Preferred carbohydrates include disaccharides and/or non-reducing sugars, such as sucrose, trehalose, and raffinose.

**[00170]** 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 hydroxanisol, butylated hydroxyltoluene, and propyl gallate. Further, amino acids that readily oxidize can be used as antioxidants, for example, cysteine, methionine, and tryptophan.

**[00171]** Examples of amino acids that may be included in the particle formulation include, but are not limited to, arginine, methionine, glycine, histidine, alanine, L-leucine, glutamic acid, iso-leucine, L-threonine, 2-phenylamine, valine, norvaline, praline, phenylalanine, tryptophan, serine, asparagines, cysteine, tyrosine, lysine, and norleucine.

**[00172]** 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.

**[00173]** Examples of inorganic compounds that may be included in the particle formulation include, but are not limited to, NaCl, $Na_2SO_4$, $NaHCO_3$, KCl, $KH_2PO_4$, $CaCl_2$, and $MgCl_2$.

**[00174]** In addition, the particle formulation may include other 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.

**[00175]** All components included in the particle formulation are typically acceptable for pharmaceutical use in mammals, particularly, in humans.

**[00176]** In summary, a selected drug or combination of drugs is formulated into dried powders in solid state, which preserve maximum chemical and biological stability of the drug. The particle formulation 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.

### 3.2.0 Vehicle Formulations and Suspension Formulations

**[00177]** 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.

**[00178]** 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.

**[00179]** 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.

**[00180]** 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.

**[00181]** 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 ($C_8$ to $C_{24}$) 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.

**[00182]** 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 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 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.

**[00183]** 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 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.

**[00184]** 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 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 type or relative amount of the solvent or polymer used.

**[00185]** 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 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.

**[00186]** The suspension vehicle may exhibit phase separation when contacted with the aqueous environment; however, typically the suspension vehicle exhibits substantially no phase separation 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.

**[00187]** 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 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 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).

**[00188]** The drug particle formulation is added to the suspension vehicle to form a suspension 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.

**[00189]** The suspension formulation may be prepared by dispersing the particle formulation in the 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 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.

**[00190]** The suspension formulation typically has an overall moisture content of less than about 10 wt %, preferably less than about 5 wt %, and more preferably less than about 4 wt %.

**[00191]** 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.

**[00192]** In summary, the components of the suspension vehicle provide biocompatibility. 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 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.

### 4.0.0 Delivery of Suspension Formulations

**[00193]** 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 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.

**[00194]** 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.

**[00195]** The 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; 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).

**[00196]** 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 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.

**[00197]** 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 sheer rate. In one embodiment of the present invention, the reservoir of the 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.

**[00198]** The release rate of the drug from the osmotic delivery device typically provides a subject 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.

**[00199]** Typically, for an osmotic delivery device, the volume of a beneficial agent chamber comprising the beneficial agent formulation is between about 100 µl to about 1000 µl, more preferably between about 120 µl and about 500 µl, more preferably between about 150 µl and about 200 µl.

**[00200]** 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).

**[00201]** 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.

**[00202]** 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.

**[00203]** 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).

## EXAMPLES

**[00204]** 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 Centigrade and pressure is at or near atmospheric.

**[00205]** The compositions used to practice the methods of the present invention meet the specifications for content and purity required of pharmaceutical products.

### Example 1: Generation of dual glucagon receptor/GLP-1 receptor agonist polypeptides

**[00206]** Glucagon polypeptides of the invention, as provided in Table 2 were synthesized on a Prelude peptide synthesizer (Protein Technologies Inc., Tucson, AZ) ) by solid-phase methods using Fmoc strategy with 2-(1 H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate (HBTU) or 2-(6-chloro-1 -H-benzotriazole- 1-yl)-1,1,3,3-tetramethyl-aminium hexafluorophosphate (HCTU) activation (5 fold molar excess) in N,N-dimethyiformamide (DMF), and N'N-Diisopropylethylamine (DIEA) as base, 20% piperidine/DMF for Fmoc deprotection. The resin was Rink Amide MBHA LL (Novabiochem) or N-α-Fmoc protected, pre-loaded Wang LL (Novabiochem), with loadings of 0.29 - 0.35 mmol/g on a 20-400 μmol scale. Final deprotection and cleavage of the peptide from the solid support was performed by treatment of the resin with (92.5% TFA, 2.5% phenol, 2.5% water and 2.5% 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, pelleted by centrifugation and lyophilized.

The lyophilized solid was re-dissolved in a 1:1 solution of acetonitrile/water, with 0.1% TFA (10-15 mL), purified via reverse phase HPLC on a Waters XBridge™ BEH 130, CIS, 10 pm, 130 Å, 30 X 250 mm ID column, using a 30 gradient within the ranges of 5 - 75% acetonitrile/water with 0.1% TFA over 30 - 45 minutes at a flow rate of 30 mL/min, λ - 215 nm. The purified product was lyophilized and analyzed by ESI-LC/MS and analytical HPLC; and were demonstrated to be pure (>98%). Mass results all agreed with calculated values.

**[00207]** 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 the three methods A or Method B or Method C.

**[00208]** LC/MS Conditions: Method A: Performed using a Phenomenex UPLC Aeris™ Peptide XB C18 35 column, 1.7 pm, 2.1 X 100 mm or ACQUiTY BEH300 or BEH130 CT8 column, 1.77 pm. 2.1 X 100 mm using 5 - 65% acetonitriie/water with 0.05% TFA over 30 minutes with a flow rate 0.5 mL/min, λ - 215 nm, 280 nm.

**[00209]** C18 HPLC Conditions: Method A: UPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 µm, 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, λ 280 nm.

**[00210]** Method B: UPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 µm, 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, λ 280 nm.

**[00211]** Method C: UPLC analysis was conducted on an Acquity BEH130, C18 column, 1.7 µm, 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, λ215 nm, λ 280 nm. 5.0 uL of sample was injected using a PLNO (partial loop w/ needle overfill) injection mode.

**[00212]** Sequences of synthesized dual glucagon receptor/GLP-1 receptor agonist peptides and their pEC50 values determined in cAMP assays are shown below in Tables 1A and 1B.

