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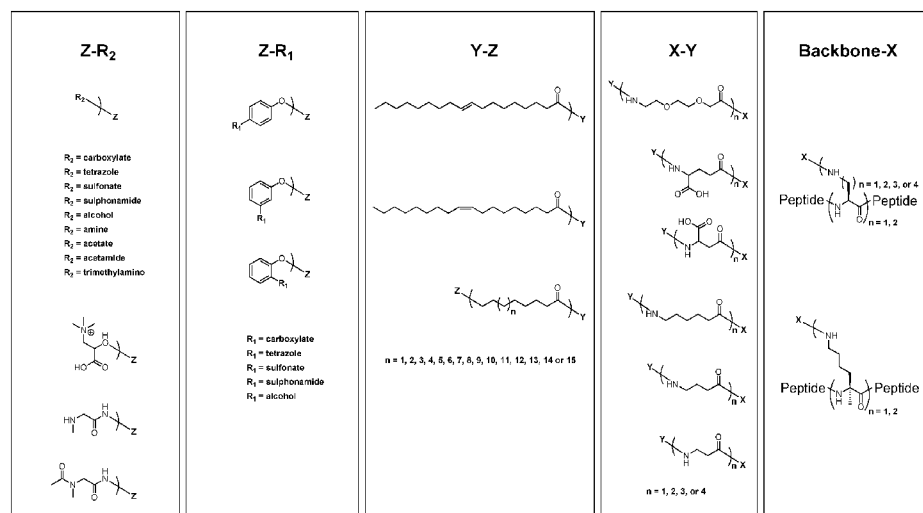


Figure 1

(57) Abstract: The present invention provides protease-resistant peptides, methods of making such peptides, as well as compositions comprising protease-resistant peptides and method of treatment utilizing such peptides. A combination of lipidation of certain amino acid residues and substitution of alternative amino acids for natural amino acids and additional amino acids added to the peptide chain has been determined to produce protease-resistant peptides.



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PROTEASE-RESISTANT MONO-LIPIDATED PEPTIDES

BACKGROUND

- [0001] The present disclosure provides protease-resistant peptides, methods of making such peptides, as well as compositions comprising protease-resistant peptides and methods of treatment utilizing such peptides. Lipid modification of amino acids at certain positions in the peptide sequence is described herein.
- [0002] The development of long-acting peptide therapeutics is hampered by factors such as short plasma half-life and poor oral bioavailability, largely a result of the natural susceptibility of peptides to enzymatic degradation. The majority of proteolytic functions are necessary, including regulating essential biomolecular processes such as turning off peptide signaling events at cell surfaces, or the gastric breakdown of proteins and peptides during digestion. Thus, the activity of the responsible proteases cannot simply be inhibited without, in many cases, causing other metabolic disturbances.
- [0003] In order to overcome degradation, increasing the enzymatic resistance of a peptide of interest is therefore desirable. Generally, two methods are utilized to increase enzymatic resistance: sequence specific modifications, *e.g.*, those affecting the primary structure of the peptide itself; and globally effective modifications, *e.g.*, those which alter certain overall physicochemical characteristics of the peptide. Introduced strategically, such modifications can reduce the effects of natural physiological processes which would otherwise eliminate or inactivate a peptide whose action is desired, *e.g.* enzymatic degradation and/or clearance by renal ultrafiltration.

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- [0004] Sequence specific modifications include incorporation of proteolysis-resistant unusual amino acids, or more involved modifications including cyclization between naturally occurring side-chain functions, e.g. disulfide formation (Cys-Cys), or lactamization (Lys-Glu or Lys-Asp). Additional modifications include cyclization between unnatural amino acid surrogates within the peptide backbone e.g. olefin metathesis stapling.
- [0005] Global modifications include processes such as peptide lipidation e.g. palmitoylation and/or PEGylation. Palmitoylation has the effect of creating a circulating reservoir of peptide which reversibly associates with naturally abundant albumin in blood serum. Peptide associated with albumin effectively escapes renal ultrafiltration since the size of the associated complex is above the glomerular filtration cutoff. As the peptide dissociates from the surface of the albumin it is again free to interact with endogenous receptors. PEGylation has the effect of physically shielding the peptide from proteolysis and imparts significant hydrophilicity which upon hydration greatly increases the hydrodynamic radius of the therapeutic molecule to overcome renal clearance.
- [0006] While these technologies can be broadly applicable to therapeutic peptides in general, and to an extent are able to extend circulatory half-life, a need still exists for methods of increasing stability of peptides and proteins to enzymatic degradation, particularly in light of the desire to produce peptides suitable for oral administration.

SUMMARY

- [0007] The present disclosure provides for an isolated polypeptide comprising the amino acid sequence: X0-H-X2-X3-GT-FTSD-X10-S-X12-X13-X14-X15-X16-X17-AA-X20-X21-

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X22-I-X24-X25-X26-X27-X28-X29-X30-X31-X32 (SEQ ID NO: 2); wherein X0 is null, A, E, F, I, L, V or T; X2 is A, Aib or d-Ser; X3 is E or I; X10 is S or a lipid modified K; X12 is S or a lipid modified K; X13 is Y or a lipid modified K; X14 is L or a lipid modified K; X15 is E or a lipid modified K; X16 is G or a lipid modified K; X17 is E, Q, or a lipid modified K; X20 is E, K, R, or a lipid modified K; X21 is E or a lipid modified K; X22 is F, Cha, 4Me-Phe, Bip, Dip, 2-CF₃-Phe, 4-CF₃-Phe, Nle or a lipid modified K; X24 is A, E, Cha, Bip, Dip, Nle, or a lipid modified K; X25 is W, Cha, Nle, NMe-Trp, α Me-Trp, or a lipid modified K; X26 is L or a lipid modified K; X27 is V or a lipid modified K; X28 is K, E, R or a lipid modified K; X29 is G or Aib; X30 is R, E, or G; X31 is G, null, or a lipid modified K; X32 is null or a lipid modified K; and wherein the polypeptide is lipidated on only one of X10, X12, X13, X14, X15, X16, X17, X20, X21, X22, X24, X25, X26, X27, X28, X31, or X32.

[0008] In certain embodiments the peptide comprises a C-terminal amide. In other embodiments, the peptide comprising a C-terminal acid.

[0009] In still further embodiments the peptide has a lipid modified K residue that is selected from the group consisting of: (γ E-Palmitoyl), K(ϵ -(PEG)2-(PEG)2- γ E-Palmitoyl), K(ϵ -(PEG)2-(PEG)2-Palmitoyl), K(ϵ -(PEG)4- γ E-Palmitoyl), K(ϵ -(PEG)4 - γ E-Stearoyl), K(ϵ -(PEG)4 -Stearoyl), K(ϵ -(PEG)2-(PEG)2- γ E-Stearoyl), K(ϵ -(PEG)2-(PEG)2- γ E- γ E-Stearoyl), K(ϵ - γ E- γ E-Stearoyl)., and any combination thereof. In other embodiments the polypeptide is substantially resistant to proteolytic degradation. In still further embodiments polypeptide is substantially resistant to DPP-IV, neprilysin, α -chymotrypsin, plasmin, thrombin, kallikrein, trypsin, elastase and/or pepsin degradation.

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[0010] In other embodiments the polypeptide at least maintains substantially the same receptor potency as a corresponding non-lipidated polypeptide. In other embodiments the polypeptide at least maintains substantially the same receptor selectivity as a corresponding non-lipidated polypeptide. In further embodiments the polypeptide exhibits increased receptor potency over a corresponding non-lipidated polypeptide.

[0011] The present disclosure also provides for an isolated polypeptide comprising the amino acid sequence: X0-HGEGT-FTSD-X10-S-X12-Q-X14-EE-X17-AV-X20-L-X22-I-X24-WLKNGGPSSGAPP-X39-X40 (SEQ ID NO: 204); wherein X0 is F or A; X10 is L or a lipid modified K; X12 is K or lipid modified K; X14 is M or a lipid modified K; X17 is E or a lipid modified K; X20 is R or a lipid modified K; X22 is F, Cha, or Phe(4Me), 4Me-phenylalanine; X24 is L or a lipid modified K; X39 is S or a lipid modified K; X40 is null or a lipid modified K; and wherein the polypeptide comprises one lipid modified K residue at X10, X12, X14, X17, X20, X24, X39, or X40.

[0012] In certain embodiments the peptide comprises a C-terminal amide. In other embodiments, the peptide comprising a C-terminal acid.

[0013] In other embodiments the peptide can have a lipid modified K residue that is selected from the group consisting of: K(γ E-Palmitoyl), K(ϵ -(PEG)2-(PEG)2- γ E-Palmitoyl), K(ϵ -(PEG)2-(PEG)2-Palmitoyl), K(ϵ -(PEG)4- γ E-Palmitoyl), K(ϵ -(PEG)4- γ E-Stearoyl), K(ϵ -(PEG)4-Stearoyl), K(ϵ -(PEG)2-(PEG)2- γ E-Stearoyl), K(ϵ -(PEG)2-(PEG)2- γ E- γ E-Stearoyl), K(ϵ - γ E- γ E-Stearoyl).

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- [0014] In some embodiments the polynucleotide encoding the polypeptide is described. In other embodiments a vector comprising the polynucleotide is disclosed. In further embodiments, a host cell comprising the polynucleotide or the vector is described.
- [0015] Presently disclosed is a method of making the polypeptide, comprising culturing the host cell under conditions allowing expression of the peptide, and recovering the peptide.
- [0016] A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 116, and a carrier. In other embodiments a kit is disclosed.
- [0017] Presently disclosed is a method of treating or preventing a disease or condition caused or characterized by hypoglycemia or impaired insulin release, comprising administering to a subject in need of treatment an effective amount of the polypeptide.
- [0018] In some embodiments the disease or condition is diabetes. In other embodiments the disease or condition is type-2 diabetes. In still other embodiments, the administration of the polypeptide further improves glycemic control, provides body weight control, improves β -cell function and mass, reduces the rate of gastric acid secretion and gastric emptying, or any combination thereof.
- [0019] In some embodiments the polypeptide or the pharmaceutical composition are administered orally or by injection. In an embodiment the polypeptide or the pharmaceutical composition is administered orally. In other embodiments the injection is administered subcutaneously or intravenously.

