GIPR-Agonist Compounds

The present invention relates to compounds having activity at the human glucose-dependent insulinotropic polypeptide (GIP) receptor. The present invention also relates to compounds having an extended duration of action at the GIP receptor. Compounds may be useful in the treatment of type 2 diabetes mellitus ("T2DM"). Also, the compounds may be useful in the treatment of obesity.

Over the past several decades, the prevalence of diabetes has continued to rise. T2DM is the most common form of diabetes, accounting for approximately 90% of all diabetes. T2DM is characterized by high blood glucose levels associated mainly with insulin resistance. The current standard of care for T2DM includes diet and exercise, treatment with oral medications, and injectable glucose-lowering drugs, including incretin-based therapies such as GLP-1 receptor agonists. A variety of GLP-1 receptor agonists are currently available for treatment of T2DM, although currently marketed GLP-1 receptor agonists are generally dose-limited by gastrointestinal side effects such as nausea and vomiting.

Subcutaneous injection is the typical route of administration for the available GLP-1 receptor agonists. When treatment with oral medications and incretin-based therapies are insufficient, insulin treatment is considered. Despite the advances in treatment available today, many patients with T2DM are unable to reach their glycemic control goals. Uncontrolled diabetes leads to several conditions associated with increased morbidity and mortality of patients.

There is a need for a treatment to enable more patients with T2DM to reach their glycemic treatment goals.

Obesity is a complex medical disorder resulting in excessive accumulation of adipose tissue mass. Today obesity is a global public health concern that is associated with undesired health outcomes and morbidities. Desired treatments for patients with obesity should reduce excess body weight, improve obesity-related co-morbidities, and maintain long-term weight reduction. Available treatments for obesity are particularly unsatisfactory for patients with severe obesity. There is a need for alternative treatment options to induce therapeutic weight loss in patients in need of such treatment.

WO2016/111971 describes peptides stated to have GLP-1R and GIPR agonist

activities. WO2013/164483 also discloses compounds stated to have GLP-1R and GIPR activities.

WO2018/181864 discloses compounds stated to have GIPR agonist activity.

There is a need for T2DM treatments capable of providing effective glucose control for a larger portion of the patients in need of such treatment. There is a further need for T2DM treatments capable of providing effective glucose control and with a favorable side effect profile. There is a need for alternate treatment options to provide therapeutic weight loss in a patient in need of such treatment, such as a patient with severe obesity. There is a desire for diabetes treatment options that may be combined with insulin therapy and/or incretin therapy to provide the patient with superior glycemic outcomes and/or more desirable side effect profiles.

Summary

In an aspect of the present disclosure, there is provided a compound, or pharmaceutically acceptable salt thereof, wherein the compound is SEQ ID NO:7 or SEQ ID NO:10.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the compound, or a pharmaceutically acceptable salt thereof, disclosed herein and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In a further aspect of the present disclosure, there is provided a use of the compound, or a pharmaceutically acceptable salt thereof, disclosed herein, in the manufacture of a medicament for treating a condition selected from the group consisting of diabetes mellitus, obesity, and metabolic syndrome.

In another aspect of the present disclosure, there is provided a use of the compound, or a pharmaceutically acceptable salt thereof, disclosed herein, in the manufacture of a medicament for treating diabetes in a patient receiving insulin therapy.

In a further aspect of the present disclosure, there is provided a compound disclosed herein, or a pharmaceutically acceptable salt thereof, for use in therapy.

Detailed Description

Compounds with extended duration of action at the GIP receptor are desirable to allow for less frequent dosing of the compound.

Accordingly, embodiment 1 is a compound of Formula I

$Z_1X_1X_2EGTX_6ISDYSIX_{13}LDX_{16}X_{17}X_{18}QX_{20}X_{21}X_{22}VX_{24}X_{25}X_{26}L$

$X_{28}X_{29}GPSSGAPPPSZ_2$ (SEQ ID NO:4) wherein:

Z_1 is a modification of the N-terminal amino group wherein the modification is selected from the group consisting of acetyl and absent;

X_1 is selected from the group consisting of Y and D-Tyr;

X_2 is selected from the group consisting of Aib, A, and D-Ala;

X_6 is selected from the group consisting of F, αMeF, Iva, L, αMeL, and αMeF(2F);

X_{13} is selected from the group consisting of αMeL, A, L, and Aib;

X_{16} is selected from the group consisting of K, E, and Orn;

X_{17} is selected from the group consisting of I and K(2-[2-(2-amino-ethoxy)-ethoxy]- acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H;

X_{18} is selected from the group consisting of H, A, and R;

X_{20} is selected from the group consisting of Aib and Q;

X_{21} is selected from the group consisting of D and E;

X_{22} is selected from the group consisting of F and αMeF;

X_{24} is selected from the group consisting of E, N, Q, and D-Glu;

X_{25} is selected from the group consisting of Y, 4-Pal, W, and αMeY;

X_{26} is selected from the group consisting of L and K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H;

X_{28} is selected from the group consisting of E and A;

X_{29} is selected from the group consisting of G, A, Q, and T; q is selected from the group consisting of 16 and 18; and

Z_2 is absent or a modification of the C-terminal group, wherein the modification is amidation;

wherein one and only one selected from X_{17} and X_{26} is K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H;

or a pharmaceutically acceptable salt thereof.

An embodiment 2 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 1 wherein Z_1 is absent and X_1 is Y.

An embodiment 3 provides a compound, or pharmaceutically acceptable salt thereof, of embodiment 1 or embodiment 2 wherein X_2 is Aib.

An embodiment 4 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 3 wherein:

X_6 is selected from the group consisting of F and αMeF(2F).

An embodiment 5 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 4 wherein:

X_{13} is selected from the group consisting of L and αMeL.

An embodiment 6 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 5 wherein X_{16} is K or Orn.

An embodiment 7 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 6 wherein X_{16} is K.

An embodiment 8 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 7 wherein X_{18} is H.

An embodiment 9 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 8 wherein X_{20} is Aib; and X_{22} is F.

An embodiment 10 provides a compound, or a pharmaceutically acceptable salt

thereof, as claimed of any one of embodiments 1 to 9 wherein X_{21} is D.

An embodiment 11 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 10 wherein X_{25} is 4-Pal or Y.

An embodiment 12 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 11 wherein:

X_{17} is K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H; and

X_{26} is L.

An embodiment 13 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 11 wherein:

X_{17} is I; and

X_{26} is K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H.

An embodiment 14 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 13 wherein q is 16.

An embodiment 15 provides a compound, or a pharmaceutically acceptable salt thereof, of any one of embodiments 1 to 13 wherein q is 18.

An embodiment 16 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 1 wherein: Z_1 is absent;

X_1 is Y;

X_2 is selected from the group consisting of Aib and D-Ala; X_6 is F;

X_{13} is selected from the group consisting of αMeL and L;

X_{16} is selected from the group consisting of K and Orn;

X_{18} is selected from the group consisting of H and A;

X_{20} is Aib;

X_{22} is F;

X_{24} is selected from the group consisting of E, N, and D-Glu;

X_{25} is selected from the group consisting of Y, 4-Pal, and W;

X_{28} is selected from the group consisting of E and A;

X_{29} is selected from the group consisting of G, A, and Q; and q is selected from the group consisting of 16 and 18.

An embodiment 17 provides a compound, or a pharmaceutically acceptable salt

thereof, of embodiment 16 wherein:

Z_1 is absent;

X_2 is Aib;

X_{13} is αMeL;

X_{18} is H;

X_{24} is selected from the group consisting of E and D-Glu;

X_{25} is selected from the group consisting of Y and 4-Pal;

X_{28} is E;

X_{29} is selected from the group consisting of G and A.

An embodiment 18 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 17 wherein: X_{17} is K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H; and

X_{26} is L.

An embodiment 19 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 17 wherein: X_{17} is I; and X_{26} is K(2-[2-(2-amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_q$-CO_2H.

An embodiment 20 provides a compound, or a pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

An embodiment 21 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:5. An embodiment 22 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:6. An embodiment 23 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:7. An embodiment 24 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:8. An embodiment 25 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:9. An embodiment 26 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ

ID NO:10. An embodiment 27 provides a compound or pharmaceutically acceptable salt thereof, of embodiment 1 wherein the compound is SEQ ID NO:11.

An embodiment provides a method of treating a condition selected from the group consisting of diabetes, obesity, and metabolic syndrome, comprising administering to a subject in need thereof, an effective amount of a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof. An embodiment provides a method of treating a condition selected from the group consisting of T2DM, obesity, and metabolic syndrome, comprising administering to a subject in need thereof, an effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof. An embodiment provides a method for providing therapeutic weight loss comprising administering to a subject in need thereof, an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof. An embodiment is a method of treating T2DM comprising administering to a subject in need thereof, and effective amount of a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof.

An embodiment provides a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in therapy. An embodiment provides a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof, for use in therapy to treat a condition selected from the group consisting of diabetes, obesity, and metabolic syndrome. An embodiment provides a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in therapy to treat a condition selected from the group consisting of T2DM, obesity, and metabolic syndrome. In an embodiment, the condition is T2DM. In an embodiment, the condition is obesity. In an embodiment, the condition is type 1 diabetes (T1DM). In an embodiment, the condition is diabetes in a patient receiving insulin therapy. In an embodiment, the condition is metabolic syndrome.

The compounds of Formula I, or a pharmaceutically acceptable salt thereof, may be useful in the treatment of a variety of symptoms or disorders. For example, certain embodiments provide a method for treatment of T2DM in a patient comprising administering to a subject in need of such treatment an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof. In an embodiment is a method for treatment of obesity in a patient comprising administering to a subject in need of such

treatment an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof. In an embodiment, the method is inducing non-therapeutic weight loss in a subject, comprising administering to a subject in need of such treatment an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention provides a method for treatment of metabolic syndrome in a patient comprising administering to a subject in need of such treatment an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof. In an embodiment, the method is treatment of diabetes in a patient receiving insulin treatment, comprising administering to a subject in need of such treatment an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof. Also provided herein is a compound of the present invention for use in simultaneous, separate and sequential combinations with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a sodium-glucose cotransporter-2 (SGLT-2) inhibitor, a growth differentiation factor 15 modulator ("GDF15"), a peptide tyrosine tyrosine modulator ("PYY"), a modified insulin, amylin, a dual amylin- calcitonin receptor agonist, and an oxyntomodulin agonist ("OXM") in the treatment of a condition selected from the group consisting of T2DM, obesity, and metabolic syndrome. Also provided herein is a compound of the present invention for use in simultaneous, separate, and sequential combinations with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a sodium-glucose cotransporter-2 (SGLT-2) inhibitor, a growth differentiation factor 15 modulator ("GDF15"), a peptide tyrosine tyrosine analog ("PYY"), a modified insulin, an amylin receptor agonist, a dual amylin-calcitonin receptor agonist, a modified urocortin-2 (UCN-2) analog, a glucagon-like-peptide-1 (GLP-1) receptor agonist, a glucagon receptor agonist, and a dual GLP-1-glucagon receptor agonist including oxyntomodulin and analogs thereof, in the treatment of a condition selected from the group consisting of T2DM, obesity, and metabolic syndrome. In an embodiment, a compound of the present invention is provided in a fixed dose combination with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a SGLT-2 inhibitor, GDF15, PYY, a

modified insulin, amylin, a dual amylin-calcitonin receptor agonist, and OXM. In an embodiment, a compound of the present invention is provided in a fixed dose combination with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a SGLT-2 inhibitor, GDF15, a PYY analog, a modified insulin, an amylin receptor agonist, a dual amylin-calcitonin receptor agonist, a modified urocortin-2 (UCN-2) analog, a glucagon-like-peptide-1 (GLP-1) receptor agonist, a glucagon receptor agonist, and a dual GLP-1-glucagon receptor agonist including OXM and analogs thereof. In an embodiment is a compound of the present invention for use in simultaneous, separate and sequential combinations with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a SGLT-2 inhibitor, GDF15, PYY, a modified insulin, amylin, a dual amylin-calcitonin receptor agonist, and OXM in the treatment of a condition selected from the group consisting of T2DM and obesity. In an embodiment is a compound of the present invention for use in simultaneous, separate and sequential combinations with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, a SGLT-2 inhibitor, GDF15, a PYY analog, a modified insulin, an amylin receptor agonist, a dual amylin-calcitonin receptor agonist, a modified urocortin-2 (UCN-2) analog, a glucagon-like-peptide-1 (GLP-1) receptor agonist, a glucagon receptor agonist, and a dual GLP-1 glucagon receptor agonist including OXM and analogs of, in the treatment of a condition selected from the group consisting of T2DM and obesity. In an embodiment is a compound of the present invention for use in simultaneous, separate and sequential combinations with one or more agents selected from metformin, a thiazolidinedione, a sulfonylurea, a dipeptidyl peptidase 4 inhibitor, and a SGLT-2 inhibitor in the treatment of a condition selected from the group consisting of T2DM and obesity.