Table 2

| Compound | SEQ ID NO: | GCGR pEC50 (Melano-phore) | GLP-1R pEC50 (Melano-phore) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | Calculated Mass (Parent MW) | Calculated Mass (Parent MW, M+3/3)) | Observed Mass (M+3/3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucagon | 21 | 10.1 | 9.6 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | R | R | A | Q | D | F | V | Q | W | L | M | N | T | OH | 3482.76 | 1161.92 | 1162.2 |
| B1 | 2 | 9.3 | 9.6 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | M | E** | T | NH2 | 3446.85 | 1149.95 | 1150.6 |
| B2 | 3 | 9.5 | 9.9 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | K* | K | A | A | E* | D | F | V | K** | W | L | Q | E** | T | OH | 3387.72 | 1130.24 | 1131.7 |
| B3 | 4 | 9.3 | 9.2 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | M | E** | T | OH | 3447.83 | 1150.28 | 1150.8 |
| B4 | 5 | 9.9 | 10.4 | N | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | Q | E** | T | NH2 | 3420.75 | 1141.25 | 1141.2 |
| B5 | 6 | 9.5 | 9.4 | Q | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | Q | E** | T | NH2 | 3434.78 | 1145.93 | 1145.7 |
| B6 | 7 | 8.9 | 9.5 | W | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | Q | E** | T | NH2 | 3493.84 | 1165.29 | 1165.2 |
| B7 | 8 | 9.1 | 9.2 | P | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | Q | E** | T | NH2 | 3403.76 | 1135.59 | 1135.6 |
| B8 | 9 | 9.0 | 9.3 | pGlu | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | W | L | Q | E** | T | NH2 | 3417.75 | 1140.25 | 1140.2 |
| B9 | 10 | 10.8 | 10.3 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | V | K** | H | L | Q | E** | T | NH2 | 3394.72 | 1132.57 | 1132.4 |
| B10 | 11 | 10.7 | 10.2 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | D-Val | K** | H | L | Q | E** | T | NH2 | 3394.72 | 1132.57 | 1132.3 |
| B11 | 12 | 9.3 | 9.1 | H | D-Ser | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K* | R | A | Q | E* | F | D-Val | K** | H | L | Q | E** | T | NH2 | 3394.72 | 1132.57 | 1132.6 |
| B12 | 13 | 10.2 | 9.8 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | K | A | A | Q | D | F | V | K** | W | L | M | E** | T | NH2 | 3365.73 | 1122.91 | 1122.6 |
| B13 | 14 | 10.6 | 10.2 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | R | R | A | Q | D | F | V | K** | W | L | Q | E** | T | NH2 | 3475.79 | 1159.6 | 1159 |
| B14 | 15 | 11.1 | 9.3 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | R | R | A | Q | D | F | V | K** | W | K | M | E** | T | NH2 | 3493.87 | 1165.62 | 1165.4 |
| B15 | 16 | 10.8 | 10.2 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | R | R | A | Q | D | F | V | K** | H | L | M | E** | T | NH2 | 3429.78 | 1144.26 | 1143.7 |
| B16 | 17 | 10.8 | 10.2 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | S | A | R | A | Q | D | F | V | K** | W | L | Q | E** | T | NH2 | 3390.68 | 1131.23 | 1130.9 |
| B17 | 18 | 10.4 | 9.8 | Y | Aib | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | K* | K | A | A | E* | D | F | V | K** | W | L | Q | E** | T | OH | 3411.78 | 1138.26 | 1139 |
| B18 | 19 | 9.2 | 9.9 | Y | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | K* | K | A | A | E* | D | F | V | K** | W | L | Q | E** | T | OH | 3413.75 | 1138.92 | 1139 |
| B19 | 20 | 9.9 | 9.5 | H | S | Q | G | T | F | T | S | D | Y | S | K | Y | L | D | K* | K | A | A | E* | D | F | V | K** | W | L | Q | E** | T | OH | 3387.72 | 1130.24 | 1131 |

**Example 2: Synthesis of glucagon analogs**

**[00213]** The synthesis, purification and analytical method was used as described in Example 1 with following modifications. Glucagon polypeptides of the invention, as provided in Table 2, were synthesized on a Prelude peptide synthesizer (Protein Technologies Inc., Tucson, AZ) ) by solid-phase methods using Fmoc strategy and using Rink Amide MBHA LL (Novabiochem) or N-α-Fmoc protected, pre-loaded Wang LL (Novabiochem), with loadings of 0.29 - 0.35 mmol/g on a 20-400 µmol scale. Fmoc amino acid (4.0 eq, 1.0 mmol) residues were activated using 4.0 eq HBTU, 4.0 eq of HOBT, 8.0 eq DIEA and coupled to the resin for 1 hour. The Fmoc group was removed by treatment with 20% (v/v) piperidine in dimethylformamide. The side chain protection groups used were Trt for Asn, Gln, Cys and His; t-Bu for Ser, Thr, and Tyr; Boc for Lys and Trp; Ot-Bu for Asp and Glu; and Pbf for Arg.

**[00214]** The Lactam Bridges between positions 17-21 and between positions 24-27 were introduced using an orthogonally protected Lysine (Alloc)17 and Glu (Allyl)21;and Glu(Allyl)24 and Lysine (Alloc)27 is represented by E in parenthesis in the sequence Lysine (Alloc) is represented by K in parenthesis in the sequence in Table 2.

**[00215]** Synthesis was carried out from C-terminal to N-terminal on the solid support. To incorporate the first lactam bridge (17-21) the synthesis was paused at Phe21. The resin was washed with DCM (6 x 10 ml) that had been flushed previously with nitrogen for 30 minutes. Next, Palladium Tetrakis, $Pd(PPh3)_3$ (3 equiv) was added to a solution of $CHCl_3$/AcOH/NMM (37:2:1). Nitrogen was bubbled through the solution until all of the solid had dissolved leaving a dark amber solution. This solution was transferred to an amino acid bottle wherein it was degassed for an additional 5 minutes with nitrogen. It was then placed on to the prelude synthesizer. 20 ml of the Palladium solution was added to each RV and the reaction mixture was allowed to agitate for 3 hours. Synthesis was continued using standard protocol. To incorporate the second lactam bridge (24-28) the synthesis was paused at Leu14 and the above procedure for lactam bride formation was repeated.