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[0020] In other embodiments the peptide or the pharmaceutical composition is administered once per day. In further embodiments the disclosure includes administering one or more additional therapies. In other embodiments the additional therapy comprises blood sugar monitoring, diet modifications, exercise, insulin, a thiazolidinedione, a sulfonylurea, an incretin, metformin, a glyburide, a dipeptidyl peptidase 4 inhibitor, a bile acid sequestrant, or any combination thereof. In some embodiments the subject is a human.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0021] FIG 1 shows representative lipid, lipid moieties, and linkers for forming lipidated polypeptides disclosed herein.

DETAILED DESCRIPTION

[0022] It should be appreciated that the particular implementations shown and described herein are examples and are not intended to otherwise limit the scope of the application in any way.

[0023] The published patents, patent applications, websites, company names, and scientific literature referred to herein are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

- [0024] As used in this specification, the singular forms “a,” “an” and “the” specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise. The term “about” is used herein to mean approximately, in the region of, roughly, or around. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20%.
- [0025] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).
- [0026] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.
- [0027] Technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which the present application pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of peptide synthesis include W.C.Chan and P.D.White., “Fmoc Solid Phase Peptide Synthesis: A Practical Approach”, Oxford University Press, Oxford (2004).

- [0028] Units, prefixes, and symbols are denoted in their *Système International de Unites* (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.
- [0029] The terms "polypeptide," "peptide," "protein," and "protein fragment" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.
- [0030] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and *O*-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, e.g., an alpha carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs can have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that

function similarly to a naturally occurring amino acid. The terms “amino acid” and “amino acid residue” are used interchangeably throughout.

[0031] The terms "fragment," "analog," "derivative," or "variant" when referring to a lipidated peptide as provided herein includes any peptide that retains at least some activity of a corresponding native peptide, e.g., GLP-1. As used herein, the term “lipidated GLP-1 peptide analog” refers to, e.g., a synthetic peptide comprising one or more lipidated amino acids, e.g., to render the peptide protease resistant, while still maintaining at least some of the GLP-1 activities of a native GLP-1 peptide. Chemical modifications intended to improve metabolic stability of peptides can involve additional chemical manipulation following synthesis of the main peptide chain. Examples of manipulation include lactamization, disulfide bridge closure, lipidation and/or PEGylation.

[0032] The terms “lipid modified amino acid” and “lipidated amino acid” are used interchangeably herein, and refer to an amino acid, typically a lysine or cysteine, which has a lipid moiety attached. The terms “lipidated polypeptide,” “lipoprotein,” and the like refer to a peptide or polypeptide that includes one or more lipid modified amino acids. Figure 1 illustrates various representative examples of lipids, lipid moieties, and linkers.

[0033] The terms "composition" or "pharmaceutical composition" refer to compositions containing a peptide or polypeptide provided herein, along with *e.g.*, pharmaceutically acceptable carriers, excipients, or diluents for administration to a subject in need of treatment.

[0034] The term "pharmaceutically acceptable" refers to compositions that are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals

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without excessive toxicity or other complications commensurate with a reasonable benefit/risk ratio.

[0035] An "effective amount" is that amount of a peptide or polypeptide provided herein, the administration of which to a subject, either in a single dose or as part of a series, is effective for treatment.

[0036] The term "subject" is meant any subject, particularly a mammalian subject, in need of treatment with a peptide or polypeptide provided herein. Mammalian subjects include, but are not limited to, humans, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, bears, cows, apes, monkeys, orangutans, and chimpanzees, and so on. In one aspect, the subject is a human subject.

[0037] Provided herein are compositions and methods that address the natural enzymatic liabilities of peptides. By lipidating selected amino acid residues, peptides are provided that demonstrate increased resistance to enzymatic degradation, while still maintaining substantially the same or exhibiting increased receptor potency and selectivity as a wild-type peptide.

Peptides Demonstrating Protease Resistance

[0038] This disclosure provides lipidated peptides with increased potency. Improvements in protease resistance and potency can be associated with the position of the lipidation on the peptide. Lipidation can include carboxyl- or amino- terminal lipidation, or main-chain lipidation. In certain embodiments, the modification is of a main-chain amino acid residue. In certain embodiments, improvements in protease resistance and increased potency are associated with the selective and strategic position of the lipidation of one or more main-chain amino acid residues. Methods of preparing peptides with lipid modified amino acids are known in the art.

[0039] In certain embodiments, a lipidated peptide comprising at least one lipidated amino acid residue is provided. In certain embodiments, the lipidated peptide comprises at least two lipidated amino acid residues. In certain embodiments, the lipidated peptide contains only one lipidated amino acid residue. As used herein, a peptide with one lipid or lipid moiety attached is referred to as a mono-lipidated peptide. In other embodiments, the lipidated peptide contains two lipidated amino acid residues. As used herein, a peptide with two lipids or lipid moieties attached is referred to as a bis-lipidated peptide.

[0040] In certain embodiments, the lipidated peptide is a synthetic peptide. *See* International Patent Application No. PCT/EP2014/077240, published as WO2015/086686A2, which is incorporated by reference herein in its entirety. In certain embodiments, the lipidated synthetic peptide comprises at least one substitution of an alternative amino acid for a native amino acid residue. In other embodiments, a lipidated synthetic peptide comprises at least two, three, four, five, six, or more substitutions of alternative amino acids functionalized amino acids for native amino acid residues. In certain embodiments the alternative amino acid can be selected from the group consisting of Aib, Arg, Bip, Cha, β,β -Dip, F5-Phe, PhG, Phe, Tyr, homoPhe, homoTyr, α -MePhe, α -Me-2F-Phe, 2Me-Phe, 3Me-Phe, 4Me-Phe, Nle, Tyr(OMe), 4I-Phe, Nal(1), Nal(2), 2F-Phe, 3F-Phe, 4F-Phe, Pro, NMe-Phe, NMe-Tyr, NMe-Trp, α -MeTrp, β,β -*di*-MeTrp, β,β -*di*-Me-Phe; α -MeTyr or β,β -*di*-MeTyr.

[0041] As described herein, “synthetic peptide” refers to a polymer of amino acid residues that has been generated by chemically coupling a carboxyl group or C-terminus of one amino acid to an amino group or N-terminus of another. Chemical peptide synthesis typically starts at the C-

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terminus of the peptide and ends at the *N*-terminus. Various methods for generating synthetic peptides are well known in the art.

[0042] As described herein “alternative amino acids” refer to amino acids that are either not the standard 20 amino acids that exist in biologically generated proteins, or modified versions of the standard 20 amino acids that exist in biologically generated proteins.

[0043] The term “native” amino acid refers to one of the standard 20 amino acids that exist in biologically generated proteins.

[0044] Substitution refers to the replacement of a native amino acid with, e.g., an alternative amino acid or side chain modified. During chemical synthesis of a synthetic peptide, the native amino acid can be readily replaced by an alpha functionalized amino acid.

[0045] The synthetic peptides described herein can be of any length, e.g., any number of amino acids in length, e.g., about 5 amino acids to about 200 amino acids in length, about 10 amino acids to about 150 amino acids in length, about 20 amino acids to about 100 amino acids in length, about 30 amino acids to about 75 amino acids in length, or about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, or about 100 amino acids in length.

[0046] Certain lipidated synthetic peptides described herein contain one or more alternative or side chain modified amino acids substituted for native amino acids, while at least maintaining substantially the same or exhibiting increased receptor potency as a corresponding synthetic peptide that does not comprise the substitutions. Improvements in protease resistance and potency can be associated with the selective and strategic position of the lipidation and use of alternative substituted for native amino acids on the peptide. In certain embodiments, synthetic

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peptides that at least maintain substantially the same or exhibit increased receptor potency and selectivity contain two or more alternative amino acids substituted for native amino acids. In some embodiments, synthetic peptides that at least maintain substantially the same or exhibiting increased receptor potency and selectivity contain three four, five, six or more alternative amino acids substituted for the native amino acids.

[0047] The term receptor “potency” refers to the inverse of the half maximum (50%) effective concentration (EC_{50}) of the peptide. The EC_{50} refers to the concentration of peptide that induces a biological response halfway between the baseline response and maximum response, after a specified exposure time, for a selected target of the peptide. Thus, peptides exhibiting a small value for EC_{50} have a corresponding high receptor potency, while peptides exhibiting a large value for EC_{50} have a corresponding low receptor potency – the more peptide required to induce a response related to a receptor, the less potent the peptide is for that receptor.

[0048] Methods for determining the receptor potency and EC_{50} are known in the art and suitably involve determining stimulation of one or more cellular receptor responses. For example, suitable cell lines expressing GLP-1 receptor (GLP-1R), glucagon receptor (GCGR) or glucose-dependent insulintropic peptide (gastric inhibitory polypeptide) receptor (GIPR) are generated by standard methods. Peptide activation of these various receptors results in downstream production of a cAMP second messenger which can be measured in a functional activity assay. From these measurements, EC_{50} values are readily determined.

[0049] As described throughout, lipidated peptides comprising one or more, e.g., one or two, attached lipids or lipid moieties and and substitution of alternative amino acids for the native amino acids can maintain “substantially the same” or exhibit increased receptor potency as compared

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to a corresponding peptide that does not comprise the lipids or lipid moieties or the non-natural amino acids. As used herein, “substantially the same” when referring to receptor potency, means that the lipidated peptide can exhibit, e.g., at least about 75% of the receptor potency, when the lipidated peptide is compared to the receptor potency of a corresponding peptide that is unlipidated or unlipidated and having different and/or fewer amino acid modifications, or other suitable comparator sequence (e.g., a control). In further embodiments, a lipidated peptide as provided herein can exhibit, e.g., about 80% of the receptor potency, about 85% of the receptor potency, about 90% of the receptor potency, about 91% of the receptor potency, about 92% of the receptor potency, about 93% of the receptor potency, about 94% of the receptor potency, about 95% of the receptor potency, about 96% of the receptor potency, about 97% of the receptor potency, about 98% of the receptor potency, about 99% of the receptor potency, about 99.1% of the receptor potency, about 99.2% of the receptor potency, about 99.3% of the receptor potency, about 99.4% of the receptor potency, about 99.5% of the receptor potency, about 99.6% of the receptor potency, about 99.7% of the receptor potency, about 99.8% of the receptor potency, about 99.9% of the receptor potency, or about 100% of the receptor potency, when the lipidated peptide is compared to the receptor potency of a corresponding peptide that is unlipidated or unlipidated and having different and/or fewer amino acid modification, or other suitable comparator sequence (e.g., a control). As used herein, “increased” when referring to receptor potency, means that the lipidated peptide exhibits greater receptor potency than the receptor potency of a corresponding peptide that is unlipidated or unlipidated and having different and/or fewer amino acid modifications, or other suitable comparator sequence (e.g., a control). In certain embodiments, increased receptor

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potency refers to, for example, 1% greater receptor potency, 2% greater receptor potency, 3% greater receptor potency, 4% greater receptor potency, 5% greater receptor potency, 6% greater receptor potency, 7% greater receptor potency, 8% greater receptor potency, 9% greater receptor potency, 10% greater receptor potency. In certain embodiments, increased receptor potency refers to for example, 1% to 10% greater receptor potency, 1% to 20% greater receptor potency, 1% to 30% greater receptor potency, 1% to 40% greater receptor potency, 1% to 50% greater receptor potency, 5% to 10% greater receptor potency, 5% to 20% greater receptor potency, 5% to 30% greater receptor potency, 5% to 40% greater receptor potency, 5% to 50% greater receptor potency, 10 to 50% greater receptor potency, 20 to 50% greater receptor potency, 30 to 50% greater receptor potency, 40% to 50% greater receptor potency, or 50% to 100% greater receptor potency.