In an embodiment is a method for treating diabetes in a patient receiving insulin therapy, comprising administering an effective amount of a Compound of Formula I or a pharmaceutically acceptable salt thereof, to a patient in need thereof. An embodiment is treatment to a patient administered insulin therapy for T1DM. An embodiment is treatment to patient administered insulin therapy for T2DM. An embodiment is once weekly dosing. An embodiment is subcutaneous treatment once weekly to a patient

administered insulin therapy. An embodiment exists wherein the insulin therapy comprises basal insulin therapy. An embodiment exists wherein the insulin therapy comprises mealtime insulin therapy. An embodiment exists wherein the insulin therapy comprises ultra-rapid insulin therapy. Insulin therapy administered with acute infusions of a compound of Formula I, or a pharmaceutically acceptable salt thereof, may enhance glucagon excursion in patients undergoing hypoglycemic clamp, thus enhancing the body's natural defense against hypoglycemia. A compound of Formula I, or a pharmaceutically acceptable salt thereof, can be dosed once weekly independent of the type of insulin used or doses of insulin used. An embodiment is a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof, administered as an effective amount to a patient receiving insulin therapy, independent of the type of insulin used or doses of insulin used. An embodiment is a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof, dosed once weekly as an effective amount to a patient receiving insulin therapy independent of the type of insulin used or doses of insulin used. As used herein "insulin therapy" means treatment of a patient with diabetes using an approved insulin treatment. Such insulin therapy is known to the skilled artisan and/or clinical health care professional. For example, insulin therapy may comprise treatment using basal insulin. Such basal insulin "insulin therapy" may be used in a dosing regimen with mealtime insulin and/or ultra-rapid insulin. As used herein, "mealtime insulin" means insulin and/or modified insulin to be administered with meals, for example, but not limited to, insulin lispro. As used herein, "basal insulin" means modified insulin with a longer duration of action, such as, for example, but not limited to, insulin glargine.

Another embodiment provides the use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a condition selected from the group consisting of T2DM, obesity, and metabolic syndrome. An embodiment provides the use of a compound of any one of embodiments 1 to 27, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a condition selected from the group consisting of diabetes, obesity, and metabolic syndrome. In an embodiment, the medicament is for the treatment of T2DM. In an embodiment, the medicament is for the treatment of obesity.

In an embodiment, the medicament is for use in the treatment of diabetes in a patient receiving insulin therapy.

Another embodiment provides a pharmaceutical composition comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and at least one selected from the group consisting of a carrier, diluent, and excipient. In an embodiment, a pharmaceutical composition for subcutaneous administration is provided.

As used herein, the term "treating" or "to treat" includes restraining, slowing, stopping, or reversing the progression or severity of a symptom, condition, or disorder.

Certain compounds of the present invention are generally effective over a wide dosage range. For example, dosages for once weekly parenteral dosing may fall within the range of 0.05 mg to about 60 mg per person per week.

The compounds of Formula I, or a pharmaceutically acceptable salt thereof, include novel amino acid sequences having affinity for the GIP receptor, with desired potency at the receptor. GIP is a 42 amino acid peptide (SEQ ID NO:1), which, like GLP-1, is known as an incretin, and it plays a physiological role in glucose homeostasis by stimulating insulin secretion from pancreatic beta cells in the presence of glucose.

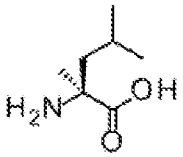
GLP-1 is a 36 amino acid peptide, the major biologically active fragment of which is produced as a 30-amino acid, C-terminal amidated peptide (GLP-1_{7-36}) (SEQ ID NO:2).

Glucagon is a 29-amino acid peptide hormone (SEQ ID NO:3) secreted by α-cells of the islet of Langerhans in the pancreas and is involved in glucose homeostasis.

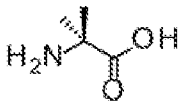
The compounds of present invention provide desired potency at the GIP receptor with high degree of selectivity against the GLP-1R and the Glucagon receptor. In an embodiment, compounds have desired GIP receptor activity with extended duration of action.

As used herein the term "amino acid" means both naturally occurring amino acids and unnatural amino acids. The amino acids are typically depicted using standard one letter codes (e.g., L = leucine), as well as alpha-methyl substituted residues of natural amino acids (e.g., α-methyl leucine, or αMeL, and α-methyl phenylalanine, or αMeF) and certain other unnatural amino acids, such as alpha-amino isobutyric acid, or "Aib," "4Pal," "Orn," and the like. The structures of these amino acids appear below:

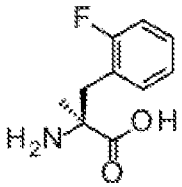
αMeL



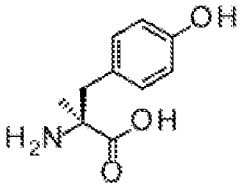
Aib



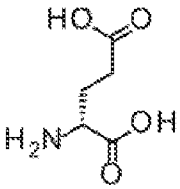
αMeF(2F)



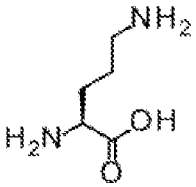
αMeY



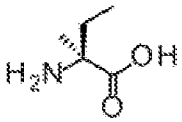
D-Glu



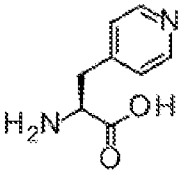
Orn



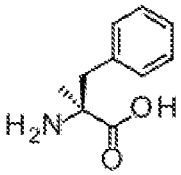
Iva



4Pal



αMeF



As used herein, "Orn" means L-ornithine. As used herein, "4Pal" means 3-(4-Pyridyl)-L- alanine. As used herein, "αMeF(2F)" means alpha-methyl 2-fluoro-L-phenylalanine. As used herein, "αMeY" and "αMeL" mean alpha-methyl-L-tyrosine and alpha-methyl-L- leucine, respectively. As used herein, "e" and "D-Glu" mean D-glutamic acid. As used herein, "D-Tyr"and "y" each mean D-tyrosine. As used herein, "D-Ala" and "a" each mean D-alanine. As used herein, "αMeF" means alpha-methyl-F and alpha-methyl-Phe. As used herein "Iva" means L-isovaline.

In an embodiment, the conjugation is an acylation. In an embodiment, the conjugation is to the epsilon-amino group of the K side-chain. In an embodiment of the compounds of the present invention, a fatty acid moiety is conjugated, via a linker, to a K at position 17. In an embodiment of the compounds of the present invention, a fatty acid moiety is conjugated, via a linker, to a K at position 26.

In an embodiment, q is selected from the group consisting of 16 and 18. In an

embodiment, q is 16. In an embodiment, q is 18.

When used herein in reference to the GIP receptor the terms "activity," "activate[s]" "activat[ing]" and the like refers to the capacity of a compound, or a pharmaceutically acceptable salt thereof, to bind to and induce a response at the receptor, as measured using assays known in the art, such as the in vitro assays described below.

The affinity of compounds of Formula I, or a pharmaceutically acceptable salt thereof, for the GIP receptor may be measured using techniques known for measuring receptor binding levels in the art, including, for example, those described in the examples below, and is commonly expressed as a K_i value. The activity of the compounds of the present invention at the GIP receptor may also be measured using techniques known in the art, including for example the in vitro activity assays described below, and is commonly expressed as an EC_{50} value, which is the concentration of compound causing half-maximal simulation in a dose response curve.

In addition, data is provided for each compound for activity and affinity at the GLP-1 and glucagon receptors, to demonstrate the degree of selectivity of the compounds of the present invention for the GIPR.

In an embodiment, a pharmaceutical composition of a compound of Formula I is suitable for administration by a parenteral route (*e.g.*, subcutaneous, intravenous, intraperitoneal, intramuscular, or transdermal). Some pharmaceutical compositions and processes for preparing same are well known in the art. (*See, e.g.*, Remington: The Science and Practice of Pharmacy (D.B. Troy, Editor, 21st Edition, Lippincott, Williams & Wilkins, 2006)).

Compounds of the present invention may react with any of a number of inorganic and organic acids/bases to form pharmaceutically acceptable acid/base addition salts. Pharmaceutically acceptable salts and common methodology for preparing them are well known in the art. (*See, e.g.*, P. Stahl, *et al.* Handbook of Pharmaceutical Salts: Properties, Selection and Use, 2nd Revised Edition (Wiley-VCH, 2011)). Pharmaceutically acceptable salts of the present invention include, but are not limited to, sodium, trifluoroacetate, hydrochloride, ammonium, and acetate salts. In an embodiment, a pharmaceutically acceptable salt is selected from the group consisting of

sodium, hydrochloride, and acetate salts.

The present invention also encompasses novel intermediates and processes useful for the synthesis of compounds of the present invention, or a pharmaceutically acceptable salt thereof. The intermediates and compounds of the present invention may be prepared by a variety of procedures known in the art. In particular, the Examples below describe a process using chemical synthesis. The specific synthetic steps for each of the routes described may be combined in different ways to prepare compounds of the present invention. The reagents and starting materials are readily available to one of ordinary skill in the art.

When used herein, the term "effective amount" refers to the amount or dose of a compound of the present invention, or a pharmaceutically acceptable salt thereof, which, upon single or multiple dose administration to the patient, provides the desired effect in the patient under diagnosis or treatment. An effective amount can be determined by a person of skill in the art using known techniques and by observing results obtained under analogous circumstances. In determining the effective amount for a subject, a number of factors are considered, including, but not limited to: the species of mammal; its size, age, and general health; the specific disease or disorder involved; the degree of or involvement or the severity of the disease or disorder; the response of the individual patient; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances.

When used herein, the term "subject in need thereof" refers to a mammal, preferably a human, with a disease or condition requiring treatment or therapy, including for example those listed in the preceding paragraphs.

As used herein, "EDTA" means ethylenediaminetetraacetic acid. As used herein, "DMSO" means dimethyl sulfoxide. As used herein, "CPM" means counts per minute. As used herein, "IBMX" means 3-isobutyl-1-methylxanthine. As used herein, "LC/MS" means liquid chromatography/mass spectrometry. As used herein, "HTRF" means homogeneous time-resolved fluorescence. As used herein, "DMF" refers to *N,N*-dimethylformamide. As used herein, "DCM" refers to dichloromethane. As used herein, "TFA" refers to trifluoroacetic acid. As used herein, "TFA salt" refers to trifluoroacetate

salt. As used herein, "RP-HPLC" means reversed-phase high performance liquid chromatography.