**[00216]** Before cleaving peptide from resin the allyl protecting groups were removed using $Pd(PPh3)_3$ in $CHCl_3$/AcOH/NMM (37:2:1). Cyclization to the lactam was effected using Pybop (6 equivs), HOBT (6 equivs), and DIEA (12 equivs). The resin was then washed with DMF and dried under vacuum for 15 minutes under nitrogen.

**[00217]** Final deprotection and cleavage of the peptide from the solid support was performed by treatment of the resin with reagent B (93% TFA, 3% phenol, 3% water 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, pelleted by centrifugation and lyophilized. The pellet was re-dissolved in water (10-15 mL), filtered purified by reverse phase HPLC on a Waters XBridge™ BEH 130, CIS, 10 pm, 130 Å, 30 X 250 mm ID column, using 30 a gradient within the ranges of 5 - 75% acetonitrile/water with 0,1% TFA over 30 - 45 minutes at a flow rate of 30 mL/min, λ - 215 nm . Alternatively, Purification was afforded using a Gilson Preparative HPLC System via reverse phase chromatography using a Waters XBridge BEH 130, C18, 10 µm, 130 Å, 30 X 250 mm ID column, with a gradient range of 5-45% acetonitrile/water with 0.1% TFA over 30-45 minutes at a flow rate of 30 mL/min, λ 215 nm. The purified product was lyophilized and analyzed by ESI-LC/MS and analytical HPLC; and were demonstrated to be pure (>98%). Mass results all agreed with calculated values.

**[00218]** Characterizations of peptides analogs were performed on UPLC or LC/MS equipment (Acquity SQD Waters Corp, Milford, MA) and UV detection provided by dual absorbance signals at 215 nm and 280 nm, using one of the three methods A or Method B or Method C.

**[00219]** The chemical structure of Compound C1 is shown in Figure 4.

**Example 3: Solubility and stability analysis of glucagon and dual glucagon receptor/GLP-1 receptor agonist polypeptides**

**[00220]** The solubility of glucagon and various dual analogs were tested for solubility in Saline 5% NaCl in Water (bioassay buffer) or in aqueous at room temperature. Samples were visually inspected for clarity of the sample, any appearance of turbidity or haziness. The results of this analysis are shown below in Table 3.

**[00221]** The stability of glucagon and various dual analogs of the disclosure were tested in Saline 5% NaCl in water (bioassay buffer) or in aqueous were incubated at 37°C and at room temperature. Samples were withdrawn at a regular interval for 30 days, and analyzed by LC/MS and HPLC for determination of purity and mass of the degradation product.

Table 3.

| Sequence | Solubility in Water | Solubility in 5% NaCl in water | Stability at 37 degrees C for 30 days 1.0 mg/ml solution |
|---|---|---|---|
| HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21) | >95% | 0.03 µg/ml -10 µg/ml | Aggregates |
| HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-$NH_2$ (SEQ ID NO: 2) | >50 mg/ml | >50 mg/ml | >90% |

**Example 4. In vitro screening of analogs peptide-receptor mediated cAMP response in melanophores assay**

**[00222]** Melanophores were transiently transfected via electroporation (500 Volts; 725 uF; 725 ohms) with 40 ug of hGCGR and hGLP1R. Electroporated cells were then placed in T225 tissue culture flasks and incubated overnight at 27°C. The following day the cells were trypsinized, counted and plated into Costar half-well 96 well tissue culture treated plates at 15,000 cells/well and incubated overnight at 27 °C. The following day peptide analogs were serial diluted four fold in 0.7x L-15 Medium Leibovitz + 1% bovine serum albumin + 3 mM L-Glutamine + 1% DMSO + 10 nM Melatonin (MAB) to create 10 point dose response curves. Media from cell plates was removed and replaced with 25 uL MAB. Cells were incubated at room temperature for 90 minutes and then read (read00) on a plate reader at 625 nM. Dose response curves of peptide analogues were added at 25 uL/well across the plate along with dose response curves for Glucagon and GLP-1 (7-36) peptides. MAB and Glucagon were added to columns 11 and 12 respectively and served as the min and max controls. Plates were incubated 60 minutes and read (read01). Transformed data was generated for each well as 1-(read01 /read00) and the resultant value then normalized using the min and max values for each plate. Normalized values were plotted using a four parameter curve fit equation and pEC50 values generated to determine both potency and efficacy.

**[00223]** Peptide-receptor activation profiles at the human glucagon receptor (GCGR) and the human GLP-1 receptor (GLP-1R) by glucagon, GLP-1 (7-36), and GCGR-GLP-1R balanced co-agonist peptide Compound C1 (SEQ ID NO: 2). Glucagon and Compound C1 activate GCGR (Figure 1A) and GLP-1R (Figure 1B) at nearly equal potencies.

**[00224]** The results of this analysis are shown in Table 2 and in Figures 1A and 1B.

**Example 5: Intravenous bolus injection: pharmacokinetic studies to assess peptide clearance from the kidney (CL)**

**[00225]** Peptides were administered as a single intravenous bolus dose to non-fasted male Wistar Han rats (n=3 per group) via jugular vein cannula. Compounds were formulated as solutions in either sterile saline, acidified saline (pH 2.0 or 4.5), or 5% DMSO in water and administered at a volume of 1.5 mL/kg and a final dose of 0.1 mg/kg. Blood samples (approximately 250 uL) were collected for pharmacokinetic analysis via a femoral vein cannula at 0.083, 0.167, 0.25, 0.33, 0.5, 1, 2, 4, 8, 12 and 24 h post-dose into microtainer tubes containing K2EDTA as anticoagulant and 25 uL of a protease inhibitor cocktail. Plasma was prepared by centrifugation and stored at -80°C until analysis. The results of this analysis are shown below in Table 4 and in Figure 2.