[0050] As described throughout, a lipidated peptide as provided herein comprising one or more, e.g., one or two, attached lipids or lipid moieties and substitution of alternative amino acids for native amino acids can also at least maintain “substantially the same selectivity” as a corresponding peptide that does not comprise the lipid or lipid moiety or alternative amino acids, or other suitable comparator sequence (e.g., a control), as described herein. As used herein, “selectivity,” refers to the ability of a peptide to bind its target (e.g., the agonist to which it is designed to bind) while not binding to other non-target proteins. A lipidated peptide as provided herein can exhibit “substantially the same selectivity” and thus exhibit, e.g., at least about 75% of the selectivity when the lipidated peptides are compared to the selectivity of peptides that do not comprise the lipid or lipid moiety, or other suitable comparator sequence (e.g., a control), as described herein. For example, a lipidated peptide as provided herein can

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exhibit about 80% of the selectivity, about 85% of the selectivity, about 90% of the selectivity, about 91% of the selectivity, about 92% of the selectivity, about 93% of the selectivity, about 94% of the selectivity, about 95% of the selectivity, about 96% of the selectivity, about 97% of the selectivity, about 98% the selectivity, about 99% of the selectivity, about 99.1% of the selectivity, about 99.2% of the selectivity, about 99.3% of the selectivity, about 99.4% of the selectivity, about 99.5% of the selectivity, about 99.6% of the selectivity, about 99.7% of the selectivity, about 99.8% of the selectivity, about 99.9% of the selectivity, or about 100% of the selectivity, when the lipidated peptide is compared to the selectivity of a corresponding peptide that does not comprise the lipid or lipid moiety, or other suitable comparator sequence (e.g., a control), as described herein. In certain embodiments, the selective and strategic incorporation of lipidation and/or alternative amino acids in GLP-1 analogues both increases GLP-1 receptor potency and accordingly, increases selectivity for this receptor.

[0051] In certain embodiments, a lipidated peptide as provided herein can also comprise one or more alternative amino acids corresponding to the substituted native amino acids in a corresponding wild-type protein. For example, the amino acid in the original, wild-type peptide sequence can be substituted with an alternative amino acid that has the same side chain, e.g., Phe, Trp, Tyr, Ser, Arg, Ala, Val, Leu, His, or Lys, can be substituted with α -MePhe, α -MeTrp, α -MeTyr, α -MeSer, α -MeArg, α -MeAla (Aib), α -MeVal, α -MeLeu, α -MeHis, or α -MeLys, respectively. In certain embodiments other amino acids can be used, for example α -cyclohexylglycine (Cha); 4-methyl-phenylalanine (4Me-Phe); norleucine (Nle); 4,4'-biphenylalanine (Bip); diphenylalanine (Dip); homophenylalanine (hPhe); phenylglycine (PhG); NMe-Phe, β,β -di-Me-Phe, α -Me-2FPhe, F5-Phe, 2Me-Phe, 4Me-Phe, 4I-Phe, 2F-Phe, 3F-Phe, 4F-Phe, homotyrosine

(hTyr), NMe-Tyr, Tyr(OMe), NMe-Trp, Nal(1), Nal(2), β,β -*di*-MeTrp, β,β -*di*-Me-Phe, or β,β -*di*-MeTyr .

[0052] In certain embodiments, an alternative amino acid in a lipidated peptide as provided herein can correspond to the same class as the substituted native amino acids. For example, aliphatic alpha-methyl functionalized amino acids can be substituted for aliphatic native amino acids; hydroxyl alpha-methyl functionalized amino acids can be substituted for hydroxyl native amino acids; sulfur-containing alpha-methyl functionalized amino acids can be substituted for sulfur-containing native amino acids; cyclic alpha-methyl functionalized amino acids can be substituted for cyclic native amino acids; aromatic alpha-methyl functionalized amino acids can be substituted for aromatic native amino acids; basic alpha-methyl functionalized amino acids can be substituted for basic native amino acids; and/or acidic alpha-methyl functionalized amino acids can be substituted for acidic native amino acids. Further, e.g., Phe, Trp, Tyr, Ser, Arg, Ala, Val, Leu, His, or Lys, can be substituted with α -MePhe, α -MeTrp, α -MeTyr, α -MeSer, α -MeArg, α -MeAla (Aib), α -MeVal, α -MeLeu, α -MeHis, or α -MeLys, respectively. In certain embodiments other amino acids can be used, for example α -cyclohexylglycine (Cha); 4-methyl-phenylalanine (4Me-Phe); norleucine (Nle); 4,4'-biphenylalanine (Bip); diphenylalanine (Dip); homophenylalanine (hPhe); phenylglycine (PhG); NMe-Phe, β,β -*di*-Me-Phe, α -Me-2FPhe, F5-Phe, 2Me-Phe, 4Me-Phe, 4I-Phe, 2F-Phe, 3F-Phe, 4F-Phe, homotyrosine (hTyr), NMe-Tyr, Tyr(OMe), NMe-Trp, Nal(1), Nal(2), β,β -*di*-MeTrp, β,β -*di*-Me-Phe, or β,β -*di*-MeTyr.

[0053] In additional embodiments, the alternative amino acid in a lipidated peptide as provided herein does not correspond to the substituted native amino acids.

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[0054] Commercial sources of alternative amino acids include, for example, Bachem AG, Switzerland.

[0055] In certain embodiments, at least one alternative amino acid in a lipidated synthetic peptide described herein is alpha-methyl alanine (Aib) or phenylalanine.

[0056] In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation.

[0057] As used herein, “proteolytic degradation” means the breakdown of peptides into smaller peptides or even amino acids, generally caused by the hydrolysis of a peptide bond by enzymes.

[0058] Lipidated peptides that are “substantially resistant” to proteolytic degradation can, for example, remain at least about 50% intact following exposure to an enzyme in conditions that the enzyme is generally active (*e.g.*, suitable pH, temperature, other environmental conditions) for a defined period of time. Lipidated peptides provided herein can be substantially resistant to proteolytic degradation for a period of at least 4 hours, at least 8 hours, at least 12 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 144 hours, at least 168 hours, at least 192 hours, at least 216 hours, at least 240 hours, or about 36 hours to about 240 hours, about 48 hours to 240 hours, about 72 hours to about 240 hours, about 96 hours to about 240 hours, about 120 hours to about 240 hours, about 144 hours to about 240 hours, about 168 hours to about 240 hours, about 192 hours to about 240 hours, or about 216 hours to about 240 hours. In certain embodiments, at least about 60% of the lipidated peptide remains intact following exposure to an enzyme in conditions that the enzyme is generally active for a defined period of time, for example, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%,

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at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8%, at least about 99.9%, or at least about 100% of the lipidated peptide remains intact following exposure to an enzyme in conditions that the enzyme is generally active for a defined period of time.

[0059] Lipidated peptides provided herein can be substantially resistant to proteolytic degradation by one or more enzymes found in a mammalian body, e.g., the human body. For example, the lipidated peptides can be substantially resistant to proteolytic degradation by one or more of dipeptidyl peptidase-IV (DPP-IV), neprilysin, α -chymotrypsin, plasmin, thrombin, kallikrein, trypsin, elastase and pepsin. In certain embodiments, the lipidated peptides can be resistant to proteolytic degradation by two or more, three or more, four or more, five or more, six or more, seven or more, or suitably all of the recited enzymes. The lipidated peptides described herein can also be substantially resistant to proteolytic degradation by other enzymes known in the art. In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation by digestive (gastric) enzymes and/or enzymes in the blood/serum.

[0060] In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation by DPP-IV and neprilysin. In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation by pepsin, trypsin, α -chymotrypsin, and elastase. In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation by plasmin, thrombin, and kallikrein. In certain embodiments, the lipidated peptides described herein can be

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substantially resistant to proteolytic degradation by pepsin, trypsin and α -chymotrypsin. In certain embodiments, the lipidated peptides described herein can be substantially resistant to proteolytic degradation by pepsin and trypsin, etc.

[0061] As described herein, including in various embodiments provided throughout, lipidation of amino acid residues and/or substitution of alternative amino acids for native amino acids can occur at native amino acid residues that are sites susceptible to proteolytic cleavage. That is, the amino acid residues that are substituted are determined to be sites where proteolytic enzymes are active in cleaving peptide bonds in the native peptides. Methods for determining sites of proteolytic cleavage are well known in the art and described herein.

[0062] Any class of peptide can be prepared according to the methods provided herein to yield lipidated peptides having the recited characteristics.

[0063] In exemplary embodiments, the lipidated peptides can be incretin class peptides. Exemplary synthetic incretin class peptides that can be prepared as described herein include, but are not limited to, glucagon-like peptide 1 (GLP-1), a glucose-dependent insulintropic peptide (GIP), an exenatide peptide, plus glucagon, secretins, tenomodulin, oxyntomodulin, insulin, or vasoactive intestinal peptide (VIP).

[0064] Additional classes of peptides can be prepared as described herein.

[0065] In certain embodiments, the lipidated peptide described herein is derived from the sequence of GLP-1 and referred to herein as a lipidated GLP-1 peptide analog.