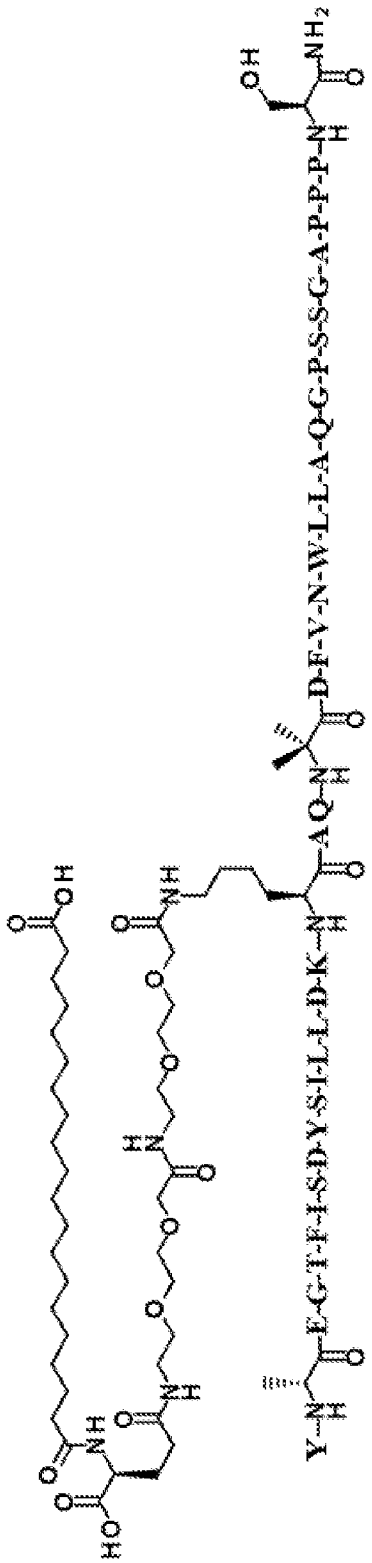
The invention is further illustrated by the following examples, which are not to be construed as limiting.

PEPTIDE SYNTHESIS

Example 1

Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)AQ-Aib-DFVNWLLAQGPSSGAPPPS-NH$_2$ (SEQ ID NO:5)

The structure of SEQ ID NO:5 is depicted below using the standard single letter amino acid codes with the exceptions of residues D-Ala2, K17, Aib20, and Ser39, where the structures of these amino acid residues have been expanded:


Y-E-G-T-F-I-S-D-Y-S-I-L-L-D-K-AQ-D-F-V-N-W-L-L-A-Q-G-P-S-S-G-A-P-P-P-N

The peptide backbone of Example 1 is synthesized using fluorenylmethyloxycarbonyl (Fmoc)/tert-butyl (t-Bu) chemistry on a Symphony multiplex peptide synthesizer (Gyros Protein Technologies. Tucson, AZ; 3.3.0.1), software version 3.3.0.

The resin consists of aminomethyl polystyrene functionalized with a Rink Amide linker (polystyrene AM RAM, RAPP polymeric GmbH, H40023, 200-400 mesh) at a substitution of 0.8 mmol/g. Standard side-chain protecting groups are used with the following exceptions: Fmoc-Lys(Mtt)-OH, where Mtt is 4-methyltrityl, is used for the lysine at position 17 and Boc-Tyr(t-Bu)-OH is used for the tyrosine at position 1. Fmoc groups are removed prior to each coupling step (2 x 10 minutes) using 20% piperidine in DMF. All standard amino acid couplings are performed using an equal molar ratio of Fmoc amino acid (0.3 M in DMF), diisopropylcarbodiimide (0.9 M in DCM) and Oxyma (0.9 M in DMF) at a 9-fold molar excess over the theoretical peptide loading. Couplings are allowed to proceed for 1.5 hours, with the following exceptions: coupling of valine, 3 hours; coupling of Cα-methylated amino acids, 6 hours; coupling to Cα-methylated amino acids, 6-10 hours. After completion of the synthesis of the peptide backbone, the resin is thoroughly washed with DCM to remove residual DMF. The Mtt protecting group on the lysine at position 17 is selectively removed from the peptide resin using three treatments of 30% hexafluoroisopropanol (Oakwood Chemicals) in DCM (3 x 20-minute treatment), and the resin is thoroughly washed with DCM and DMF.

Subsequent attachment of the fatty acid-linker moiety is accomplished by coupling of 2-[2-(2-Fmoc-amino-ethoxy)-ethoxy]-acetic acid (Fmoc-AEEA-OH, ChemPep, Inc.) and Fmoc-glutamic acid α-t-butyl ester (Fmoc-Glu-OtBu, Ark Pharm, Inc.) following the procedures described above for standard coupling and deprotection reactions. After removal of the final Fmoc protecting group, mono-OtBu-eicosanedioic acid (WuXi AppTec, Shanghai, China) is coupled for 1 hour using a 4-fold excess of the diacid, PyBOP, and diisopropylethylamine (1: 1 :1 mol/mol/mol) in 1:1 DCM/DMF.

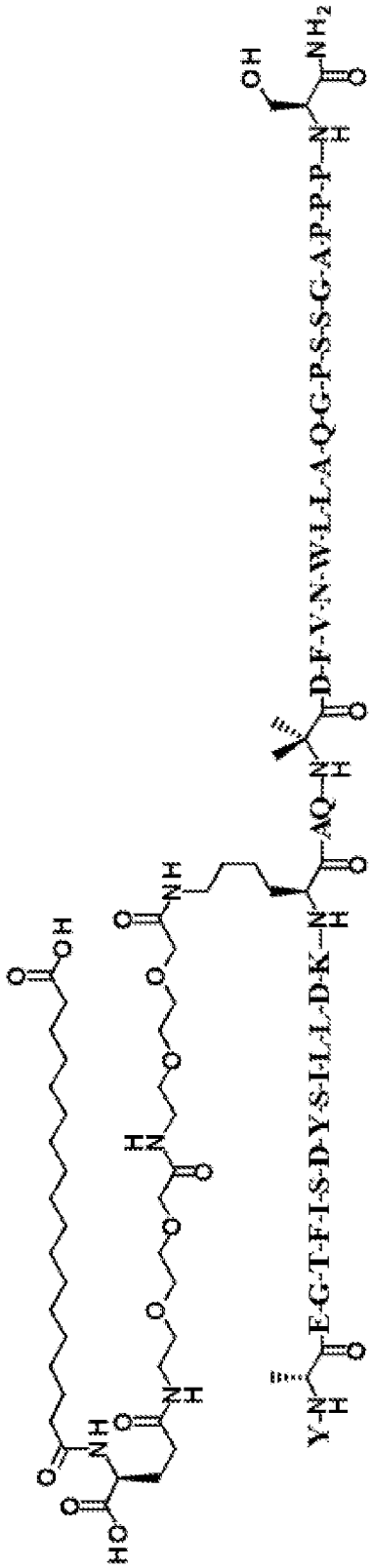
After the synthesis is complete, the peptide-resin is washed with DCM and then thoroughly dried over vacuum. The dry peptide-resin is treated with cleavage cocktail (10 mL TFA, 0.5 mL triisopropylsilane, 0.5 mL water, and 0.5 mL 1,2-ethanedithiol) for

2 hours at room temperature. The peptide resin solution is filtered into a 50-mL conical centrifuge tube and treated with 5-fold excess volume of cold diethyl ether (-20°C) to precipitate the crude peptide. The peptide/ether suspension is centrifuged at 3000 rcf for 1.5 min. to form a solid pellet and the supernatant is decanted. The pellet is washed further two times with cold diethyl ether, centrifuging for 1 min. each time, then dried in vacuo. The crude peptide is solubilized in 20% acetic acid/80% water and purified by RP-HPLC on a SymmetryPrep 7 μm C18 preparative column (18 x 300 mm, Waters) with linear gradients of 100% acetonitrile and 0.1% TFA/water buffer system (25-45% acetonitrile in 65 min). The purity of peptide is assessed using analytical RP-HPLC and pooling criteria is >95%. The main pool purity of compound of example 1 is found to be 96.8%. Subsequent lyophilization of the final main product pool yielded the lyophilized peptide TFA salt. The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1638.4; Calculated $[M+3H]^{3+}$=1638.53; Found MW (avg)=4912.2; Calc. MW (avg): 4912.58).

Example 2

Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-Aib-DFVNWLLAQGPSSGAPPPS-NH$_2$ (SEQ ID NO:6)

The structure of SEQ ID NO:6 is depicted below using the standard single letter amino acid codes with the exceptions of residues D-Ala2, K17, Aib20, and Ser39, where the structures of these amino acid residues have been expanded:

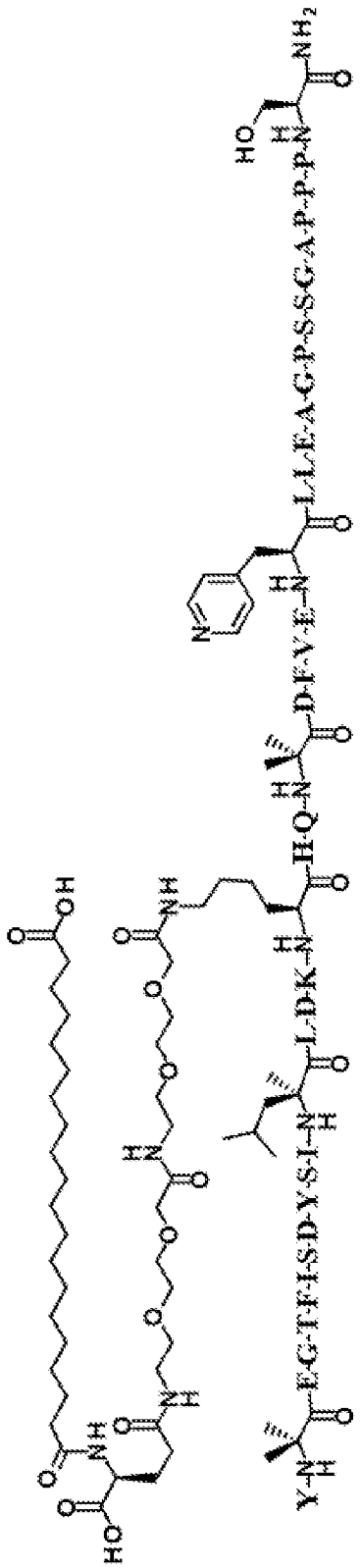

Y-E-G-T-F-I-S-D-Y-S-I-L-A-D-K-AQ-D-F-V-N-W-L-L-A-Q-G-P-S-S-G-A-P-P-P-NH2

The compound according to SEQ ID NO:6 is prepared substantially as described by the procedures of Example 1, where instead the protected diacid is mono-OtBu-octadecanedioic acid (WuXi AppTec, Shanghai, China). The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1629.15; Calc. $[M+3H]^{3+}$=1629.18; Found MW (avg)=4884.45; Calc. MW (avg)=4884.53).

Example 3

Y-Aib-EGTFISDYSI-αMeL-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH_2)$_{18}$-CO_2H)HQ-Aib-DFVE-4-Pal-LLEAGPSSGAPPPS-NH_2 (SEQ ID NO:7)

The structure of SEQ ID NO:7 is depicted below using the standard single letter amino acid codes with the exceptions of residues Aib2, αMeL13, K17, Aib20, 4-Pal25, and Ser39, where the structures of these amino acid residues have been expanded:

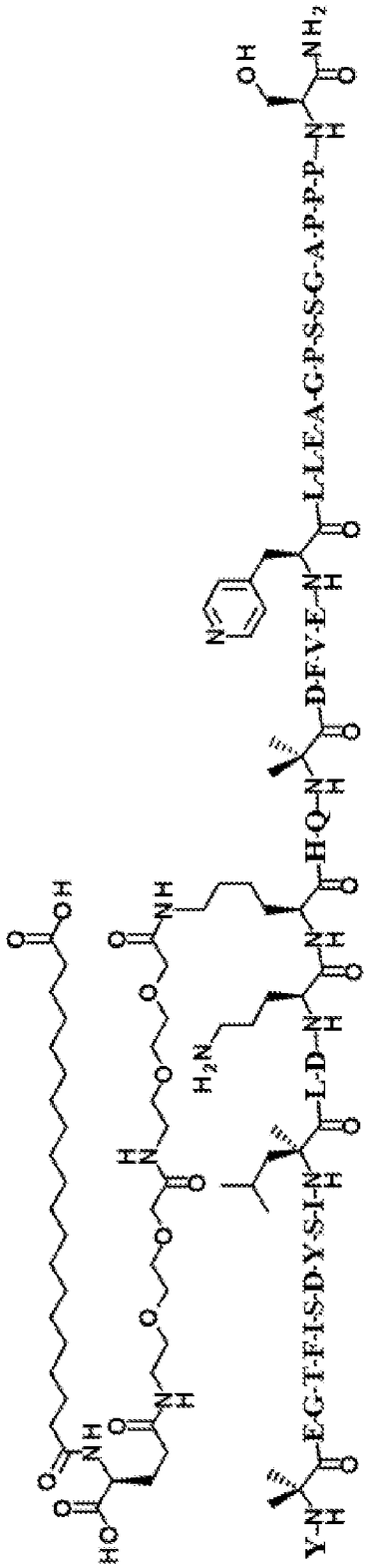


The compound according to SEQ ID NO:7 is prepared substantially as described by the procedures of Example 1. The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1662.52; Calc. $[M+3H]^{3+}$=1662.55; Found MW (avg)=4984.55; Calc. MW (avg)=4984.64).