Table 4.

| Compound | Sequence | Clearance via Kidney (CL) (mL/min/kg) |
|---|---|---|
| Glucagon | HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH (SEQ ID NO: 21) | 94 |
| 8 | (pGlu)SQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 9) | 40 |
| 1 | HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-$NH_2$ (SEQ ID NO: 2) | 7 |

**Example 6: Generic method of plasma sample preparation for pharmacokinetic studies**

**[00226]** Protein Precipitation: All 96-well plates were coated with a blocking agent to mitigate non-specific binding of peptides. Plasma samples (75 uL) were added to a 96-well plate containing 200 uL of 2:1 ethanol : acetonitrile containing 0.1% TFA and mixed well via aspiration. Plates were capped, vortex-mixed and centrifuged. Supernatants (215 uL) were transferred to a clean 96-well plate, evaporated to dryness under nitrogen flow and then reconstituted in 75 µL of 20% acetonitrile in water containing 0.1% formic acid.

**[00227]** Solid-Phase Extraction: Samples were diluted 3-fold with 5% $NH_4OH$ (aq) and loaded onto an Oasis MAX microElution plate (Waters Corp, Milford, MA) that had been pre-conditioned with 200 uL each of methanol and 5% $NH_4OH$ (aq). The plate was washed with 200 uL 5% $NH_4OH$ (aq), followed by 200 uL 20% acetonitrile in water.

Peptides were eluted using 200 uL 5% formic acid in methanol and evaporated under nitrogen flow. Samples were reconstituted in 80 uL 0.1% formic acid in water.

**Example 7: LC/MS quantification of peptides in plasma**

**[00228]** All calibration standards were prepared in control rat plasma containing K2EDTA and protease inhibitor cocktail. Samples and standards were analyzed by TurboIonSpray™ 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), and either an AB Sciex API 5600 TripleTOF™ or Sciex API 4000QTrap mass spectrometer (Framingham, MA). Samples were injected onto a 2.1 × 50 mm reverse phase C18 analytical column, typically a Waters ACQUITY UPLC™ HSS T3, 1.8 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 linearly to 95% B over a period of 3-4 minutes, depending on the peptide. Typical flow rates were 600 µL/min. The column temperature was held constant at 40 or 45°C. Peptides were quantified my monitoring one or more product ions produced from a multiply charged parent ion.

**Example 8: In vivo efficacy of glucagon analogs: reduction in body weight**

**[00229]** Chronic (13 days) in vivo efficacy studies were conducted in a rodent model for obesity (diet- induced obese (DIO) Long Evans rat) to investigate the efficacy and durability of examples 11 and 12 singly and in combination with exendin-4 as anti-obesity agents.

**[00230]** Male Diet-Induced Obese (DIO) Long Evans (LE) rats were used (Harlan Laboratories, Inc., Indianapolis, IN) and beginning at weaning (about 3 weeks of age), the rats were fed a high fat chow (Teklad TD 95217, 40% kcal from fat, Harlan Laboratories, Madison, WI). Rats were 15-17 weeks old at the start of the study. The rats were housed 1 per cage and given ad libitum access to high fat diet (Harlan TD.95217, 4.3 kcal/g) and water, maintained on a 12 hr light/dark cycle from 5:00 AM to 5:00 PM at 21° C and 50% relative humidity and allowed to acclimate for at least 10 days prior to the surgeries. Baseline fat mass and non-fat mass measurements were taken 3 days before the start of peptide infusion using a QMR instrument (Echo Medical Systems, Houston, TX). Body

weight measurements were taken 2 times/week starting three days before the surgery. Rats were randomized according to their percent body fat mass and/or body weight into the various treatment groups (n=4-6 rats/group). Alzet mini-osmotic pumps (2 week; Model 2002, Durect Corporation, Cupertino, CA) were filled under sterile condition with either vehicle or peptide one day prior to the surgery. On the day of surgery, rats were anesthetized under isoflurane and the dorsal skin surface was shaved and cleansed. Rats were injected SC with Flunexin (2.5 mg/kg). A 1-2 cm surgical incision was made between the scapulae. Using blunt dissection, a 2-3 cm subcutaneous tunnel was created into which the sterile, filled, mini-osmotic pump was introduced. The skin opening was closed with a skin staple. Each rat was implanted with either one or two osmotic pumps containing vehicle or peptide according to their treatment group. All the data are presented as mean ± SEM. The data were analyzed in Excel and/or Prism (GraphPad Software, Inc., La Jolla, CA) using one-way ANOVA to compare each group to the appropriate control group. P-values $< 0.05$ were considered to indicate a significant difference between treatment groups.

**[00231]** Animals were housed and maintained in an AAALAC, international accredited care and use programs. All procedures were performed in compliance with the Animal Welfare Act, USDA regulations and approved by the GlaxoSmithKline and Use Committees.

**Example 9. Efficacy of Compound C1 singly or in combination with exendin-4 in DIO LE rats**

**[00232]** Continuous dosing of Compound C1 led to dose-dependent decreases in body weight after 13 days of dosing in DIO LE rats. Significant efficacy of 6.8% and 18.9% weight loss was achieved at the 0.1 and 0.3 mg/kg/day doses when compared to vehicle control ($p<0.05$), respectively (Figure 3, left panel). In combination with Exendin-4 at 0.01 mg/kg/d (2.5% weight loss singly), doses of 0.03, 0.1 and 0.3 mg/kg/d Compound C1 achieved 5.3, 11 and 29% weight loss vs. vehicle control ($p<0.05$) after 13 days in DIO LE rats (Figure 3, right panel.)

**Other Embodiments**

**[00233]** 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.

What is claimed is:

1. An isolated polypeptide comprising the amino acid sequence: $X_1X_2$QGTFTSDYSKYLD$X_{16}X_{17}X_{18}AX_{20}X_{21}FVX_{24}WX_{26}X_{27}X_{28}TX_{30}$ (SEQ ID NO: 1), wherein:

$X_1$ is H, W, pyroglutamate (pGlu), Q, P, Y, or N;

$X_2$ is S, D-Ser, or 2-Aminoisobutyric acid (Aib);

$X_{16}$ is S or K*, where K* at position 16 is connected with E* at position 20 via lactam bridge;

$X_{17}$ is R, A, K, or K*, where K* at position 17 is connected with E* at position 21 via lactam bridge;

$X_{18}$ is R or A;

$X_{20}$ is Q or E*, where E* at position 20 is connected with K* at position 16 via lactam bridge;

$X_{21}$ is D or E*, where E* at position 21 is connected with K* at position 17 via lactam bridge;

$X_{23}$ is V or D-Val;

$X_{24}$ is K**, where K** at position 24 is connected with E** at position 28 via lactam bridge;

$X_{25}$ is W or H;

$X_{26}$ is L or K;

$X_{27}$ is M or Q;

$X_{28}$ is E**, where E** at position 28 is connected with K** at position 24 via lactam bridge; and

$X_{30}$ is OH or $NH_2$.