[0066] Sequences for the native (*wild type*) peptides of the various peptides and classes of peptides described herein that can be prepared to yield synthetic peptides having the recited characteristics are well known in the art.

[0067] The native amino acid sequence for GLP-1 (7-36) is known in the art as set forth below:

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR (SEQ ID NO: 1).

[0068] In certain embodiments, the modified GLP-1 agonist is extended by one or more amino acids.

Representative examples of these embodiments are shown in TABLE 1.

TABLE 1: Extended GLP-1 Agonists

SEQ ID NO:	Peptide
205	X ⁰ -HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
206	G HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
207	A HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
208	V HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
209	L HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
210	I HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
211	M HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
212	E HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
213	D HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
214	N HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
215	Q HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
216	R HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
217	H HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
218	K HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
219	S HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
220	T HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
221	P HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
222	F HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
223	Y HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG
224	W HAEGT FTSDV SSYLE GQAAK EFIAW LVKGRG

[0069]

TABLE 2: GLP1 Agonists with Modified Peptide Sequences

SEQ ID NO	Peptide
1	HAEGT ⁵ FTSDV ¹⁰ SSYLE ¹⁵ QQA ²⁰ EFIAW ²⁵ LVKGR ³⁰ [GLP-1 (7-36)]
2	X ⁰ -H-X ² -X ³ -GT-FTSD-X ¹⁰ -S-X ¹² -X ¹³ -X ¹⁴ -X ¹⁵ -X ¹⁶ -X ¹⁷ -AA-X ²⁰ -X ²¹ -X ²² -I-X ²⁴ -X ²⁵ -X ²⁶ -X ²⁷ -X ²⁸ -X ²⁹ -X ³⁰ -X ³¹ -X ³²
3	F HAEGT FTSDK ((PEG) 2- (PEG) 2-stea ^r) SSYLE QQA ^k EFIAW LVKGRG
4	F HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVKGRG
5	F HAEGT FTSDK (γE-palm) SSYLE GEA ^k EFIAW LVKGRG
6	F HAEGT FTSDK ((PEG) 2- (PEG) 2-stea ^r) SSYLE GEA ^k EFIAW LVKGRG
7	F HAEGT FTSDK ((PEG) 2- (PEG) 2-γE-palm) SSYLE GEA ^k EFIAW LVKGRG
8	F HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVEGRG
9	F HAEGT FTSDK ((PEG) 2- (PEG) 2-γE-stea ^r) SSYLE GEA ^k EFIAW LVKGRG
10	F HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVEGEG
11	I HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVKGRG
12	I HAEGT FTSDK (γE-palm) SSYLE GEA ^k EFIAW LVKGRG
13	I HAEGT FTSDK ((PEG) 2- (PEG) 2-stea ^r) SSYLE GEA ^k EFIAW LVKGRG
14	T HAEGT FTSDK (γE-palm) SSYLE GEA ^k EFIAW LVKGRG
15	E HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVEGR G
16	A HAEGT FTSDK (γE-palm) SSYLE QQA ^k EFIAW LVEGRG
17	A HAEGT FTSDK (γE-palm) SSYLE QQA ^k E (Cha) IAW LVEGRG
18	A HAEGT FTSDK ((PEG) 2- (PEG) 2-stea ^r) SSYLE QQA ^k E (Cha) IAW LVEGRG
19	A HAEGT FTSDK (γE-γE-stea ^r) SSYLE QQA ^k E (Cha) IAW LVEGRG
20	A HAEGT FTSDK (γE-γE-stea ^r) SSYLE QQA ^k E (Cha) IA (Cha) LVEGRG
21	A HAEGT FTSDK (γE-palm) SSYLE QQA ^k E (Cha) IAW LVKGRG
22	A HAEGT FTSDK (γE-γE-stea ^r) SSYLE QQA ^k E (4Me-Phe) IAW LVEGRG
23	A HAEGT FTSDK ((PEG) 2- (PEG) 2-stea ^r) SSYLE QQA ^k E (4Me-Phe) IAW LVKGRG
24	F HAEGT FTSDV SK ((PEG) 2- (PEG) 2-stea ^r) YLE QQA ^k EFIAW LVKGRG
25	F HAEGT FTSDV SK (γE-palm) YLE QQA ^k EFIAW LVKGRG
26	F HAEGT FTSDV SK (γE-palm) YLE GEA ^k EFIAW LVKGRG
27	F HAEGT FTSDV SK ((PEG) 2- (PEG) 2-stea ^r) YLE GEA ^k EFIAW LVKGRG
28	I HAEGT FTSDV SK (γE-palm) YLE GEA ^k EFIAW LVKGRG
29	T HAEGT FTSDV SK (γE-palm) YLE GEA ^k EFIAW LVKGRG

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30	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-stearyl)LE	GQAAK	EFIAW	LVKGRG
31	F	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	EFIAW	LVKGRG
32	F	HAEGT	FTSDV	SSK(γE-palm)LE	GEAAK	EFIAW	LVEGRG
33	F	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	EFIAW	LVEGRG
34	F	HAEGT	FTSDV	SSK(γE-γE-palm)LE	GQAAK	EFIAW	LVEGRG
35	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-γE-γE-stearyl)LE	GQAAK	EFIAW	LVEGRG
36	F	HAEGT	FTSDV	SSK(γE-γE-stearyl)LE	GQAAK	EFIAW	LVEGRG
37	F	HAEGT	FTSDV	SSK((PEG)4-γE-stearyl)LE	GQAAK	EFIAW	LVEGRG
38	F	HAEGT	FTSDV	SSK((PEG)4-γE-palm)LE	GQAAK	EFIAW	LVEGRG
39	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-stearyl)LE	GQAAK	EFIAW	LVKGEG
40	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-stearyl)LE	GEAAK	EFIAW	LVKGEG
41	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-γE-stearyl)LE	GEAAK	EFIAW	LVEGRG
42	F	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-γE-stearyl)LE	GEAAK	EFIAW	LVEGEG
43	F	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	EFIAW	LVKGE G
44	L	HAEGT	FTSDV	SSK(γE-palm)LE	GEAAK	EFIAW	LVEGRG
45	T	HAEGT	FTSDV	SSK(γE-palm)LE	GEAAK	EFIAW	LVEGRG
46	A	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	EFIAW	LVEGRG
47	A	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	E(Cha)	IAW LVEGRG
48	A	HAEGT	FTSDV	SSK((PEG)2-(PEG)2-stearyl)LE	GQAAK	E(Cha)	IAW LVEGEG
49	A	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	E(Cha)	IAW LVKGEG
50	A	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	E(4Me-Phe)	IAW LVEGRG
51	E	HAEGT	FTSDV	SSK(γE-palm)LE	GQAAK	EFIAW	LVEGRG
52	F	HAEGT	FTSDV	SSYK(γE-stearyl)E	GQAAK	EFIAW	LVKGRG
53	F	HAEGT	FTSDV	SSYK((PEG)2-(PEG)2-stearyl)E	GQAAK	EFIAW	LVKGEG
54	F	HAEGT	FTSDV	SSYK((PEG)2-(PEG)2-stearyl)E	GEAAK	EFIAW	LVKGEG
55	F	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAK	EFIAW	LVKGRG
56	F	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAE	EFIAW	LVEGEG
57	F	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAK	EFIAW	LVEGEG
58	L	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAE	EFIAW	LVEGEG
59	L	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAK	EFIAW	LVEGEG
60	T	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAE	EFIAW	LVEGEG
61	A	HAEGT	FTSDV	SSYK(γE-palm)E	GQAAK	E(Cha)	IAW LVEGRG

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62	A	HAEGT	FTSDV	SSYK	(γ E-palm)E	GQAAK	E(Cha)	IAW	LVKGEG
63	F	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	EFIAW	LVKGRG	
64	F	HAEGT	FTSDV	SSYLE	GK((PEG)2-(PEG)2-stea	r)AAK	EFIAW	LVKGRG	
65	F	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAE	EFIAW	LVEGEG	
66	F	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	EFIAW	LVEGEG	
67	F	HAEGT	FTSDV	SSYLE	GK((PEG)2-(PEG)2- γ E-stea	r)AAK	EFIAW	LVEGEG	
68	F	HAEGT	FTSDV	SSYLE	GK((PEG)4- γ E-palm)	AAK	EFIAW	LVEGEG	
69	F	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	E(4Me-Phe)	IAW	LVEGRG
70	I	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	EFIAW	LVKGRG	
71	I	HAEGT	FTSDV	SSYLEGK	(γ E- γ E-stea	r)AAK	EFIAW	LVEGEG	
72	I	HAEGT	FTSDV	SSYLE	GK((PEG)4- γ E-stea	r)AAK	EFIAW	LVKGRG	
73	L	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAE	EFIAW	LVEGRG	
74	T	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAE	EFIAW	LVEGRG	
75	E	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAE	EFIAW	LVEGRG	
76	E	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAR	EFIAW	LVEGRG	
77	E	HAEGT	FTSDV	SSYLE	GK((PEG)4- γ E-stea	r)AAR	EFIAW	LVEGRG	
78	E	HAEGT	FTSDV	SSYLE	GK((PEG)2-(PEG)2-palm)	AAR	EFIAW	LVEGRG	
79	E	HAEGT	FTSDV	SSYLE	GK(γ E- γ E-stea	r)AAR	EFIAW	LVEGRG	
80	E	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	E(4Me-Phe)	IAW	LVEGRG
81	A	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	EFIAW	LVEGEG	
82	A	HAEGT	FTSDV	SSYLE	GK((PEG)2-(PEG)2-stea	r)AAK	EFIAW	LVEGEG	
83	A	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	E(Cha)	IAW	LVEGRG
84	A	HAEGT	FTSDV	SSYLE	GK((PEG)2-(PEG)2-stea	r)AAK	E(Cha)	IAW	LVEGRG
85	A	HAEGT	FTSDV	SSYLE	GK(γ E-palm)	AAK	E(Cha)	IAW	LVKGEG
86	F	HAEGT	FTSDV	SSYLE	GQAA	K((PEG)2-(PEG)2-stea	r)	EFIAW	LVKGRG
87	F	HAEGT	FTSDV	SSYLE	GQAA	K(γ E-palm)	EFIAW	LVKGRG	
88	I	HAEGT	FTSDV	SSYLE	GQAA	K(γ E-palm)	EFIAW	LVKGRG	
89	E	HAEGT	FTSDV	SSYLE	GQAA	K(γ E-palm)	EFIAW	LVRGRG	
90	E	HAIGT	FTSDV	SSYLE	GQAA	K(γ E-palm)	EFIAW	LVEGRG	
91	F	HAEGT	FTSDV	SSYLE	GQAAK	K((PEG)2-(PEG)2-stea	r)	FIAM	LVKGRG
92	F	HAEGT	FTSDV	SSYLE	GQAAK	K(γ E-palm)	FIAM	LVKGRG	
93	F	HAEGT	FTSDV	SSYLE	GQAAR	K(γ E-palm)	FIAM	LVRGRG	