Example 4

Y-Aib-EGTFISDYSI-αMeL-LD-Orn-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH_2)$_{18}$-CO_2H)HQ-Aib-DFVE-4-Pal-LLEAGPSSGAPPPS-NH_2 (SEQ ID NO:8)

The structure of SEQ ID NO:8 is depicted below using the standard single letter amino acid codes with the exceptions of residues Aib2, αMeL13, Orn16, K17, Aib20, 4- Pal25, and Ser39, where the structures of these amino acid residues have been expanded:

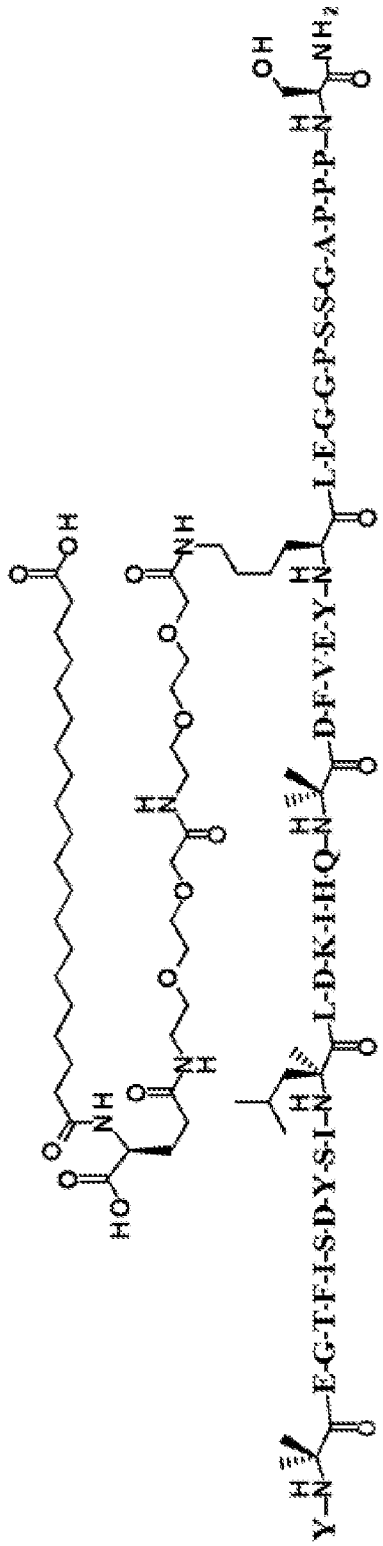


The compound according to SEQ ID NO:8 is prepared substantially as described by the procedures of Example 1. The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1657.82; Calc. $[M+3H]^{3+}$=1657.87; Found MW (avg)=4970.46; Calc. MW (avg)=4970.62).

Example 5

Y-Aib-EGTFISDYSI-αMeL-LDKIHQ-Aib-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$ (SEQ ID NO:9)

The structure of SEQ ID NO:9 is depicted below using the standard single letter amino acid codes with the exceptions of residues Aib2, αMeL13, Aib20, K26, and Ser39, where the structures of these amino acid residues have been expanded:

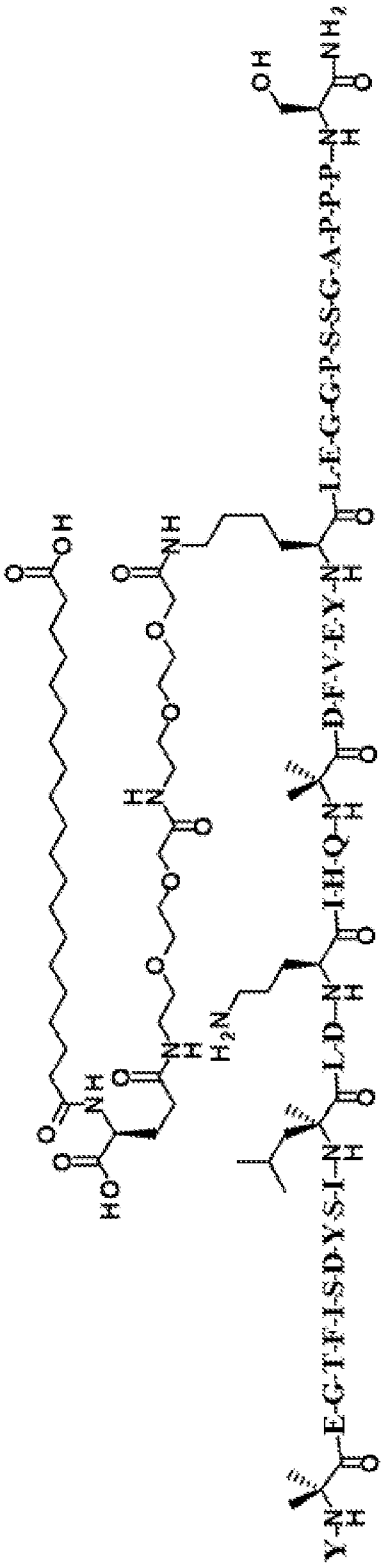


The compound according to SEQ ID NO:9 is prepared substantially as described by the procedures of Example 1, where Fmoc-Lys(Mtt)-OH is used for the lysine at position 26 rather than at position 17. The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1662.8; Calc. $[M+3H]^{3+}$=1662.88; Found MW (avg)=4985.4; Calc. MW (avg)=4985.63).

Example 6

Y-Aib-EGTFISDYSI-αMeL-LD-Orn-IHQ-Aib-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)LEGGPSSGAPPPS-NH_2 (SEQ ID NO:10)

The structure of SEQ ID NO:10 is depicted below using the standard single letter amino acid codes with the exceptions of residues Aib2, αMeL13, Orn16, Aib20, K26, and Ser39, where the structures of these amino acid residues have been expanded:

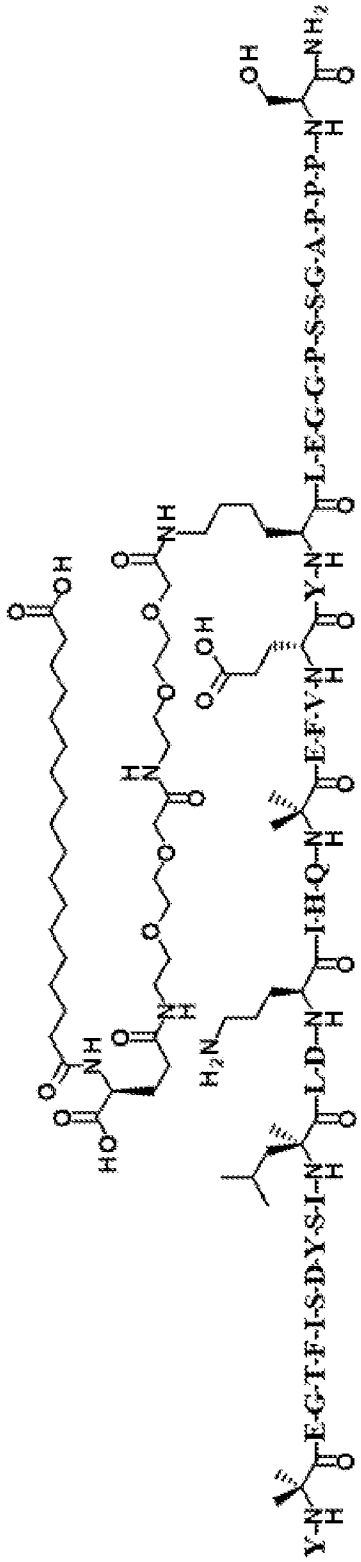

Y-N-E-G-T-F-I-S-D-Y-S-I-N-L-D-K-H-Q-K-D-F-V-E-Y-N-L-E-G-G-P-S-S-G-A-P-P-P-N-NH2

The compound according to SEQ ID NO:10 is prepared substantially as described by the procedures of Example 1, where Fmoc-Lys(Mtt)-OH is used for the lysine at position 26 rather than at position 17. The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1658.1; Calc. $[M+3H]^{3+}$=1658.20; Found MW (avg)=4971.3; Calc. MW (avg)=4971.6).

Example 7

Y-Aib-EGTFISDYSI-αMeL-LD-Orn-IHQ-Aib-EFV-(D-Glu)-YK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{16}$-CO_2H)LEGGPSSGAPPPS-NH_2 (SEQ ID NO:11)

The structure of SEQ ID NO:11 is depicted below using the standard single letter amino acid codes with the exceptions of residues Aib2, αMeL13, Orn16, Aib20, D-Glu24, K26, and Ser39, where the structures of these amino acid residues have been expanded:


E-G-T-F-I-S-D-Y-S-I-
L-D-
I-H-Q-
E-F-V-N-
Y-N-
L-E-G-G-P-S-S-G-A-P-P-P-N-
NH2

The compound according to SEQ ID NO:11 is prepared substantially as described by the procedures of Example 1, where Fmoc-Lys(Mtt)-OH is used for the lysine at position 26 rather than at position 17 and the protected diacid is mono-OtBu-octadecanedioic acid (WuXi AppTec, Shanghai, China). The molecular weight is determined by LC/MS (Found: $[M+3H]^{3+}$=1653.4; Calc. $[M+3H]^{3+}$=1653.52; Found MW (avg)=4957.2; Calc. MW (avg)=4957.57).

The compounds according to Example 8 (SEQ ID NO:12) through Example 122 (SEQ ID NO:126) are prepared substantially as described by the procedures of Example 1.