2. The isolated polypeptide of claim 1, wherein the isolated polypeptide comprises an amino acid sequence selected from the group consisting of

HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-$NH_2$ (SEQ ID NO: 2);

HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 3);

HSQGTFTSDYSKYLDSK*RAQE*FVK**WLME**T-OH (SEQ ID NO: 4);

NSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 5);

QSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 6);
WSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 7);
PSQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 8);
(pGlu)SQGTFTSDYSKYLDSK*RAQE*FVK**WLQE**T-$NH_2$ (SEQ ID NO: 9);
HSQGTFTSDYSKYLDSK*RAQE*FVK**HLQE**T-$NH_2$ (SEQ ID NO: 10);
HSQGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 11); H(D-Ser)QGTFTSDYSKYLDSK*RAQE*F(D-Val)K**HLQE**T-$NH_2$ (SEQ ID NO: 12);
HSQGTFTSDYSKYLDSKAAQDFVK**WLME**T- $NH_2$ (SEQ ID NO: 13);
HSQGTFTSDYSKYLDSRRAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 14);
HSQGTFTSDYSKYLDSRRAQDFVK**WKME**T-$NH_2$ (SEQ ID NO: 15);
HSQGTFTSDYSKYLDSRRAQDFVK**HLME**T-$NH_2$ (SEQ ID NO: 16);
HSQGTFTSDYSKYLDSARAQDFVK**WLQE**T-$NH_2$ (SEQ ID NO: 17);
Y(Aib)QGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 18);
YSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 19); and
HSQGTFTSDYSKYLDK*KAAE*DFVK**WLQE**T-OH (SEQ ID NO: 20).

3. The isolated polypeptide of claim 1, wherein the isolated polypeptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-20.

4. A pharmaceutical composition comprising the isolated polypeptide of any one of claims 1 to 3.

5. The pharmaceutical composition of claim 4, wherein the additional agent is a second polypeptide.

6. The pharmaceutical composition of claim 5, wherein the second polypeptide is an insulinotrophic polypeptide.

7. The pharmaceutical composition of claim 5, wherein the second polypeptide is selected from the group consisting of exenatide, a derivative of exenatide, an analog of exenatide, glucagon-like peptide-1 (GLP-1), a derivative of GLP-1, and an analog of GLP-1.

8. The pharmaceutical composition of claim 5, wherein the second polypeptide is exenatide.

9. The pharmaceutical composition of claim 8, wherein the exenatide is synthetic exenatide.

10. The pharmaceutical composition of claim 9, wherein the synthetic exenatide comprises the amino acid sequence of SEQ ID NO: 23.

11. The pharmaceutical composition of claim 5, wherein the second polypeptide is a GLP-1 analog.

12. The pharmaceutical composition of claim 10, wherein the GLP-1 analog is a synthetic GLP-1 analog.

13. The pharmaceutical composition of any one of claims 4 to 12 further comprising at least one stabilizing component.

14. The pharmaceutical composition of claim 13, wherein the stabilizing component is selected from the group consisting of one or more carbohydrates, one or more antioxidants, one or more amino acids, one or more buffers, one or more inorganic compounds, one or more surfactants, and combinations thereof.

15. A particle formulation comprising the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 and at least one stabilizing component.

16. The particle formulation of claim 15, wherein the stabilizing component is selected from the group consisting of one or more carbohydrates, one or more antioxidants, one or more amino acids, one or more buffers, one or more inorganic compounds, one or more surfactants, and combinations thereof.

17. A suspension formulation comprising the particle formulation of claim 15 or claim 16 and a suspension vehicle.

18. The suspension formulation of claim 17, wherein the suspension vehicle comprises one or more surfactants, one or more antioxidants, one or more soluble compounds, or combinations thereof.

19. An osmotic delivery device, comprising the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18.

20. The osmotic delivery device of claim 19, 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.

21. The osmotic delivery device of claim 20, wherein the reservoir comprises titanium or a titanium alloy.

22. The osmotic delivery device of claim 20, wherein the suspension formulation comprising the particle formulation of claim 15 or claim 16 and a suspension vehicle.

23. The osmotic delivery device of claim 22, wherein the suspension vehicle comprises one or more surfactants, one or more antioxidants, one or more soluble compounds, or combinations thereof.

24. A method of treating or alleviating a symptom of a disease or disorder associated with aberrant glucagon activity in a subject in need thereof, the method comprising administering the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18.

25. The method of claim 24, wherein the disease or disorder is type 2 diabetes mellitus.

26. The method of claim 24 or claim 25, wherein the subject is a human.

27. The method of claim 24, wherein the isolated polypeptide or pharmaceutical composition is formulated for administration by a route selected from the group consisting of parenteral, oral, transdermal, transmucosal, and rectal administration.

28. The method of claim 24, wherein the isolated polypeptide or pharmaceutical composition is formulated for administration by intravenous administration, intradermal administration, subdermal administration, subcutaneous administration, or combinations thereof.

29. A method of treating a metabolic disorder, said method comprising administering the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18 to a subject in need thereof.

30. A method of treating obesity, said method comprising administering the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of

claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18 to a subject in need thereof.

31. A method of treating or preventing a metabolic disease or disorder associated with elevated blood glucose in a subject, comprising administering to said subject a therapeutically or prophylactically effective amount of the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18.