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94	F	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVEGRG
95	F	HAEGT	FTSDV	SSYLE	GEAAK	K	((PEG) 2- (PEG) 2-stea	r) FIAW	LVKGEG
96	F	HAEGT	FTSDV	SSYLE	GQAAE	K	(YE-palm)	FIAM	LVEGRG
97	F	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVKGEG
98	I	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVKGRG
99	I	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVKGRG
100	I	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVEGRG
101	A	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVKGRG
102	A	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-YE-palm)	FIAM	LVKGRG
103	A	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-YE-stea	r) FIAW	LVKGRG
104	A	HAEGT	FTSDV	SSYLE	GQAAK	K	((PEG) 4-YE-stea	r) FIAW	LVKGRG
105	A	HAEGT	FTSDV	SSYLE	GQAAK	K	((PEG) 4-YE-palm)	FIAM	LVKGRG
106	A	HAEGT	FTSDV	SSYLE	GQAAK	K	((PEG) 2- (PEG) 2-stea	r) FIAW	VKGRG
107	A	HAEGT	FTSDV	SSYLE	GQAAK	K	((PEG) 2- (PEG) 2-YE-YE-stea	r) FIAW	LVKGRG
108	A	HAEGT	FTSDV	SSYLE	GQAAK	K	((PEG) 2- (PEG) 2-stea	r) FIAW	LVEGEG
109	A	HAEGT	FTSDV	SSYLE	GQAAK	K	(YE-palm)	FIAM	LVEGEG
110	F	HAEGT	FTSDV	SSYLE	GQAAK	EK	((PEG) 2- (PEG) 2-stea	r) IAW	LVKGRG
111	F	HAEGT	FTSDV	SSYLE	GQAAK	EK	(YE-palm)	IAW	LVKGRG
112	F	HAEGT	FTSDV	SSYLE	GQAAK	EFI	K ((PEG) 2- (PEG) 2-stea	r) W	LVKGRG
113	F	HAEGT	FTSDV	SSYLE	GQAAK	EFI	K (YE-palm)	W	LVKGRG
114	F	HAEGT	FTSDV	SSYLE	GQAAE	EFIK	(YE-palm)	W	LVEGRG
115	F	HAEGT	FTSDV	SSYLE	GEAAE	EFIK	(YE-palm)	W	LVEGRG
116	F	HAEGT	FTSDV	SSYLE	GQAAK	EFI	K ((PEG) 2- (PEG) 2-stea	r) W	LVKGEG
117	I	HAEGT	FTSDV	SSYLE	GQAAK	EFI	K (YE-palm)	W	LVKGRG
118	L	HAEGT	FTSDV	SSYLE	GQAAE	EFIK	(YE-palm)	W	LVEGRG
119	T	HAEGT	FTSDV	SSYLE	GQAAE	EFK	(YE-palm)	W	LVEGRG
120	E	HAEGT	FTSDV	SSYLE	GQAAE	EFI	K (YE-palm)	W	LVEGRG
121	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIA	K ((PEG) 2- (PEG) 2-stea	r) LVKGRG	
122	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIA	K (YE-palm)	LVKGRG	
123	F	HAEGT	FTSDV	SSYLE	GQAAE	EFIA	K (YE-palm)	LVRGRG	
124	A	HAEGT	FTSDV	SSYLE	GQAAK	EFIA	K ((PEG) 2- (PEG) 2-stea	r) LVEGRG	
125	A	HAEGT	FTSDV	SSYLE	GQAAK	E	(Cha) IAK (YE-palm)	LVKGEG	

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126	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	K((PEG)2-(PEG)2-stea	r)	LVKGRG				
127	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	K(γE-palm)	LVKGRG					
128	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	LK(γE-palm)	KGRG					
129	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	LVK(γE-palm)	GRG					
130	A	HAEGT	FTSDV	SSYLE	GQAAK	E(Cha)	IAW	LVKGE	K(γE-palm)				
131	F	HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	LVKGR	G	K(γE-palm)				
132	A	HAEGT	FTSDV	SSYLE	GQAAK	E(Cha)	IAW	LVEGR	GK(γE-palm)				
133	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(Cha)	IAW	LVKGRG				
134	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GEAAK	E(Cha)	IAW	LVKGRG				
135	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GEAAK	E(Cha)	IAW	LVEGRG				
136	H	Aib	EGT	FTSDK((PEG)2-(PEG)2-γE-γE-stea	r)	SSYLE	GEAAK	E(Cha)	IAW	LVEGEG			
137	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GEAAK	E(Cha)	IAW	LVEGG				
138	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GEAAK	E(Cha)	IA	(Cha)	LVEGE			
139	H	Aib	EGT	FTSDK((PEG)2-(PEG)2-γE-palm)	SSYLE	GQAAK	E(Cha)	IAW	LVEGG				
140	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(Bip)	IAW	LVKGRG				
141	H	Aib	EGT	FTSD	K(γE-palm)	SSYLE	GQAAK	E(Dip)	IAW	LVKGRG			
142	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(4MePhe)	IAW	LVKGRG				
143	H	Aib	EGT	FTSD	K(γE-γE-stea	r)	SSYLE	GQAAK	E(4MePhe)	IAW	LVKGRG		
144	H	Aib	EGT	FTSD	K((PEG)4-γE-stea	r)	SSYLE	GQAAK	E(4MePhe)	IAW	LVKGRG		
145	H	Aib	EGT	FTSD	K((PEG)2-(PEG)2-stea	r)	SSYLE	GQAAK	E(4MePhe)	IAW	LVKGE		
146	H	Aib	EGT	FTSD	K((PEG)2-(PEG)2-stea	r)	SSYLE	GEAAK	E(4MePhe)	IAW	LVKGE		
147	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(2MePhe)	IAW	LVKGRG				
148	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(2CF3-Phe)	IAW	LVKGRG				
149	H	Aib	EGT	FTSDK(γE-palm)	SSYLE	GQAAK	E(4CF3-Phe)	IAW	LVKGRG				
150	H	Aib	EGT	FTSDV	SK(γE-palm)	YLE	GQAAK	E(Cha)	IAW	LVKGRG			
151	H	Aib	EGT	FTSDV	SS	K((PEG)2-(PEG)2-stea	r)	LE	GQAAK	EFIAW	LVKGRG		
152	H	Aib	EGT	FTSDV	SS	K(γE-palm)	LE	GQAAK	EFIAW	LVKGRG			
153	H	Aib	EGT	FTSDV	SS	K((PEG)2-(PEG)2-stea	r)	LE	GQAAK	E(4Me	Phe)	IAW	LVKGE
154	H	Aib	EGT	FTSDV	SSK(γE-palm)	LE	GQAAK	E(Cha)	IAW	LVKGRG			
155	H	Aib	EGT	FTSDV	SSK(γE-palm)	LE	GEAAK	E(Cha)	IAW	LVKGRG			
156	H	Aib	EGT	FTSDV	SSK(γE-palm)	LE	GEAAK	E(Cha)	IAW	LVEGRG			
157	H	Aib	EGT	FTSDV	SSK(γE-palm)	LE	GEAAK	E(Cha)	IAW	(Cha)	LVEGE		

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158	H Aib	EGT	FTSDV	SSK((PEG)2-(γ E-palm)LE	GQAAK E(Cha)IA(Cha) LVEGE
159	H Aib	EGT	FTSDV	SSK((PEG)2-(γ E-palm)LE	GQAAK E(Cha)IAW LVEGE
160	H Aib	EGT	FTSDV	SSK((PEG)2-(γ E-palm)LE	GQAAK E(Cha)IAW LVEGG
161	H Aib	EGT	FTSDV	SSYK(γ E-palm)	EGQAAK E(Cha)IAW LVKGRG
162	H Aib	EGT	FTSDV	SSYL K(γ E-palm)	GQAAK E(Cha)IAW LVKGRG
163	H Aib	EGT	FTSDV	SSYLE K(γ E-palm)QAAK E(Cha)IAW LVKGRG	
164	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Nle)IAW LVKGRG	
165	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Cha)IAW LVKGRG	
166	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Cha)IEW LVKGRG	
167	H Aib	EGT	FTSDV	SSYLE GAAK E(Nle)IAW LVKGRG	
168	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Cha)IAW LVEGREG	
169	H Aib	EGT	FTSDV	SSYLE GK((PEG)2-(γ E- γ E-stearyl)AAK E(Cha)IAW LVEGEG	
170	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Cha)IA(Cha) LVEGE	
171	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(Cha)IAW LVEGREG	
172	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK EFIA(Cha) LVKGRG	
173	H Aib	EGT	FTSDV	SSYLE GK(γ E-palm)AAK E(4Me-Phe)IAW LVKGRG	
174	H Aib	EGT	FTSDV	SSYLE GQAAK(γ E-palm) E(Cha)IAW LVKGRG	
175	H Aib	EGT	FTSDV	SSYLE GQAA K(γ E-palm) E(Nle)IAW LVK(Aib)R	
176	H Aib	EGT	FTSDV	SSYLE GQAAK K((PEG)2-(γ E- γ E-stearyl)FIW LVKGRG	
177	H Aib	EGT	FTSDV	SSYLE GQAAK K((PEG)2-(γ E- γ E-stearyl)FIW LVKGRG	
178	H Aib	EGT	FTSDV	SSYLE GEAAK K((PEG)2-(γ E- γ E-stearyl)FIW LVEGEG	
179	H Aib	EGT	FTSDV	SSYLE GQAAK K(γ E-palm)FIW LVKGRG	
180	H Aib	EGT	FTSDV	SSYLE GQAAK EFIA K((PEG)2-(γ E- γ E-stearyl) LVKGRG	
181	H Aib	EGT	FTSDV	SSYLE GQAAK EFIA K((PEG)2-(γ E- γ E-stearyl) LVKGRG	
182	H Aib	EGT	FTSDV	SSYLE GQAAK EFIA K(γ E-palm) LVKGRG	
183	H Aib	EGT	FTSDV	SSYLE GQAAK EFIAW K((PEG)2-(γ E- γ E-stearyl)VKGRG	
184	H Aib	EGT	FTSDV	SSYLE GQAAK EFIAW K(γ E-palm)VKGR G	
185	H Aib	EGT	FTSDV	SSYLE GQAAK EFIAW LK((PEG)2-(γ E- γ E-stearyl)KGRG	
186	H Aib	EGT	FTSDV	SSYLE GQAAK EFIAW LK(γ E-palm)KGRG	
187	H Aib	EGT	FTSDV	SSYLE GQAAK E(Cha)IAW LVKGRK(γ E-palm)	