Example	SEQ ID NO	Compound Name	Calculated (avg) MW	Found (avg) MW
8	12	Y-(Aib)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)AQ-(Aib)-DFVNWLLAQGPSSGAPPPS-NH_2	4926.61	4926.6
9	13	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)HQ-(Aib)-DFVNWLLAQGPSSGAPPPS-NH_2	4978.64	4978.4
10	14	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)HQQDFVNWLLAGGPSSGAPPPS-NH_2	4950.59	4950.4
11	15	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)AQQDFVNWLLAQGPSSGAPPPS-NH_2	4955.61	4955.2
12	16	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-$(CH_2)_{18}$-CO_2H)AQQDFVNWLLAGGPSSGAPPPS-NH_2	4884.53	4884.4

13	17	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQQEFVNWLLAQGPSSGAPPPS-NH$_2$	5035.7	5035.2
14	18	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQQDFVEWLLAQGPSSGAPPPS-NH$_2$	5036.68	5036.8
15	19	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQQDFVNYLLAQGPSSGAPPPS-NH$_2$	4998.63	4998.4
16	20	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-DFVEWLLAQGPSSGAPPPS-NH$_2$	4899.54	4899.3
17	21	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-DFVNWLLEQGPSSGAPPPS-NH$_2$	4942.56	4941.9
18	22	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-DFVN-(4-Pal)-LLAQGPSSGAPPPS-NH$_2$	4846.48	4846.5
19	23	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-DFVNWLLAGGPSSGAPPPS-NH$_2$	4813.45	4813.2
20	24	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-EFVNWLLAQGPSSGAPPPS-NH$_2$	4898.55	4898.4
21	25	Y-(Aib)-EGTFISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4828.42	4827.9
22	26	Y-(Aib)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4870.5	4870.5

23	27	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4842.45	4842.3
24	28	Y-(Aib)-EGTFISDYSI-(Aib)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4842.45	4842.3
25	29	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVNWLLAGGPSSGAPPPS-NH$_2$	4907.56	4907.1
26	30	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVNWLLAGGPSSGAPPPS-NH$_2$	4925.56	4925.4
27	31	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-EFVQWLLAGGPSSGAPPPS-NH$_2$	4841.5	4841.4
28	32	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-EFVNWLLEGGPSSGAPPPS-NH$_2$	4885.51	4885.2
29	33	Y-(D-Ala)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-EFVEWLLAGGPSSGAPPPS-NH$_2$	4842.49	4842.45
30	34	Acetyl-(D-Tyr)-AEGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-EFVNYLLAGGPSSGAPPPS-NH$_2$	4902.47	4902.0
31	35	Y-(Aib)-EGTFISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVNYLLAAGPSSGAPPPS-NH$_2$	4884.53	4884.6
32	36	Y-(D-Ala)-EGTLISDYSILLDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)AQ-(Aib)-DFVNWLLAQGPSSGAPPPS-NH$_2$	4850.51	4850.4

33	37	Acetyl-(D-Tyr)-AEGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVNYLLEGGPSSGAPPPS-NH$_2$	4988.56	4988.1
34	38	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVNYLLATGPSSGAPPPS-NH$_2$	4946.57	4946.4
35	39	Y-(Aib)-EGT-(αMeL)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVNYLLATGPSSGAPPPS-NH$_2$	4894.56	4894.2
36	40	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLETGPSSGAPPPS-NH$_2$	5005.59	5005.8
37	41	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLEGGPSSGAPPPS-NH$_2$	4961.54	4961.4
38	42	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	4990.58	4990.2
39	43	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4946.53	4946.4
40	44	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEWLLETGPSSGAPPPS-NH$_2$	5028.63	5028.6
41	45	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	4972.59	4972.5

42	46	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEQGPSSGAPPPS-NH$_2$	4999.61	4999.8
43	47	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEAGPSSGAPPPS-NH$_2$	4942.56	4942.2
44	48	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4928.54	4928.1
45	49	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	5014.67	5014.65
46	50	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEQGPSSGAPPPS-NH$_2$	5041.69	5041.35
47	51	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4970.62	4970.4
48	52	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4912.58	4912.8
49	53	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4884.51	4884.6
50	54	Y-(Aib)-EGT-(αMeF)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4926.61	4926.9
51	55	Y-(Aib)-EGT-αMeF(2F)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4944.6	4944.6

52	56	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4926.61	4926.3
53	57	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLAGGPSSGAPPPS-NH$_2$	4927.59	4927.5
54	58	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEWLLAGGPSSGAPPPS-NH$_2$	4950.63	4950.6
55	59	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4864.54	4864.8
56	60	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-D-(αMeF)-VE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4926.61	4926.6
57	61	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLAQGPSSGAPPPS-NH$_2$	4997.69	4997.5
58	62	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLATGPSSGAPPPS-NH$_2$	4970.66	4970.4
59	63	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLAAGPSSGAPPPS-NH$_2$	4940.63	4940.4
60	64	Y-(Aib)-EGT-(αMeF)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLAQGPSSGAPPPS-NH$_2$	5011.71	5011.5
61	65	Y-(Aib)-EGT-(αMeF)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLATGPSSGAPPPS-NH$_2$	4984.69	4984.8

62	66	Y-(Aib)-EGT-(αMeF)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVE-(4-Pal)-LLAAGPSSGAPPPS-NH$_2$	4954.66	4954.2
63	67	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVEYLLEQGPSSGAPPPS-NH$_2$	5070.73	5070.9
64	68	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVEYLLETGPSSGAPPPS-NH$_2$	5043.71	5043.9
65	69	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVEYLLEAGPSSGAPPPS-NH$_2$	5013.68	5013.9
66	70	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLETGPSSGAPPPS-NH$_2$	5029.68	5029.2
67	71	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLEQGPSSGAPPPS-NH$_2$	5056.71	5056.8
68	72	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLEAGPSSGAPPPS-NH$_2$	4999.65	4999.2
69	73	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEYLLEGGPSSGAPPPS-NH$_2$	4985.63	4985.7
70	74	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLATGPSSGAPPPS-NH$_2$	4956.63	4956.3
71	75	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVEWLLETGPSSGAPPPS-NH$_2$	5052.72	5052.6
72	76	Y-(Aib)-EGTLISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVEYLLETGPSSGAPPPS-NH$_2$	5009.69	5009.7

73	77	Y-(Aib)-EGTLISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-EFVEYLLEGGPSSGAPPPS-NH$_2$	4965.64	4965.3
74	78	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4922.57	4922.4
75	79	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	4966.63	4966.8
76	80	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4908.55	4908.6
77	81	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	4952.6	4952.4
78	82	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAAGPSSGAPPPS-NH$_2$	4926.61	4926.3
79	83	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAQGPSSGAPPPS-NH$_2$	4983.66	4983.3
80	84	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLETGPSSGAPPPS-NH$_2$	5000.64	5000.4
81	85	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEQGPSSGAPPPS-NH$_2$	5027.67	5027.7

82	86	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEGGPSSGAPPPS-NH$_2$	4956.59	4956.45
83	87	Y-(Aib)-EGTFISDYSI-(αMeL)-LDEK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLATGPSSGAPPPS-NH$_2$	4957.57	4957.8
84	88	Y-(Aib)-EGTFISDYSI-(αMeL)-LDEK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAQGPSSGAPPPS-NH$_2$	4984.6	4984.2
85	89	Y-(Aib)-EGTFISDYSI-(αMeL)-LDEK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAAGPSSGAPPPS-NH$_2$	4927.55	4927.2
86	90	Y-(Aib)-EGTFISDYSI-(αMeL)-LDEK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLAGGPSSGAPPPS-NH$_2$	4913.52	4913.1
87	91	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEAGPSSGAPPPS-NH$_2$	4942.56	4942.8
88	92	Y-(Aib)-EGT-(Iva)-ISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEAGPSSGAPPPS-NH$_2$	4936.6	4936.2
89	93	Y-(Aib)-EGTLISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(4-Pal)-LLEAGPSSGAPPPS-NH$_2$	4950.63	4950.6
90	94	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(αMeY)-LLEAGPSSGAPPPS-NH$_2$	5013.64	5013.6
91	95	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)HQ-(Aib)-DFVE-(αMeY)-LLEGGPSSGAPPPS-NH$_2$	4999.61	4999.5

92	96	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIHQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEQGPSSGAPPPS-NH$_2$	5056.71	5057.1
93	97	Y-(D-Ala)-EGTFISDYSILLDKIAQ-(Aib)-DFVNWK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)LAQGPSSGAPPPS-NH$_2$	4884.53	4884.3
94	98	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-EFVNYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)LAGGPSSGAPPPS-NH$_2$	4874.46	4874.4
95	99	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-EFVNYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4960.55	4960.8
96	100	Y-(Aib)-EGTFISDYSIALDKIHQ-(Aib)-EFVNYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LAGGPSSGAPPPS-NH$_2$	4870.5	4870.2
97	101	Y-(Aib)-EGT-(αMeF)-ISDYSIALDKIHQ-(Aib)-EFVNYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LAGGPSSGAPPPS-NH$_2$	4884.53	4884.6
98	102	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-EFVNYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LAGGPSSGAPPPS-NH$_2$	4902.52	4902.15
99	103	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4961.54	4961.4
100	104	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIHQ-(Aib)-EFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4984.64	4984.2
101	105	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-DFV-(D-Glu)-YK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4971.6	4971.75

102	106	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-EFV-(D-Glu)-YK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4985.63	4985.1
103	107	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4943.55	4943.4
104	108	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-EFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEAGPSSGAPPPS-NH$_2$	4999.65	4999.2
105	109	Y-(Aib)-EGTFISDYSI-(αMeL)-LD -(Orn)-IHQ-(Aib)-EFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LETGPSSGAPPPS-NH$_2$	5029.68	5029.2
106	110	Y-(Aib)-EGTFISDYSI-(αMeL)-LD -(Orn)-IHQ-(Aib)-EFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEQGPSSGAPPPS-NH$_2$	5056.71	5056.8
107	111	Y-(Aib)-EGTFISDYSI-(αMeL)-LD -(Orn)-IHQ-(Aib)-EFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4985.63	4985.4
108	112	Y-(Aib)-EGTFISDYSI-(αMeL)-LD -(Orn)-IHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4956.59	4956.3
109	113	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4970.62	4970.4
110	114	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4942.56	4942.2
111	115	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{16}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4928.54	4928.4

112	116	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4946.53	4946.4
113	117	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H) LEAGPSSGAPPPS-NH$_2$	4960.55	4960.8
114	118	Y-(Aib)-EGT-αMeF(2F)-ISDYSIALDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H) LEQGPSSGAPPPS-NH$_2$	5017.6	5017.2
115	119	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIRQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	5004.63	5004.6
116	120	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IRQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4990.61	4990.2
117	121	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIHQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEAGPSSGAPPPS-NH$_2$	4999.65	4999.8
118	122	Y-(Aib)-EGTFISDYSI-(αMeL)-LDKIRQ-(Aib)-DFVEYK((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEAGPSSGAPPPS-NH$_2$	5018.66	5018.4
119	123	Y-(Aib)-EGT-αMeF(2F)-ISDYSILLDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEGGPSSGAPPPS-NH$_2$	4988.57	4988.4
120	124	Y-(Aib)-EGT-αMeF(2F)-ISDYSILLDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH$_2$)$_{18}$-CO$_2$H)LEAGPSSGAPPPS-NH$_2$	5002.59	5002.5

121	125	Y-(Aib)-EGT-αMeF(2F)-ISDYSILLDKIHQ-(Aib)-DFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH_2)$_{18}$-CO_2H)LEQGPSSGAPPPS-NH_2	5059.64	5059.8
122	126	Y-(Aib)-EGTFISDYSI-(αMeL)-LD-(Orn)-IHQ-(Aib)-EFVE-(4-Pal)-K((2-[2-(2-Amino-ethoxy)-ethoxy]-acetyl)$_2$-(γ-Glu)-CO-(CH_2)$_{18}$-CO_2H)LEAGPSSGAPPPS-NH_2	4984.64	4984.80

Binding Assays

Glucagon (referred to as Gcg or hGcg) is a Reference Standard prepared at Eli Lilly and Company. GLP-1(7-36)-NH_2 (referred to as GLP-1 or hGLP-1) is obtained from CPC Scientific (Sunnyvale, CA, 97.2% purity, 100 μM aliquots in 100% DMSO). GIP(1-42)-NH_2 (referred to as GIP) is prepared at Lilly Research Laboratories using peptide synthesis and HPLC chromatography as described above (>80% purity, 100 μM aliquots in 100% DMSO). [^{125}I]-radiolabeled Gcg, GLP-1, or GIP is prepared using [^{125}I]-lactoperoxidase and obtained from Perkin Elmer (Boston, MA).

Stably transfected cell lines are prepared by subcloning receptor cDNA into a pcDNA3 expression plasmid and transfected into human embryonic kidney (HEK) 293 (hGcgR and hGLP-1R) or Chinese Hamster Ovary (CHO) (hGIPR) cells followed by selection with Geneticin (hGLP-1R and hGIPR) or hygromycin B (hGcgR).

Two methods are used for the preparation of crude cell membranes.