32. A method of treating, preventing, delaying the onset of, delaying the progression of, or otherwise ameliorating a symptom of a disease or disorder in which co-agonism at both the glucagon and GLP-1 receptors is desired, comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of the isolated polypeptide of any one of claims 1 to 3 or the pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18, wherein the disease or disorder is selected from the group consisting of chronic pain, a blood disorder, hemophilia, an endocrine disorder, a metabolic disorder, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Alzheimer's disease, a cardiovascular disease, hypoglycemia unawareness, restrictive lung disease, chronic obstructive pulmonary disease, lipoatrophy, metabolic syndrome, leukemia, hepatitis, renal failure, an autoimmune diseases, shock, a wasting disorder, and combinations thereof.

33. The method of claim 32, wherein the autoimmune disease is Grave's disease, systemic lupus erythematosus, multiple sclerosis, or rheumatoid arthritis.

34. A method of treating, preventing, delaying the onset of, delaying the progression of, or otherwise ameliorating a symptom of an infectious disease requiring chronic treatment, comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of the isolated polypeptide of any one of claims 1 to 3 or the

pharmaceutical composition of any one of claims 4 to 14 or the particle formulation of claim 15 or claim 16 or the suspension formulation of claim 17 or claim 18.

35. The method of any one of claims 29 to 33, wherein the subject is a human.

36. The method of any one of claims 29 to 35, wherein the isolated polypeptide or pharmaceutical composition is formulated for administration by a route selected from the group consisting of parenteral, oral, transdermal, transmucosal, and rectal administration.

37. The method of claim 36, wherein the isolated polypeptide or pharmaceutical composition is formulated for administration by intravenous administration, intradermal administration, subdermal administration, subcutaneous administration, or combinations thereof.

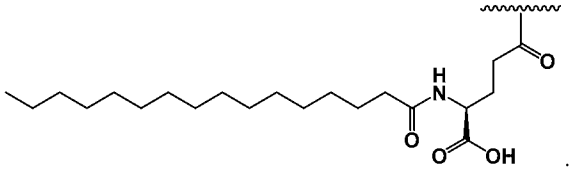
38. The pharmaceutical composition of claim 4, further comprising a pharmaceutically acceptable salt.

39. The pharmaceutical composition of claim 4, wherein the pharmaceutically acceptable salt is a trifluoroacetate salt, acetate salt or hydrochloride salt.

40. The isolated polypeptide of any one of claims 1-3, wherein the isolated polypeptide is covalently attached to one or more lipophilic substituents, each optionally via spacer.

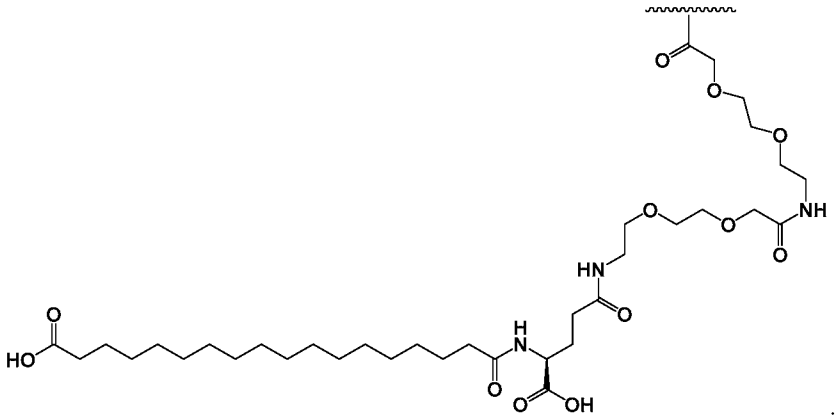
41. The isolated polypeptide of claim 40, wherein each of the one or more lipophilic substituents is attached, optionally via a spacer, to the ε-amino group of a lysine residue in the isolated polypeptide.

42. The isolated polypeptide of claim 41, wherein each of the one or more lipophilic substituents is attached, via a spacer, to the ε-amino group of a lysine residue in the isolated polypeptide and the lipophilic substituent and spacer are of Formula II:



Formula II

43. The isolated polypeptide of claim 41, wherein each of the one or more lipophilic substituents is attached, via a spacer, to the ε-amino group of a lysine residue in the isolated polypeptide and the lipophilic substituent and spacer are of Formula III:



Formula III

FIG. 1A

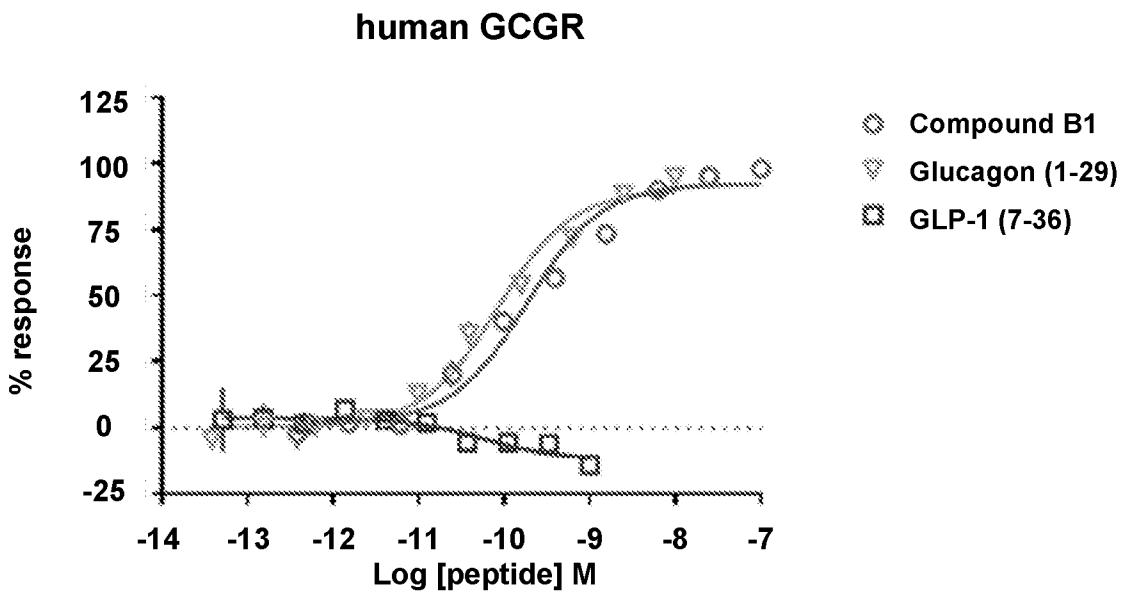

human GCGR
% response
125
100
75
50
25
0
-25
-14
-13
-12
-11
-10
-9
-8
-7
Log [peptide] M
Compound B1
Glucagon (1-29)
GLP-1 (7-36)