Table 3— Modified GLP1 Agonists

SEQ ID NO	Peptide
204	X ⁰ -HGEGT-FTSD-X ¹⁰ -S-X ¹² -Q-X ¹⁴ -EE-X ¹⁷ -AV-X ²⁰ -L-X ²² -I-X ²⁴ -WLKNGGPSSGAPP-X ³⁹ -X ⁴⁰
188	F HGEGT FTSDL SKQME EEAVR LFIEW LKNGG PSSGA PPPS
189	F HGEGT FTSDL SK(γE-palm)QME EEAVR LFIEW LKNGG PSSGA PPPS
190	F HGEGT FTSDL SKQK((PEG)2-(PEG)2-γE-palm)E EEAVR L(Cha)IEW LKNGG PSSGA PPPS
191	F HGEGT FTSDL SKQME EEAVK(γE-palm) LFIEW LKNGG PSSGA PPPS
192	F HGEGT FTSDL SKQME EEAVR LFIK((PEG)2-(PEG)2-γE-palm)W LKNGG PSSGA PPPS
193	F HGEGT FTSDL SKQME EEAVR LFIEK(γE-palm)W LKNGG PSSGA PPPS
194	F HGEGT FTSDL SKQME EEAVR LFIEW LKNGG PSSGA PPPSK(γE-palm)
195	F HGEGT FTSDL SKQME EEAVR L(Cha)IEW LKNGG PSSGA PPPSK((PEG)2-(PEG)2-γE-palm)
196	A HGEGT FTSDL SKQME EEAVR L(Cha)IEW LKNGG PSSGA PPPSK((PEG)2-(PEG)2-γE-palm)
197	HGEGT FTSDK(γE-palm) SKQME EEAVR L(Cha)IEW LKNGG PSSGA PPPS
198	HGEGT FTSDK((PEG)2-(PEG)2-γE-γE-palm) SKQME EEAVR LPhe(4Me)IEW LKNGG PSSGA PPPS
199	HGEGT FTSDK((PEG)2-(PEG)2-γE-γE-stear) SKQME EEAVR LPhe(4Me)IEW LKNGG PSSGA PPPS
200	HGEGT FTSDK((PEG)2-(PEG)2-γE-γE-palm) SKQME EEAVR LFIEW LKNGG PSSGA PPPS
201	HGEGT FTSDK((PEG)2-(PEG)2-γE-γE-stear) SKQME EEAVR LFIEW LKNGG PSSGA PPPS
202	HGEGT FTSDL SKQME EK(γE-palm)AVR L(Cha)IEW LKNGG PSSGA PPPS
203	HGEGT FTSDL SKQME EEAVR L(Cha)IEW LKNGG PSSGA PPPK(γE-palm)

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[0070] Note that in Tables 2 and 3 above stear is the abbreviation for stearoyl and palm is the abbreviation for palmitoyl.

[0071] The *C*-terminus of a peptide is generally either a free carboxylic acid or an amide. Thus, in certain aspects, any one of the peptides in Tables 1 and 2 3 can either have a *C*-terminal acid or a *C*-terminal amide. In certain aspects, any one of the peptides in Tables 1 and 2 comprises a *C*-terminal acid. In certain aspects, any one of the peptides in Tables 1 and 2 comprises a *C*-terminal amide.

[0072] Linkers used in various polypeptides provided herein can facilitate formation of a structure. In some aspects, a polypeptide linker can comprise 1-50 amino acids, 1-25 amino acids, 25-50 amino acids, or 30-50 amino acids. Generally longer linkers correlate with higher activity (more flexible), but also decreased stability as the peptide becomes more exposed. Linkers can comprise, e.g., (Gly-Ser)*n*, residues, where *n* is an integer of at least one, and up to, e.g., 4, 5, 6, 10, 20, 50, 100, or more, optionally with some Glu or Lys residues dispersed throughout to increase solubility. Alternatively, certain linkers do not comprise any Serine residues, e.g., where the linker is subject to O-linked glycosylation. In some aspects, linkers can contain cysteine residues, for example, if dimerization of linkers is used to bring two or more agonist polypeptides into a dimeric configuration. In some aspects, an agonist polypeptide can comprise at least one, two, three, four, or more linkers. The length and amino acid sequence of a linker can be readily selected and optimized.

Methods of Preparing Lipidated Peptides

- [0073] While various methods of attaching lipids and lipid moieties to peptides are known, provided herein is at least one representative method of preparing lipidated peptides.
- [0074] In certain embodiments, lipidated peptides can be prepared as C-terminal carboxamides, such as on NovaSyn[®] TGR resin. In certain embodiments, amino acids (both natural and unnatural) can be coupled at ambient temperature, such as by using HCTU/DIPEA in NMP, capping residual functionality with a solution of acetic anhydride and pyridine. In such methods, the N-Fmoc group can be deblocked using piperidine in DMF (20% v/v) at ambient temperature and the C-terminal residue incorporated as the N-Boc-protected form, e.g. Boc-His(Trt)-OH or Boc-Tyr(tBu)-OH or equivalent. At the position(s) of lipidation Fmoc-Lys(Mmt)-OH can be incorporated into the peptide backbone during automated assembly and upon completion the Mmt protecting group(s) can be removed manually and selectively by treatment of the synthesis resin with 1% TFA, 2% TIPS, DCM (10 x 1 minute, 20.0 mL/g). The acidified resin can be quenched, such as with 5% DIPEA/NMP, and the exposed lysine amino-function(s) acylated, PEGylated or lipidated as required prior to peptide cleavage.
- [0075] Crude peptides can be cleaved from the resin support by treatment with a suitable cleavage cocktail. In certain embodiments the cocktail consists of TFA (95% v/v), TIPS (2.5% v/v), and water (2.5% v/v) with agitation (3 x 1 hour at ambient temperature). Cleavage aliquots can be combined, concentrated by rotary evaporation and precipitated by addition of cold diethyl ether, isolating the solids by centrifugation. The crude peptides can be dried under a flow of dry nitrogen, reconstituted in a suitable aqueous buffer and filtered prior to chromatographic purification.

[0076] Crude mono-lipidated peptides can be dissolved in a solution of acetic acid/acetonitrile/water (1:5:50 v/v) and filtered. The crude filtrates can be chromatographed, such as over an Agilent Polaris C8-A stationary phase (21.2 x 250 mm, 5 micron) eluting with a linear solvent gradient of 10-70%, 15-80% or 20-90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 30 minutes using a Varian SD-1 PrepStar binary pump system, monitoring by UV absorption at 210 nm. The peptide-containing fractions can then be pooled, frozen (dry-ice/acetone) and lyophilized.

[0077] Crude lipidated peptides can be dissolved, such as in 0.1M ammonium bicarbonate solution (1:5 acetonitrile/water v/v, pH 8.0) and filtered. The crude filtrates can be chromatographed, such as over a Waters X-Bridge C18 stationary phase (19.0 x 250 mm, 5 micron) eluting with a linear solvent gradient of 20-90% B against A over 30 minutes using a Varian SD-1 PrepStar binary pump system, monitoring by UV absorption at 210 nm. (A = 0.1M ammonium bicarbonate in water, B = 0.1M ammonium bicarbonate in 1:2 water/acetonitrile). The peptide-containing fractions can then be pooled, frozen (dry-ice/acetone) and lyophilized.

The peptide sequence can be a GLP-1 analog sequence such as those disclosed in Tables 2 or

3. The lipid or lipid moiety can be any such as disclosed herein, including but not limited to:

K(ϵ -(PEG)₂-(PEG)₂- γ E-Stearate); K(ϵ - γ E-Palmitoyl); K(ϵ -(PEG)₂-(PEG)₂- γ E-Stearate);
 K(ϵ -(PEG)₂-(PEG)₂- γ E-Palmitoyl); K(ϵ -(PEG)₂-(PEG)₂-Palmitoyl); K(ϵ -(PEG)₄- γ E-Palmitoyl);
 K(ϵ -(PEG)₂-(PEG)₂-(PEG)₂-Stearoyl); K(ϵ - γ E-Lauroyl); K(ϵ - γ E- γ E-Lauroyl);
 K(ϵ - γ E- γ E- γ E-Lauroyl); K(ϵ -Ahx-Lauroyl); K(ϵ -Ahx-Ahx-Lauroyl); K(ϵ -Ahx-Ahx-Ahx-Lauroyl);
 K(ϵ -(PEG)₂-Lauroyl); K(ϵ -(PEG)₂-(PEG)₂-Lauroyl); K(ϵ -(PEG)₂-(PEG)₂-(PEG)₂-Lauroyl);
 K(ϵ - γ E-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ - γ E- γ E-12-(4-

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carboxyphenoxy)dodecanoyl); K(ϵ - γ E- γ E- γ E-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -Ahx-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -Ahx-Ahx-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -Ahx-Ahx-Ahx-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -(PEG)₂-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -(PEG)₂-(PEG)₂-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ -(PEG)₂-(PEG)₂-(PEG)₂-12-(4-carboxyphenoxy)dodecanoyl); K(ϵ - γ E-Stearoyl); K(ϵ -(PEG)₂-(PEG)₂- γ E- γ E-Stearoyl); K(ϵ -(PEG)₂-(PEG)₂- γ E-Stearoyl); K(ϵ - γ E- γ E- γ E-Stearoyl); K(ϵ - γ E- γ E-Stearoyl); K(ϵ -Ahx-Stearoyl); K(ϵ -Ahx-Ahx-Stearoyl); K(ϵ -Ahx-Ahx-Ahx-Stearoyl); K(ϵ -(PEG)₂-Stearoyl); K(ϵ -(PEG)₄-Stearoyl); K(ϵ -(PEG)₂-(PEG)₂-Stearoyl); K(ϵ -(PEG)₂-(PEG)₂-(PEG)₂-Stearoyl); K(ϵ - γ E-Stearate); K(ϵ - γ E- γ E-Stearate); K(ϵ - γ E- γ E- γ E-Stearate); K(ϵ -Ahx-Stearate); K(ϵ -Ahx-Ahx-Stearate); K(ϵ -Ahx-Ahx-Ahx-Stearate); K(ϵ -(PEG)₂-Stearate); K(ϵ -(PEG)₂-(PEG)₂-Stearate); K(ϵ -(PEG)₂-(PEG)₂-(PEG)₂-Stearate), palmitoyl, stearoyl, myristoyl, lauroyl, palmitate, stearate and any combination thereof. In addition, the peptides of Tables 2 and 3 can be fused or conjugated to proteins at the positions of lipidation. For example, the peptides can be fused to an Fc region of an antibody, an antibody, and scfv portion of an antibody, or to proteins like albumin.