Method 1: Frozen cell pellets are lysed on ice in hypotonic buffer containing 50 mM Tris HCl, pH 7.5, and Roche Complete™ Protease Inhibitors with EDTA. The cell suspension is disrupted using a glass Potter-Elvehjem homogenizer fitted with a Teflon® pestle for 25 strokes. The homogenate is centrifuged at 4°C at 1100 x g for 10 minutes. The supernatant is collected and stored on ice while the pellets are resuspended in homogenization buffer and rehomogenized as described above. The homogenate is centrifuged at 1100 x g for 10 minutes. The second supernatant is combined with the first supernatant and centrifuged at 35000 x g for 1 hour at 4°C. The resulting membrane pellet is resuspended in homogenization buffer containing

protease inhibitors at approximately 1 to 3 mg/mL, quick frozen in liquid nitrogen and stored as aliquots in a -80°C freezer until use.

Method 2: Frozen cell pellets are lysed on ice in hypotonic buffer containing 50 mM Tris HCl, pH 7.5, 1 mM $MgCl_2$, Roche Complete™ EDTA-free Protease Inhibitors and 25 units/mL DNAse I (Invitrogen). The cell suspension is disrupted using a glass Potter- Elvehjem homogenizer fitted with a Teflon® pestle for 20 to 25 strokes. The homogenate is centrifuged at 4°C at 1800 x g for 15 minutes. The supernatant is collected and stored on ice while the pellets are resuspended in homogenization buffer (without DNAse I) and rehomogenized as described above. The homogenate is centrifuged at 1800 x g for 15 minutes. The second supernatant is combined with the first supernatant and centrifuged an additional time at 1800 x g for 15 minutes. The overall supernatant is then centrifuged at 25000 x g for 30 minutes at 4°C. The resulting membrane pellet is resuspended in homogenization buffer (without DNAse I) containing protease inhibitors at approximately 1 to 3 mg/mL and stored as aliquots in a -80°C freezer until use.

Binding Determination Methods

The equilibrium binding dissociation constants (K_d) for the various receptor/radioligand interactions are determined from homologous competition binding analysis instead of saturation binding due to high propanol content in the [^{125}I] stock material. The K_d values determined for the receptor preparations were as follows: hGcgR (3.9 nM), hGLP-1R (1.2 nM) and hGIPR (0.14 nM).

[^{125}I]-Glucagon Binding

The human Gcg receptor binding assays are performed using a Scintillation Proximity Assay (SPA) format with wheat germ agglutinin (WGA) beads (Perkin Elmer). The binding buffer contains 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% (w/v) bacitracin (Research Products), 0.003% (w/v) Polyoxyethylenesorbitan monolaurate (TWEEN®-20), and Roche Complete™ Protease Inhibitors without EDTA. Peptides and Gcg are thawed and 3-fold serially diluted in 100% DMSO (10

point concentration response curves). Next, 5 µL serially diluted compound or DMSO is transferred into Corning® 3632 clear bottom assay plates containing 45 µL assay binding buffer or unlabeled Gcg control (non-specific binding or NSB, at 1 µM final). Then, 50 µL [^{125}I]-Gcg (0.15 nM final), 50 µL human GcgR membranes (1.5 µg/well) and 50 µL of WGA SPA beads (80 to 150 µg/well) are added with a Biotek Multiflo dispenser. Plates are sealed and mixed on a plate shaker (setting 6) for 1 minute and read with a PerkinElmer Trilux MicroBeta® scintillation counter after 12 hours of incubation/settling time at room temperature. Final assay concentration ranges for peptides tested in response curves is typically 1150 nM to 0.058 nM and for the control Gcg from 1000 nM to 0.05 nM.

[^{125}I]-GLP-1 Binding

The human GLP-1 receptor binding assay is performed using an SPA format with WGA beads. The binding buffer contains 25 mM HEPES, pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% (w/v) bacitracin, 0.003% (w/v) TWEEN®-20, and Roche Complete™ Protease Inhibitors without EDTA. Peptides and GLP-1 are thawed and 3-fold serially diluted in 100% DMSO (10 point concentration response curves). Next, 5 µL serially diluted compound or DMSO is transferred into Corning® 3632 clear bottom assay plates containing 45 µL assay binding buffer or unlabeled GLP-1 control (non-specific binding or NSB, at 0.25 µM final). Then, 50 µL [^{125}I]-GLP-1 (0.15 nM final), 50 µL human GLP-1R membranes (0.5 µg/well and 50 µL of WGA SPA beads (100 to 150 µg/well) are added with a Biotek Multiflo dispenser. Plates are sealed and mixed on a plate shaker (setting 6) for 1 minute and read with a PerkinElmer Trilux MicroBeta® scintillation counter after 5 to 12 hours of incubation/settling time at room temperature. Final assay concentration ranges for peptides tested in response curves are typically 1150 nM to 0.058 nM and for the control GLP-1, 250 nM to 0.013 nM.

[^{125}I]-GIP Binding

The human GIP receptor binding assay is performed using an SPA format with WGA beads. The binding buffer contains 25 mM HEPES, pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% (w/v) bacitracin, 0.003% (w/v) TWEEN®-20, and Roche Complete™ Protease Inhibitors without EDTA. Peptides and GIP are thawed and 3-

fold serially diluted in 100% DMSO (10 point concentration response curves). Next, 5 µL serially diluted compound or DMSO is transferred into Corning® 3632 clear bottom assay plates containing 45 µL assay binding buffer or unlabeled GIP control (non-specific binding or NSB, at 0.25 µM final). Then, 50 µL [^{125}I]-GIP (0.075-0.15 nM final), 50 µL human GIPR membranes (3 µg/well) and 50 µL of WGA SPA beads (100 to 150 µg/well) are added with a Biotek Multiflo dispenser. Plates are sealed and mixed on a plate shaker (setting 6) for 1 minute and read with a PerkinElmer Trilux MicroBeta® scintillation counter after 2.5 to 12 hours of incubation/settling time at room temperature. Final assay concentration ranges for peptides tested in response curves is typically 1150 to 0.058 nM or 115 nM to 0.0058 nM and for the control GIP, 250 nM to 0.013 nM.

Binding Assay Data Analysis

Raw CPM data for concentration curves of peptides, Gcg, GLP-1, or GIP are converted to percent inhibition by subtracting nonspecific binding (binding in the presence of excess unlabeled Gcg, GLP-1, or GIP, respectively) from the individual CPM values and dividing by the total binding signal, also corrected by subtracting nonspecific binding. Data are analyzed using four-parameter (curve maximum, curve minimum, IC_{50}, Hill slope) nonlinear regression routines (Genedata Screener, version 12.0.4, Genedata AG, Basal, Switzerland). The affinity constant (K_i) is calculated from the absolute IC_{50} value based upon the equation $K_i = IC_{50}/(1 + D/K_d)$ where D is the concentration of radioligand used in the experiment, IC_{50} is the concentration causing 50% inhibition of binding and K_d is the equilibrium binding dissociation constant of the radioligand (described above). Values for K_i are reported as the geometric mean, with error expressed as the standard error of the mean (SEM) and n is equal to the number of independent replicates (determined in assays performed on different days). Geometric Means are calculated as follows:

Geometric Mean = $10^{(\text{Arithmetic Mean of Log Ki Values})}$

n=y/x means that only a subset of replicates (y) out of the total number of replicates (x) is used to express the mean. SEM is only calculated when y=2 or greater. Means are expressed as geometric means with the standard error of the

mean (SEM) and the number of replicates (n) indicated in parentheses.

Table 1. In vitro Binding Affinity (K_i) of indicated Examples and comparator molecules for human GcgR, GIPR, and GLP-1R in the presence of 0.1% bacitracin.

Example or Comparator	**hGcgR Ki (nM) (SEM, n)**	**hGIPR Ki (nM) (SEM, n)**	**hGLP1R Ki (nM) (SEM, n)**
hGIP-NH_2	1150 (18.3, n=4)	0.125 (0.00511, n=319)	1100 (143, n=4)
hGlucagon	3.05 (0.120, n=457)	>2420 (n=3)	>4940 (n=5)
hGLP-1 (7-36) NH_2	>4590 (n=5)	>2300 (n=3)	0.785 (0.0252, n=489)
1	228 (46.6, n=5)	0.0667 (0.0566, n=5)	57.8 (16.7, n=5)
2	379 (218, n=3)	0.0373 (0.000430, n=3)	77.9 (20.2, n=3)
3	338 (220, n=8)	0.0525 (0.0215, n=8)	381 (226, n=8)
4	431 (253, n=6)	0.0620 (0.0259, n=6)	423 (235, n=6)
5	619 (140, n=9)	0.0771 (0.0342, n=9)	>913 (n=9)
6	453 (125, n=7)	0.104 (0.0866, n=7)	571 (368, n=7)
7	>961 (n=5)	0.193 (0.157, n=5)	>913 (n=5)
8	101	0.027	38.1
9	>230	0.110	168
10	>230	0.159	60.7
11	163	0.0584	85.9
12	134	0.0876	46.8
13	>230	0.237	>213
14	>230	0.460	>213
15	>254	0.471	>236
16	549	0.0438	99.2
17	274	0.0571	106
18	463	0.0331	158
19	335	0.0469	15.2
20	216	0.0477	79.8

21	>1530	1.46	801
22	>1380	0.219	388
23	323	0.0341	251
24	>960	0.254	729
25	101	0.0417	15.8
26	74.1	0.045	16.5
27	401	0.0354	23.2
28	86.8	0.0732	6.46
29	133	0.0569	22.7
30	407	0.037	26.5
31	>959	0.0473	266
32	>959	0.0343	85.2
33	63.5	0.0369	31.2
34	143	0.034	201
35	>960	0.0351	177
36	201	0.0683	393
37	51.4	0.026	83.9
38	182 (59.8, n=2)	0.0588 (0.0112, n=2)	776 (134, n=2)
39	76.0 (19.3, n=2)	0.0444 (0.00439, n=2)	235 (8.34, n=2)
40	50.9	0.0594	302
41	758	0.143	>906
42	99.1	0.0392	563
43	285 (121, n=2)	0.0366	605
44	50	0.059	209
45	660 (257, n=3)	0.0663 (0.0241, n=3)	>909 (n=3)
46	435 (89.6, n=3)	0.0319 (0.00724, n=3)	744 (58.2, n=3)
47	175 (69.7, n=3)	0.0452 (0.0127, n=3)	320 (77.8, n=3)
48	267	0.0498	211
49	>960	0.0512	596
50	23.4	0.0501	34.9
51	26.7	0.0386	54.1
52	114	0.0372	128
53	152	0.0184	62.9
54	386	0.0326	49.9
55	>960	0.0331	262

56	193	0.0487	530
57	422	0.0154	280
58	418 (1.17, n=2)	0.0324 (0.00694, n=2)	459 (68.3, n=2)
59	230	0.0148	109
60	26.2	0.0390	51.4
61	80.4	0.0665	135
62	31.3	0.0414	47.6
63	185	0.0248	327
64	196	0.022	477
65	279	0.0316	305
66	279	0.0866	326
68	290	0.0948	421
69	114	0.0406	144
70	857	0.0421	570
71	102	0.0422	347
72	837	0.0594	437
73	292	0.036	117
74	511	0.0287	395
75	>958	0.0626	>902
76	358	0.0293	263
77	>958 (n=2)	0.0841 (0.0207, n=2)	>905 (n=2)
78	505	0.0236	394
79	888	0.0229	486
80	>958 (n=2)	0.0828 (0.0284, n=2)	>906 (n=2)
81	699 (35.4, n=2)	0.0374 (0.0106, n=2)	714 (39.8, n=2)
82	305 (112, n=2)	0.0617 (0.00946, n=2)	388 (140, n=2)
83	>958	0.116	452
84	>958	0.0584	325
85	800	0.0529	220
86	462	0.0533	108
87	576	0.0538	849
88	788	0.0830	261
89	611 (n=3)	0.0720 (0.0169, n=4)	294 (72.9, n=4)
90	112	0.129	102
91	54.4	0.122	37.4