FIG. 1B

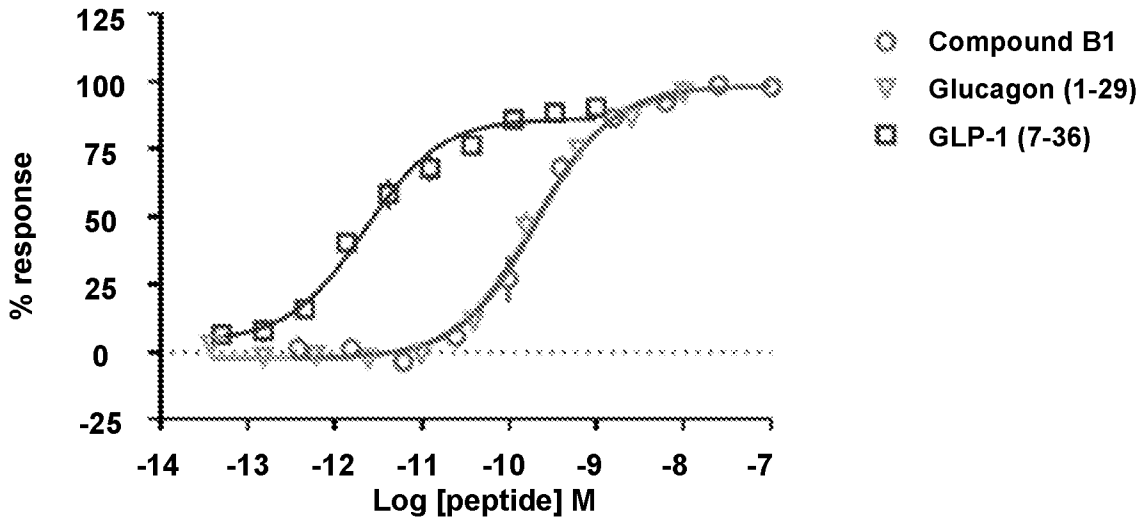
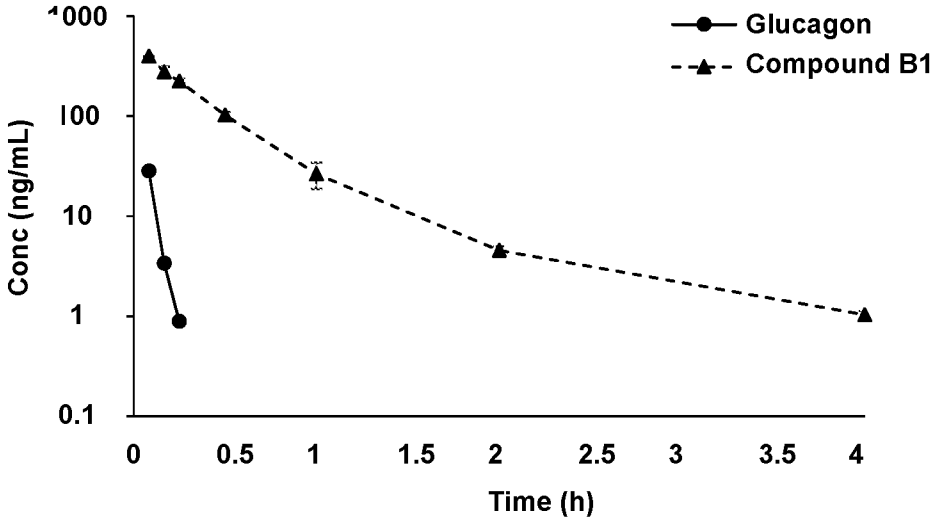

human GLP-1R
% response
125
100
75
50
25
0
-25
-14
-13
-12
-11
-10
-9
-8
-7
Log [peptide] M
Compound B1
Glucagon (1-29)
GLP-1 (7-36)

FIG. 2




| Peptide | CL (mL/min/kg) |
|---|---|
| Glucagon | 94.1 ± 16.1 |
| Compound B1 | 7.17 ± 0.51 |