Methods of Preparing Synthetic Peptides

[0078] Also provided are methods of preparing synthetic peptides.

[0079] In some embodiments, the methods suitably comprise identifying at least one native amino acid residue in the peptide for substitution. In other embodiments, the methods suitably comprise identifying at least two native amino acid residues in the peptide for substitution. Alternative amino acids can then substituted for the identified native amino acid residues.

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- [0080] As described throughout, the synthetic peptides prepared by the methods provided herein suitably maintain substantially the same or exhibit increased receptor potency and in some cases selectivity as a corresponding synthetic peptide that does not comprise the substitutions. In addition, the synthetic peptides prepared according to the methods described herein can also be substantially resistant to proteolytic degradation.
- [0081] Suitably in the methods provided herein the substituted alternative amino acids correspond to the substituted native amino acid residues, and in additional embodiments, the substituted alternative amino acids correspond to the same class as the substituted native amino acid residues.
- [0082] In certain embodiments, the synthetic peptides prepared according to the methods described herein can be substantially resistant to one or more of DPP-IV, neprilysin, α -chymotrypsin, plasmin, thrombin, kallikrein, trypsin, elastase and pepsin degradation.
- [0083] In embodiments, synthetic peptides can be prepared as C-terminal carboxamides on NOVASYN[®] TGR resin. Amino acids (both natural and unnatural) can be coupled at ambient temperature using HCTU/DIPEA in NMP, capping residual functionality with a solution of acetic anhydride and pyridine. Fmoc is suitably deblocked in using piperidine in DMF at ambient temperature.
- [0084] As described herein, identifying at least one native amino acid residue in the peptide for substitution suitably comprises identifying amino acids at sites susceptible to enzymatic cleavage. Exemplary methods of identifying amino acids at sites susceptible to enzymatic cleavage are well known in the art. In certain embodiments, methods of identifying amino acids at sites susceptible to enzymatic cleavage suitably comprise exposing a natural peptide

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(e.g., a wild-type peptide) to a single enzyme under conditions in which the enzyme is active (e.g., suitable pH, buffer conditions, temperature, etc.) for a pre-determined amount of time and measuring the enzymatic degradation products of the peptide. Exemplary methods for measuring the enzymatic degradation products include, for example, reverse-phase liquid chromatography-mass spectrometry.

[0085] Peptide solutions can be added to solutions of a protease. The peptide and enzyme can be co-incubated, suitably at about 37°C. Aliquots of the incubated peptide-enzyme mixture can be withdrawn periodically, quenched to arrest proteolytic activity, and analyzed by liquid chromatography-mass spectrometry (LC/MS). Analytes can be detected by both UV absorption (e.g., at 210 nm) and by ionization using a mass detector (ESI+ mode). Peptidic species (fragments) deriving from enzymatic cleavage of peptides can be analyzed post-process, and their molecular masses can be used to identify the precise cleavage position (highlighting the scissile bond in each case).

[0086] In certain embodiments, the methods described herein can be used to prepare any class of peptide having the recited characteristics.

[0087] In certain embodiments, the methods can be used to prepare incretin class peptides. Synthetic incretin class peptides that can be prepared as described herein include, but are not limited to, glucagon-like peptide 1 (GLP-1), a glucose-dependent insulintropic peptide (GIP), an exenatide peptide, plus glucagon, secretins, tenomodulin and oxyntomodulin.

[0088] Additional classes of peptides can be prepared as described herein.

[0089] In embodiments, the methods can be used to prepare synthetic GLP-1 peptides. In further embodiments, the methods can be used to prepare synthetic insulin.

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[0090] In further embodiments, methods of preparing a proteolytically stable peptide are provided.

Suitably, such methods comprise exposing a peptide to one or more proteases, identifying at least two native amino acid residues which are sites susceptible to proteolytic cleavage, and substituting alternative amino acids for the identified amino acid residues.

[0091] As described throughout, suitably such methods provide a synthetic peptide that maintains substantially the same or exhibits increased receptor potency and in some cases selectivity as a corresponding synthetic peptide that does not comprise the substitution(s). In further embodiments, the methods also provide a synthetic peptide that is substantially resistant to proteolytic degradation.

[0092] Suitably in the methods provided herein, the substituted alternative amino acids correspond to the substituted native amino acid residues, and in additional embodiments, the substituted alpha-methyl functionalized amino acids correspond to the same class as the substituted native amino acid residues. Further embodiments in this application involve various derivatives of phenylalanine that are modified/substituted in the phenyl ring and modified on the Nitrogen, alpha carbon, or beta carbon of the residue.

[0093] In certain embodiments, the synthetic peptides prepared according to the methods described herein can be substantially resistant to one or more of DPP-IV, neprilysin, α -chymotrypsin, plasmin, thrombin, kallikrein, trypsin, elastase and pepsin degradation.

Formulations Comprising Lipidated Peptides

[0094] Also provided are formulations (or pharmaceutical compositions) comprising a lipidated peptide described herein. Suitably such formulations comprise a lipidated peptide as described herein and a carrier. Such formulations can be readily administered in the various methods

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described throughout. In some embodiments, the formulation comprises a pharmaceutically acceptable carrier.

[0095] The term "pharmaceutically acceptable carrier" means one or more non-toxic materials that do not interfere with the effectiveness of the biological activity of the lipidated peptides. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the lipidated peptide is combined to facilitate the application.

[0096] Formulations as described herein can be formulated for a particular dosage. Dosage regimens can be adjusted to provide the optimum response. It can be useful to formulate parenteral compositions in dosage unit forms for ease of administration and uniformity of dosage. Dosage unit forms as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of a lipidated peptide calculated to produce a therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by, and directly dependent on, (a) the unique characteristics of the lipidated peptide and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a lipidated peptide.

[0097] Formulations described herein can be formulated for particular routes of administration, such as oral, nasal, pulmonary, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations can conveniently be presented in unit dosage form and can be prepared by any methods known in the art of pharmacy. The amount of lipidated peptide that can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of lipidated peptide that can be combined with a carrier material to produce a single

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dosage form will generally be that amount of the composition which produces a therapeutic effect.

Methods of Treatment Utilizing Lipidated Peptides

[0098] Also provided herein are methods of treating a patient comprising administering a lipidated peptide, e.g., the formulations, described herein to a subject in need thereof.

[0099] Suitably subjects that can be administered the lipidated peptides in the various methods described herein are mammals, such as for example, humans, dogs, cats, primates, cattle, sheep, horses, pigs, etc.

[00100] Methods by which the lipidated peptides can be administered to the subject in any of the various methods described herein include, but are not limited to, injection, intravenous (IV), intratumoral (IT), intralesional (IL), aerosol, percutaneous, oral, endoscopic, topical, intramuscular (IM), intradermal (ID), intraocular (IO), intraperitoneal (IP), transdermal (TD), intranasal (IN), intracerebral (IC), intraorgan (e.g. intrahepatic), slow release implant, or subcutaneous administration, or via administration using an osmotic or mechanical pump. Administration can be once per day, once per week, or once per month. Moreover, administration can accompany one or more additional therapies. In some embodiments, the therapies can be blood sugar monitoring, diet modifications, exercise, insulin, a thiazolidinedione, a sulfonylurea, an incretin, metformin, a glyburide, a dipeptidyl peptidase 4 inhibitor, a bile acid sequestrant, or any combination thereof.

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- [00101] Suitably, the lipidated peptides can be administered as soon as possible after a suitable diagnosis, e.g., within hours or days.
- [00102] As described herein, suitably the various methods can be carried out on mammalian subjects that are humans, including adults of any age and children.
- [00103] In certain embodiments, the methods of treatment comprise treating a subject (also referred to herein as a patient) diagnosed with diabetes comprising administering a therapeutically effective amount of a suitable lipidated peptide as described herein, suitably a lipidated GLP-1 peptide as described herein.
- [00104] As used herein, the term "therapeutically effective amount" refers to the amount of a lipidated peptide, or formulation, that is sufficient to reduce the severity of a disease or disorder (or one or more symptoms thereof), ameliorate one or more symptoms of such a disease or disorder, prevent the advancement of such a disease or disorder, cause regression of such a disease or disorder, or enhance or improve the therapeutic effect(s) of another therapy. In some embodiments, the therapeutically effective amount cannot be specified in advance and can be determined by a caregiver, for example, by a physician or other healthcare provider, using various means, for example, dose titration.
- [00105] In embodiments, methods are provided of treating a patient diagnosed with diabetes comprising administering a therapeutically effective amount of lipidated insulin to a patient.
- [00106] In certain embodiments, the condition is type-2 diabetes. Further, in other embodiments, the administration of the peptides disclosed can further improve glycemic control, provides body weight control, improves β -cell function and mass, reduces the rate of gastric acid secretion and gastric emptying, or any combination thereof.

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[00107] As described herein, in certain embodiments the methods of administration of the lipidated peptides or formulations described herein can be delivered orally. As described herein, lipidated peptides can be substantially resistant to proteolytic degradation, *e.g.*, degradation by enzymes in the stomach following oral administration.

[00108] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of any of the embodiments. The following examples are included herewith for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1: Chemical Synthesis and Testing of Proteolytic-Resistant Lipidated Peptides

1. Introduction

[00109] The following provides exemplary methods for preparing proteolytic-resistant peptides as described herein.