92	>958	0.0331	>902
93	>959	0.0430	>909
94	114	0.0310	>909
95	85.0	0.0255	832
96	>960	0.232	>908
97	77.5	0.0249	570
98	38.4	0.0195	311
99	174	0.0341	>901
100	572 (182, n=4)	0.0674 (0.0265, n=3)	>913 (n=3)
101	>961 (n=2)	0.139 (0.0585, n=2)	>913 (n=2)
102	>961 (n=3)	0.164 (0.113, n=3)	>913 (n=3)
103	>960	0.0405	>913
104	>960	0.134	>910
105	>960	0.174	>910
106	304	0.0918	>910
107	347	0.0722	>911
108	>960	0.134	>910
109	>960	0.117	>908
110	>960	0.339	>908
111	>960	0.0871	>908
112	257	0.0742	>908
113	821 (3.12, n=4)	0.108 (0.0287, n=5)	>921 (n=5)
114	>971 (n=3)	0.121 (0.0496, n=4)	>921 (n=4)
115	373 (n=2)	0.0997 (0.0472, n=2)	>912 (n=2)
116	178 (n=2)	0.166 (0.0753, n=2)	>912 (n=2)
117	685	0.0722	591
118	NA	0.0696	>920
119	68.8 (n=2)	0.129 (0.0552, n=2)	723 (119, n=2)
120	575 (n=2)	0.171 (0.231, n=2)	>912 (n=2)
121	94.9 (n=2)	0.103 (0.0473, n=2)	>912 (n=2)
122	957	0.0311	>913

As demonstrated in Table 1, examples of the present invention are very potent

binders of the human GIPR, and have lower affinity for the GLP-1R and GcgR.

cAMP Pharmacological Functional Assay in presence of 0.1% casein

A set of cAMP assays are conducted in HEK293 cells expressing the human GLP-1 receptor (GLP-1R), glucose-dependent insulinotropic peptide receptor (GIPR), or glucagon receptor (GcgR). Each receptor over-expressing cell line (20 µl) is treated with the test peptide in DMEM (Gibco Cat# 31053) supplemented with 0.1% Casein (Sigma Cat# C4765), 250 µM IBMX, 1X GlutaMAXTM (Gibco Cat# 35050), and 20 mM HEPES (HyClone Cat# SH30237.01) in a 20 µl assay volume. After incubating for 60 minutes at room temperature, the resulting increase in intracellular cAMP is quantitatively determined using the CisBio cAMP Dynamic 2 HTRF Assay Kit (62AM4PEJ). The Lysis buffer containing cAMP-d2 conjugate (20 µl) and the antibody anti-cAMP-Eu3+-Cryptate (20 µl) are then added to determine the cAMP level. After incubating 60 minutes at room temperature, HTRF signal is detected with an Envision 2104 plate reader (PerkinElmer). Fluorescent emission at 620 nm and at 665 nm is measured and the ratio between 620 nm and at 665 nm is calculated and then are converted to nM cAMP per well using a cAMP standard curve. Dose response curves of compounds are plotted as the percentage of stimulation normalized to minimum (buffer only) and maximum (maximum concentration of each control ligand) values and analyzed using a four parameter non-linear regression fit with a variable slope (Genedata Screener 13). EC50 is the concentration of compound causing half-maximal simulation in a dose response curve. A relative EC_{50} value is derived by non-linear regression analysis using the percent maximal response vs. the concentration of peptide added, fitted to a four-parameter logistic equation.

Using Homogeneous Time Resolved Fluorescence methods, assays are conducted to determine the intrinsic potency of Example and comparator molecules performed in the presence of casein (instead of serum albumin) as a nonspecific blocker, which does not interact with the fatty acid moieties of the analyzed molecules.

Intracellular cAMP levels are determined by extrapolation using a standard curve. Dose response curves of compounds are plotted as the percentage of stimulation normalized to minimum (buffer only) and maximum (maximum concentration of each

control ligand) values and analyzed using a four-parameter non-linear regression fit with a variable slope (Genedata Screener 13). EC_{50} is the concentration of compound causing half-maximal simulation in a dose response curve. Each relative EC50 value for the geometric mean calculation is determined from a curve fitting.

Concentration response curves of compounds are plotted as the percentage of stimulation normalized to minimum (buffer only) and maximum (maximum concentration of each control ligand) values and analyzed using a four-parameter non-linear regression fit with a variable slope (Genedata Screener 13). EC50 is the concentration of compound causing half-maximal simulation in a dose response curve. The EC_{50} summary statistics are computed as follows: Geometric mean:

GM = $10^{(\text{arithmetic mean of } \log_{10} \text{ transformed } EC_{50} \text{ values})}$. The standard error of the mean is reported:

SEM = geometric mean x (standard deviation of $\log_{10}$ transformed EC_{50} values / square root of the # of runs) x $\log_e$ of 10.

The log transform accounts for the EC_{50} values falling on a multiplicative, rather than an arithmetic scale.

Each time the assay is performed, the test peptides are run plus the native ligands GIP, GLP-1, and glucagon, buffer only as baseline (minimum) and the highest concentration of the respective GIP, GLP-1, and glucagon standard is used as maximum for calculations. For illustration, as shown by Example 1, the peptide is tested in 8 runs of the hGIPR cAMP assay. For avoidance of doubt, hGIP amide, hGLP-1 amide, and glucagon EC50 in Table 2 are illustrative of geometric mean values from a series of 18 assay values, and values will vary each day compared to the zero buffer. Accordingly, each Example will use the geometric mean of those values to normalize the Example assay runs.

Table 2. Functional activation of hGcgR, hGIPR, and hGLP-1R in the presence of 0.1% Casein.

Example or Comparator	**hGcgR cAMP Rel EC50 nM (SEM, n)**	**hGIPR cAMP Rel EC_{50} nM (SEM, n)**	**hGLP1R cAMP Rel EC_{50} nM (SEM, n)**
hGIP-NH_2	>5000 (n=5)	0.122 (0.00449, n=494)	>500 (n=3)
hGlucagon	0.0116 (0.000315, n=306)		9.79 (1.83, n=3)
hGLP-1(7-36) NH_2	>500 (n=4)		0.0549 (0.00149, n=490)
1	575 (369, n=4)	0.0476 (0.0253, n=8)	>5000 (n=5)
2	608 (n=11)	0.0123 (0.00751, n=15)	>5000 (n=11)
3	1250 (398, n=6)	0.0178 (0.00680, n=9)	>5000 (n=6)
4	1690 (n=5)	0.0182 (0.00783, n=5)	>5000 (n=5)
5	>5000 (n=5)	0.0148 (0.00434, n=5)	>5000 (n=5)
6	>5000 (n=4)	0.0176 (0.00714, n=14)	>5000 (n=4)
7	>5000 (n=2)	0.0219 (0.00294, n=2)	>5000 (n=2)
8	295 (62.9, n=2)	0.0268 (0.000153, n=3)	>5000 (n=2)
9	>5000	0.165	>100
10	>5000	0.611	>100
11	445	0.0738	>100
12	>5000	0.0911	>100
13	>5000	0.129	>100
14	>5000	0.340	>100
15	>5000	0.282	>100
16	>5000 (n=2)	0.00937 (0.00210, n=2)	>5000 (n=2)
17	>5000 (n=2)	0.0135 (0.00227, n=2)	>5000 (n=2)
18	>5000 (n=2)	0.00656 (0.00228, n=2)	>5000 (n=2)
19	>5000 (n=2)	0.00992 (0.00148, n=3)	>5000 (n=2)

20	>5000 (n=2)	0.00933 (0.00112, n=3)	>5000 (n=2)
21	>5000 (n=3)	0.291 (0.0766, n=4)	>5000 (n=3)
22	1250 (487, n=3)	0.0345 (0.00839, n=4)	>5000 (n=3)
23	>5000 (n=5)	0.0192 (0.00529, n=4)	206 (13.8, n=4)
24	>5000 (n=2)	0.0699 (0.0155, n=3)	>5000 (n=2)
25	>5000 (n=2)	0.0155 (0.00237, n=3)	8.39 (2.46, n=4)
26	>5000 (n=2)	0.00991 (0.00277, n=2)	5.69 (2.48, n=4)
27	>5000 (n=2)	0.0132 (0.000266, n=2)	>5000 (n=2)
28	>5000 (n=2)	0.0118 (0.00124, n=2)	>5000 (n=2)
29	>5000 (n=4)	0.00679 (0.00193, n=4)	>5000 (n=4)
30	>5000 (n=4)	0.00572 (0.00160, n=4)	>5000 (n=4)
31	1750 (886, n=3)	0.0148 (0.00426, n=3)	>5000 (n=3)
32	>5000 (n=2)	0.00469 (0.00199, n=3)	>5000 (n=2)
33	>5000	0.0551	>5000
34	913 (586, n=2)	0.0177 (0.00478, n=2)	462 (321, n=2)
35	>5000	0.0125	>5000
36	>5000	0.0543	1080
37	>5000	0.0360	61.1
38	>5000	0.0368	1390
39	>5000	0.0112	3330
40	>5000	0.0330	2330
41	>5000	0.0281	1800
42	>5000	0.0091	815
43	>5000	0.0154	1020
44	>5000	0.0084	1180
45	1690 (n=2)	0.0490 (0.000420, n=2)	>5000 (n=2)
46	863 (n=2)	0.0294 (0.0166, n=2)	>5000 (n=2)
47	757 (492, n=2)	0.0234 (0.00230, n=2)	>5000 (n=2)

48	1360	0.0166	>5000
49	2860 (620, n=6)	0.0156 (0.00569, n=7)	>5000 (n=6)
50	371.0	0.0212	41.0
51	308.0	0.0166	24.7
52	337.0	0.0194	>5000
53	344 (136, n=2)	0.0194 (0.00603, n=3)	>5000 (n=2)
54	>5000	0.0540	>5000
55	>5000	0.0170	>5000 (n=2)
56	>5000	0.0169	>5000 (n=2)
57	886 (430, n=2)	0.0177 (0.00819, n=3)	>5000 (n=2)
58	>5000	0.0183 (0.00544, n=2)	>5000
59	>5000	0.0202	>5000
60	>5000	0.0369	71
61	>5000	0.0167	192
62	>5000	0.0116	58.2
63	1170	0.0398	>5000
64	3070	0.0448	>5000
65	850	0.0346	>5000
66	>5000	0.0786	>5000
67	>5000	0.0627	>5000
68	3030 (n=2)	0.0768	>5000 (n=2)
69	803 (237, n=2)	0.0302 (0.00976, n=2)	>5000
70	3560	0.0254	>5000
71	581	0.0721	>5000
72	>5000	0.0182	>5000
73	>5000	0.0151	>5000
74	627	0.0167	>5000
75	2170	0.0182	>5000
76	1200	0.0154	>5000
77	2660	0.0265	>5000
78	3000 (n=2)	0.0125 (0.00185, n=2)	>5000 (n=2)
79	>5000	0.0316	>5000
80	>5000 (n=2)	0.0777 (0.0223, n=2)	>5000 (n=2)

81	>5000 (n=2)	0.0282 (0.00791, n=2)	>5000 (n=2)
82	3790 (n=2)	0.0391 (0.00658, n=2)	>5000 (n=2)
83	>5000	0.0432	>5000
84	>5000	0.0340	>5000
85	>5000	0.0359	>5000
86	>5000	0.0300	>5000
87	>5000	0.0107	>5000
88	1670	0.0031	>5000
89	>5000 (n=2)	0.00687 (0.00245, n=2)	>5000 (n=2)
90	>5000	0.0272	>5000
91	289	0.0321	530
92	>5000 (n=2)	0.0191 (0.00110, n=2)	>5000 (n=2)
93	>5000 (n=2)	0.00482 (0.000315, n=2)	884 (n=2)
94	>5000 (n=4)	0.00436 (0.00186, n=4)	1090 (442, n=4)
95	>5000	0.0272 (0.0110, n=2)	>5000
96	>5000	0.0251	>5000
97	>5000	0.0090	2510
98	>5000 (n=2)	0.00718 (0.00331, n=3)	649 (369, n=2)
99	>5000	0.00454 (0.000120, n=2)	>5000
100	>5000	0.0224	>5000
101	>5000 (n=2)	0.0396 (0.00639, n=2)	>5000 (n=2)
102	>5000 (n=2)	0.0166 (0.00337, n=2)	>5000 (n=2)
103	>5000 (n=2)	0.0129 (0.00901, n=2)	>5000 (n=2)
104	>5000	0.0165	>5000
105	>5000	0.0179	>5000
106	>5000	0.0140	>5000
107	>5000	0.0199	>5000
108	>5000	0.0088	>5000
109	>5000	0.0113	>5000
110	>5000	0.0071	>5000
111	>5000	0.0065	>5000
112	>5000	0.0041	>5000

113	>5000 (n=3)	00.0142 (0.0108, n=3)	>5000 (n=3)
114	>5000	0.0075	>5000
115	>5000	0.0372	>5000
116	>5000	0.0280	>5000
117	>5000	0.0617	>5000
118	>5000	0.0611	>5000
119	>5000	0.0220	>5000
120	>5000	0.0228	>5000
121	>5000	0.0196	>5000
122	>5000	0.0100	>5000

As demonstrated by data in Table 2, Example compounds of the present invention are very potent stimulating cAMP from human GIPR in the presence of 0.1% casein.