FIG. 3

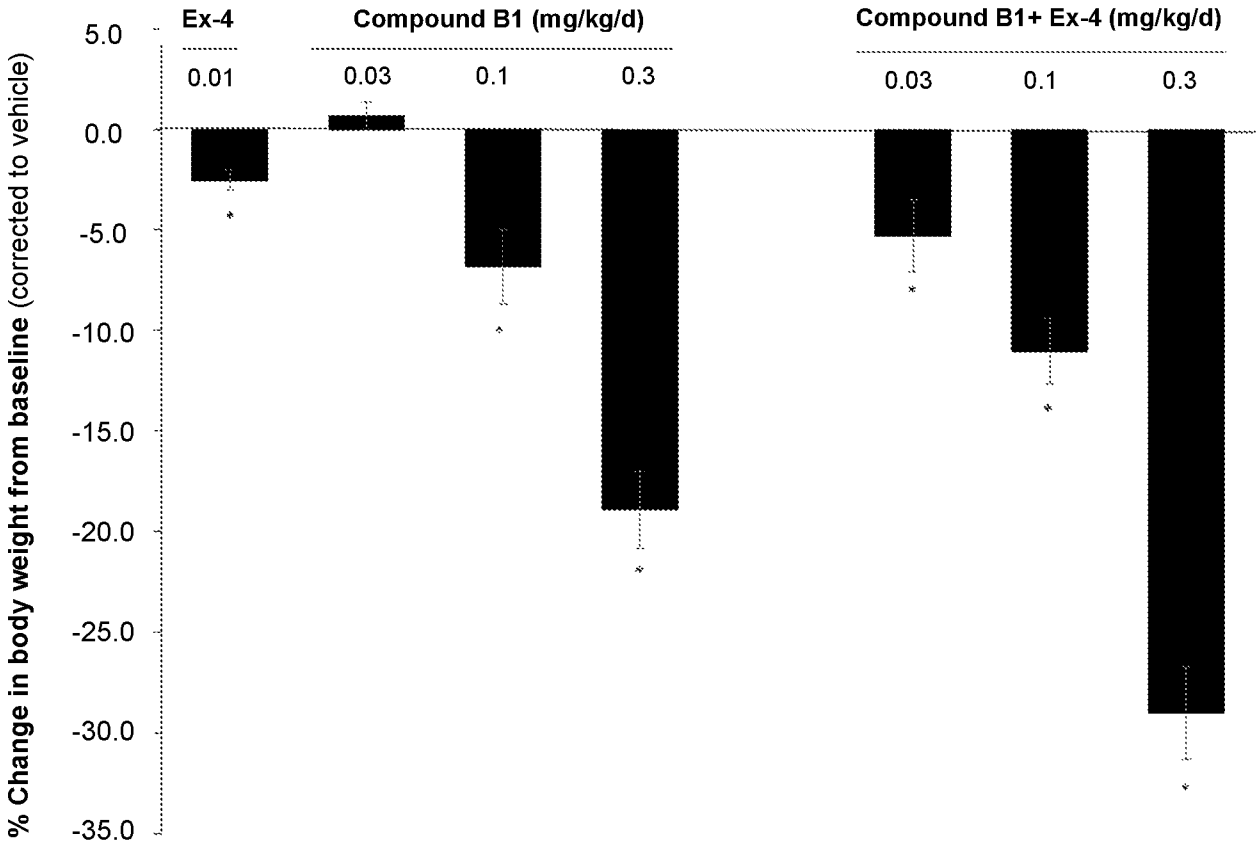
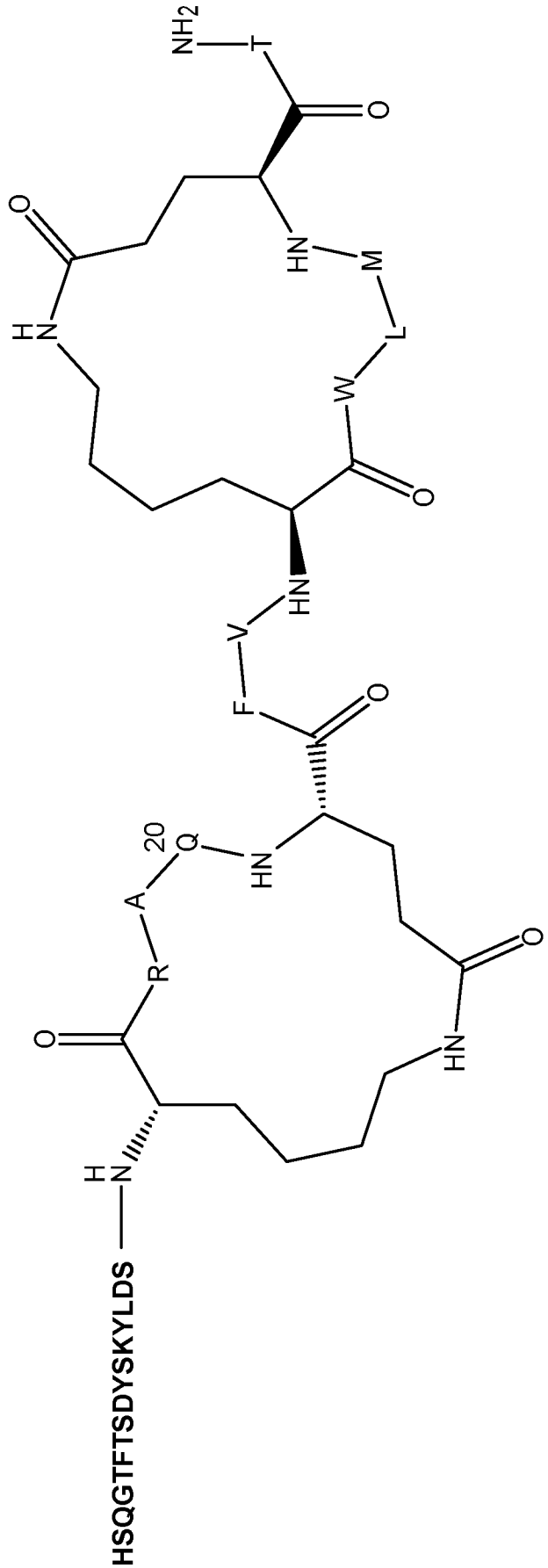

Ex-4
Compound B1 (mg/kg/d)
Compound B1+ Ex-4 (mg/kg/d)
0.01
0.03
0.1
0.3
0.03
0.1
0.3
5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
-35.0
% Change in body weight from baseline (corrected to vehicle)

FIG. 4



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/032717

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/032717

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/26 C07K14/605
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 2008/086086 A2 (INDIANA UNIVERSITY)<br>17 July 2008 (2008-07-17)<br>compound 38<br>----- | 1-43 |
| A | J AHN ET AL.: "A new approach to search for the bioactive conformation of glucagon. positional cyclization scanning",<br>JOURNAL OF MEDICINAL CHEMISTRY,<br>vol. 44, no. 19,<br>16 August 2001 (2001-08-16), pages 3109-3116, XP2610716,<br>American Chemical Society<br>ISSN: 0022-2623<br>table 1<br>----- | 1-43 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 July 2017 | 03/08/2017 |

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

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/032717

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | CA 2674354 A1 | 17-07-2008 |
| | | CN 101578102 A | 11-11-2009 |
| | | EP 2124974 A2 | 02-12-2009 |
| | | IL 199263 A | 30-09-2013 |
| | | JP 5890085 B2 | 22-03-2016 |
| | | JP 2010515686 A | 13-05-2010 |
| | | RU 2009129961 A | 10-02-2011 |
| | | SG 177953 A1 | 28-02-2012 |
| | | US 2010190699 A1 | 29-07-2010 |
| | | WO 2008086086 A2 | 17-07-2008 |