IN VIVO STUDIES

Pharmacokinetics in male Cynomolgus Monkeys

The pharmacokinetics of select Examples are evaluated following a single subcutaneous administration of 50 nmol/kg to male cynomolgus monkeys. Blood samples are collected over 504 hours and resulting individual plasma concentrations are used to calculate pharmacokinetic parameters. Peptide plasma (K_3 EDTA) concentrations are determined using a qualified LC/MS method that measured the intact mass of the compound. Each peptide and an analog as an internal standard are extracted from 100% cynomolgus monkey plasma using a protein precipitation method. Instruments are combined for LC/MS detection. Mean pharmacokinetic parameters are shown in **Table 3**.

Table 3. Mean Pharmacokinetic Parameters of peptides Following a Single Subcutaneous Administration of 50 nmol/kg to Male Cynomolgus Monkeys.

Example	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max}/Dose (kg*nmol/L/nmol)	AUC_{Inf}/Dose (hr*kg*nmol/L/nmol)	CL/F (mL/hr/kg)
1	71.1	24	7.20	786	1.27

2	51.8	6	6.92	624	1.61
3	88.8	60	12.1	1764	0.57
4	124	6	8.71	1387	0.72
5	128	120	12.0	2262	0.44
6	129	6	7.83	1382	0.77
7	109	9	10.1	1603	0.63

Abbreviations: $T_{1/2}$ = half-life, T_{max} = time to maximal concentration, C_{max}/dose = maximal plasma concentration divided by dose, AUC_{Inf}/Dose = AUC_{Inf} divided by dose, CL/F = clearance/bioavailability. Notes: Data are the mean, where n=2/group.

As seen in **Table 3**, results from this study for Example peptides tested are consistent with an extended pharmacokinetic profile.

Pharmacokinetics in male Sprague Dawley rats following subcutaneous administration

The pharmacokinetics of select Examples are evaluated following a single subcutaneous (SC) administration of 100 nmol/kg to male Sprague Dawley rats. Blood samples are collected over 168 hours following SC administration. Pharmacokinetic parameters are calculated using individual plasma concentrations. A qualified LC/MS method that measures the intact mass of the Example is used to determine plasma (K_3 EDTA) concentrations. Each peptide and an analog as an internal standard are extracted from 100% rat plasma using a protein precipitation method. Instruments are combined for LC/MS detection. Mean pharmacokinetic parameters for the Examples are shown in **Table 4**.

Table 4. Mean (+/- SD) Pharmacokinetic Parameters of peptides Following a Single Subcutaneous Administration of 100 nmol/kg to Male Sprague Dawley rats.

Example	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max}/Dose (kg*nmol/L/nmol)	AUC_{Inf}/Dose (hr*kg*nmol/ L/nmol)	CL/F (mL/hr/kg)
1	37.4	24	3.62	280	3.57
2	26.9	12	5.47	304	3.30
3	24.9	12	3.03	130	7.67
5	27.1	24	4.61	239	4.20
6	34.8	24	4.07	271	3.69

Abbreviations: $T_{1/2}$ = half-life, T_{max} = time to maximal concentration, C_{max}/Dose = maximal plasma concentration divided by dose, AUC_{Inf}/Dose = AUC_{Inf} divided by dose, CL/F = clearance/bioavailability. Notes: Data are the mean, where n=3/group, except for example 3, where the data is from n=1 animal.

As seen in table 4, results from this study using these Example peptides are consistent with an extended pharmacokinetic profile.

In Vivo Effect on Insulin Secretion in Male Wistar Rats

Male Wistar rats with femoral artery and femoral vein canulas (Envigo, Indianapolis, IN) (280-320 grams) are single-housed in polycarbonate cages with filter tops. Rats maintained on a 12:12 h light-dark cycle (lights on at 6:00 A.M.) at 21°C and receive food and deionized water ad libitum. Rats are randomized by body weight and dosed 1.5 mL/kg subcutaneously (s.c.) at doses of 0.3, 1.0, 3, 10, 30, and 100 nmol/kg 16 hours prior to glucose administration then fasted. Animals are weighed and anesthetized with sodium pentobarbital dosed intraperitoneally (i.p.) (65 mg/kg, 30 mg/mL). At time 0, a blood sample is collected into EDTA tubes after which glucose is administered intravenously (i.v.) (0.5 mg/kg, 5 mL/kg). Blood samples are collected for glucose and insulin levels at 2, 4, 6, 10, 20 and 30 min post-intravenous administration of glucose. Plasma glucose levels are determined using a clinical chemistry analyzer. Plasma insulin is determined using an electrochemiluminescence assay (Meso Scale, Gaithersburg, MD). Glucose and insulin AUC are examined compared to the vehicle control with n = 5 animals per group. Results are presented (SEM)(N).

Table 5: The effect of Example compounds on insulin secretion during intravenous glucose tolerance test.

	Dose (nmol/kg, s.c.)						
Example	0.0	0.3	1.0	3.0	10	30	100
3	38.3 (8.8)(5)	43.3 (0.8)(5)	45.1 (8.8)(5)	53.3 (6.5)(5)	64.9 (9.3)(5)	116.3 (28.6)(4)	133.6 (16.1)(5)
5	35.9 (7.5)(5)	36.2 (4.4)(5)	45.3 (6.1)(5)	80.6 (6.4)(5)	122.0 (5.9)(5)	144 (16.4)(5)	212.7 (19.4)(5)
6	39.5 (2.2)(5)	34.7 (3.6)(5)	54.2 (6.8)(5)	75.8 (10.3)(5)	100.2 (15.6)(5)	135.4 (22.9)(5)	166.5 (21.6)(5)

The data provided by Table 5 demonstrate a dose dependent increase in insulin secretion.

Table 6: ivGTT Insulin Secretion shown by the following data:

Insulin secretion (ivGTT)	
Example	(ED_{50}, nmol/kg) (SEM, n)
3	17.1 (n = 1)
5	18.4 (n = 1)
6	12.9 (n = 1)

The data provided by Table 6 demonstrate dose dependent increase in insulin secretion.

Immunogenicity assessment of the Compounds of Examples 1, 2, 3, 5, and 6

The purpose of this study is to determine the relative potential for clinical immunogenicity of Example compounds 1, 2, 3, 5, and 6.

Methods:

CD4+ T Cell Assay: The CD4+ T cell assay is used to compare the compounds of Examples 1, 2, 3, 5, and 6 for a potential to induce an immune response in vivo according to methods known in the art (*see*, *e.g.*, Jones *et al.* (2004) *J. Interferon*

Cytokine Res. 24:560-572; and Jones *et al.* (2005) *J. Thromb. Haemost.* 3:991-1000), where an assessment of clinically tested monoclonal antibodies and peptides shows some degree of correlation between T cell proliferation observed in vitro and immunogenicity in the clinic. Protein therapeutics that induce less than 30% positive response in the CD4+ T cell proliferation assay are associated with a low risk of immunogenicity. Briefly, to assess the propensity for a clinical immunogenic response to the compounds of Examples 1, 2, 3, 5, and 6, CD8+ T cell- depleted peripheral blood mononuclear cells (PBMCs) are prepared and labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen) from a cohort of 10 healthy donors with diverse human leukocyte antigen (HLA) class II haplotypes. Each donor is tested in triplicate with 2.0 mL media control, keyhole limpet hemocyanin (KLH; 0.33 μM), and the compounds of Examples 1, 2, 3, 5, and 6 (0.33 μM). Cultures are incubated for 7 days at 37°C with 5% CO_2. On day 7, samples are analyzed by flow cytometry using a BD LSR II Fortessa (Becton Dickinson; Franklin Lakes, NJ), equipped with a high throughput sampler (HTS). Data is analyzed using FlowJo® Software (FlowJo, LLC/TreeStar; Ashland, OR).

Results and Discussion

All donors produce a positive T cell response against KLH (100%). Analysis of the frequency and magnitude of the CD4+ T cell response for Example compounds is shown in Table 7.

Table 7: CD4+ T Cell Responses for Example compounds and Positive Control (KLH).

Example or Comparator	**% Donor Response (n=10)**	**Median Response Strength in positive donors (CDI)**
KLH	100%	164.285 (n=10)
Example 1	0%	NA (n=0)
Example 2	10%	2.69 (n=1)
Example 3	0%	NA (n=0)
Example 5	0%	NA (n=0)
Example 6	0%	NA (n=0)

Cell Division Index ("CDI"): proportion of divided CD4+ T cells to the total number of CD4+ T cells in stimulated versus unstimulated samples.

These data show that the frequency of positive CD4+ T cell response (CDI>2.5) was low for the tested Example compounds, and the magnitude of the response in the one positive donor from the Example 2 group was low (CDI<3). Thus, based on this assay, these compounds have a low risk of immunogenicity.

Amino Acid Sequences

SEQ ID NO:1

GIP 1-42 (Human)

YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ

SEQ ID NO:2

GLP-1 (7-36) amide (Human)

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH_2

SEQ ID NO:3

Glucagon (Human) HSQGTFTSDYSKYLDSRRAQDFVQWLMNT

SEQ ID NO:4

$Z_1X_1X_2EGTX_6ISDYSIX_{13}LDX_{16}X_{17}X_{18}QX_{20}X_{21}X_{22}VX_{24}X_{25}X_{26}LX_{28}X_{29}$

GPSSGAPPPSZ_2

WE CLAIM:

1. A compound, or pharmaceutically acceptable salt thereof, wherein the compound is SEQ ID NO:7 or SEQ ID NO:10.
2. The compound, or a pharmaceutically acceptable salt thereof, as claimed by Claim 1 wherein the compound is SEQ ID NO: 7.
3. The compound, or a pharmaceutically acceptable salt thereof, as claimed by Claim 1 wherein the compound is SEQ ID NO:10.
4. A pharmaceutical composition comprising the compound, or a pharmaceutically acceptable salt thereof, as claimed by any one of claims 1-3 and at least one pharmaceutically acceptable carrier, diluent, or excipient.
5. Use of the compound, or a pharmaceutically acceptable salt thereof, as claimed by any one of claims 1-3, in the manufacture of a medicament for treating a condition selected from the group consisting of diabetes mellitus, obesity, and metabolic syndrome.
6. Use of the compound, or a pharmaceutically acceptable salt thereof, as claimed by any one of claims 1-3 in the manufacture of a medicament for treating diabetes in a patient receiving insulin therapy.
7. The use as claimed by Claim 5 wherein the condition is obesity.
8. The use as claimed by Claim 5 wherein the condition is type 2 diabetes mellitus.
9. A compound as claimed in any one of claims 1-3, or a pharmaceutically acceptable salt thereof, for use in therapy.