2. Abbreviations

[00110] Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; EK, enterokinase; ESI, electrospray ionisation; Fmoc, 9-fluorenylmethyloxycarbonyl; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; HCTU, *O*-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; RP-HPLC,

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reversed-phase high-performance liquid chromatography; EC₅₀, half maximal (50%) effective concentration; LC/MS, liquid chromatography-coupled mass spectrometry; MeCN, acetonitrile; Mmt, 4-methoxytrityl; NMP, *N*-methylpyrrolidinone; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PBS, phosphate buffered saline; ^tBu, tertiary-butyl; TFA, trifluoroacetic acid; TIS, triisopropylsilane; Tris, Tris(hydroxymethyl)aminomethane; Trt, triphenylmethyl; UV, ultraviolet.

3. Experimental

3.1. Peptide Synthesis

3.1.1. Materials

[00111] *N*- α -Fmoc-*L*-amino acids were obtained from Bachem AG (Switzerland). Unnatural amino acids were obtained from Iris Biotech AG (Germany), prepared by Pharmaron (China), or Peptech corporation (USA). NovaSyn[®] TGR (TentaGel Rink) and NovaSyn[®] TGA (TentaGel Wang) synthesis resins were obtained from Novabiochem, Merck Biosciences (Germany). Peptides were prepared by automated synthesis (PTI Prelude) using the Fmoc/^tBu protocol. Asparagine (Asn) and glutamine (Gln) were incorporated as their sidechain trityl (Trt) derivatives. Tryptophan (Trp) and lysine (Lys) were incorporated as their sidechain Boc derivatives. Serine (Ser), threonine (Thr) and tyrosine (Tyr) were incorporated as sidechain ^tBu ethers, and aspartate (Asp) and glutamate (Glu) as their sidechain O^tBu esters. Arginine (Arg) was incorporated as the sidechain Pbf derivative. Synthesis reagents were obtained from Sigma-Aldrich, Dorset, United Kingdom. Solvents were obtained from Merck, Darmstadt, Germany at the highest grade available and used without further purification.

3.1.2. Chemical synthesis of lipidated peptides containing α -methyl amino acids

[00112] Unless otherwise stated, peptides were prepared as C-terminal carboxamides on NovaSyn® TGR resin (initial substitution 0.24 mmole/g). Amino acids (both natural and unnatural) were coupled at ambient temperature using HCTU/DIPEA in NMP, capping residual functionality with a solution of acetic anhydride and pyridine. The *N*-Fmoc group was deblocked using piperidine in DMF (20% v/v) at ambient temperature. The C-terminal residue was incorporated as the *N*-Boc-protected form, e.g. Boc-His(Trt)-OH or Boc-Tyr(tBu)-OH or equivalent. At the position(s) of lipidation Fmoc-L-Lys(Mmt)-OH was incorporated into the peptide backbone during automated assembly and upon completion the Mmt protecting group(s) were removed manually by treatment of the synthesis resin with 1% TFA, 2% TIPS, DCM (10 x 1 minute, 20.0 mL/g). The acidified resin was quenched with 5% DIPEA/NMP and the exposed lysine amino-function(s) acylated, PEGylated or lipidated as required prior to peptide cleavage.

3.1.3. Cleavage of lipidated peptides

[00113] Crude peptides were cleaved from the resin support by treatment with a cocktail consisting of TFA (95% v/v), TIPS (2.5% v/v), water (2.5% v/v) with agitation (3 x 1 hour at ambient temperature). Cleavage aliquots were combined, concentrated by rotary evaporation and precipitated by addition of cold diethyl ether, isolating the solids by centrifugation. Crude peptides were dried under a flow of dry nitrogen, reconstituted in a suitable buffer and filtered prior to chromatographic purification.

3.1.4. Purification of crude mono-lipidated peptides

[00114] Crude mono-lipidated peptides were dissolved in a solution of acetic acid/acetonitrile/water (1:5:50 v/v) and filtered. The crude filtrates were chromatographed over an Agilent Polaris C8-A stationary phase (21.2 x 250 mm, 5 micron) eluting with a linear solvent gradient of 10-70%, 15-80% or 20-90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 30 minutes using a Varian SD-1 PrepStar binary pump system, monitoring by UV absorption at 210 nm. The peptide-containing fractions were pooled, frozen (dry-ice/acetone) and lyophilized.

[00115]

3.1.6. Peptide analysis and characterization

[00116] Purified peptides were characterized by single quadrupole LC/MS using a Waters Mass Lynx 3100 platform. Analytes were chromatographed by elution over a Waters X-Bridge C18 stationary phase (4.6 x 100 mm, 3 micron) using a generic linear binary gradient of 10-90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 10 minutes at 1.5 mL min⁻¹ at ambient temperature. Analytes were detected by both UV absorption at 210 nm and ionization using a Waters 3100 mass detector (ESI⁺ mode), verifying molecular mass against calculated theoretical values. Analytical RP-HPLC spectra were recorded using an Agilent 1260 Infinity binary gradient system. Analytes were chromatographed by elution over an Agilent Polaris C8-A stationary phase (4.6 x 100 mm, 3 micron) at 1.5 mL min⁻¹ using a linear binary gradient of 10-90% MeCN (0.1% TFA v/v) in water (0.1% TFA v/v) over 15 minutes at 40°C.

4.0 cAMP Assays

[00117] The biological activities/receptor potencies of the lipidated GLP-1 analog peptides described herein are suitably tested for biological activity, e.g., stimulation of one or more

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cellular receptor responses. Stable cell lines expressing human, mouse, rat, or dog GLP-1 receptor (GLP-1R), glucagon receptor (GCGR) or glucose-dependent insulintropic peptide (gastric inhibitory polypeptide) receptor (GIPR) are generated in HEK293 cells or CHO cells by standard methods. Peptide activation of these various receptors results in downstream accumulation of cAMP second messenger which can be measured in a functional activity assay.

[00118] cAMP assays were performed using "assay buffer": Assay Buffer: 0.1% BSA (Sigma # A3059) in HBSS (Sigma # H8264) with 25mM HEPES, pH 7.4 and containing 0.5mM IBMX (Sigma # I7018).

[00119] Low protein binding 384-well plates (Greiner # 781280) are used to perform eleven 1 in 5 serial dilutions of test samples which are made in assay buffer. Sample dilutions are made in duplicate.

[00120] A frozen cryo-vial of cells expressing the receptor of interest is thawed rapidly in a water-bath, transferred to pre-warmed assay buffer and spun at 240xg for 5 minutes. Cells are re-suspended in assay buffer at a batch-dependent optimized concentration (e.g. hGCGR cells at 2×10^5 cells/ml, hGLP-1R and hGIPR cells at 1×10^5 cells /ml).

[00121] From the dilution plate, a 5 μ L replica is stamped onto a black shallow-well u-bottom 384-well plate (Corning # 3676). To this, 5 μ L cell suspension is added and the plates incubated at room temperature for 30 minutes.

[00122] cAMP levels are measured using a commercially available cAMP dynamic 2 HTRF kit (Cisbio, Cat # 62AM4PEJ), following the two step protocol as per manufacturer's recommendations. In brief; anti-cAMP cryptate (donor fluorophore) and cAMP-d2 (acceptor

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fluorophore) are made up separately by diluting each 1/20 in conjugate & lysis buffer provided in the kit. 5µL anti-cAMP cryptate is added to wells of the assay plate, and 5µL cAMP-d2 is added to wells except non-specific binding (NSB) wells, to which conjugate and lysis buffer are added. Plates are incubated at room temperature for one hour and then read on an Envision (Perkin Elmer) using excitation wavelength of 320nm and emission wavelengths of 620nm & 665nm. EC₅₀ values of the synthetic peptides determined in cAMP assays are then determined.

[00123] In additional experiments for determining biological activity/receptor potency, CHO cells with stable recombinant expression of the human, mouse or rat GCGR or GLP-1 receptor are cultured in assay buffer as above). Cryopreserved cell stocks are prepared in 1x cell freezing medium-DMSO serum free (Sigma Aldrich) at either 1x10⁷ or 2x10⁷/vial and stored at -80°C. Cells are rapidly thawed at 37°C and then diluted into assay buffer (buffer as above) containing serum albumin at 4.4, 3.2 and 3.2% for human, rat, and mouse serum albumin respectively. Peptides are serially diluted in 100% DMSO and then diluted 100 fold into assay buffer as above containing serum albumin at stated final concentration. Diluted peptides are then transferred into 384 black shallow well microtitre assay plates. Cells are added to the assay plates and incubated for 30 min at room temperature. Following incubation the assay is stopped and cAMP levels measured using the HTRF® dynamic d2 cAMP assay kit available from CisBio Bioassays, as per the manufacturer's guidelines. Plates are read on Perkin Elmer ENVISION® fluorescence plate readers. Human and rat serum albumin are purchased from Sigma Aldrich and mouse serum albumin from Equitech Bio Ltd.

[00124] Data is transformed to % Delta F as described in the manufacturer's guidelines and analyzed by 4-parameter logistic fit to determine EC₅₀ values. EC₅₀ values determined are

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dependent on both the potency of the peptides tested at the GLP-1 and glucagon receptors in the recombinant cell lines and on the affinity of the peptide for serum albumin, which determines the amount of free peptide. Association with serum albumin increases the EC₅₀ value obtained. The fraction of free peptide at plasma concentrations of albumin and the EC₅₀ at 0% serum albumin (SA) can be calculated based on the variation in cAMP generation with the SA concentration. To compare the balance of activities at the GLP-1R and GCGR between different peptides and across different conditions, these can be correlated, where the EC₅₀'s are related to those of comparator peptides. Tables 4 and 5 show the results of these experiments.

Table 4 – cAMP Results of Extended GLP-1 Agonists

SEQ ID NOL	cAMP (EC ₅₀ nM)
206	0.04
207	0.016
208	0.012
209	0.009
210	0.004
211	0.02
212	0.03
213	0.3
214	0.02
215	0.033
216	0.1
217	0.43
218	1.3
219	0.033
220	0.02
221	0.03
222	0.005
223	0.01
224	0.06

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Table 5 – cAMP and INS1 Results for Modified GLP-1 Agonists

SEQ ID NO.	cAMP	INS1
	(nM)	(nM)
1	0.002	0.033
3	0.001	0.003
4	0.001	0.017
5	0.003	0.044
6	0.001	0.004
7	0.002	0.037
8	0.002	0.08
9	0.002	0.02
10	0.006	0.22
11	0.005	0.04
12	0.004	0.05
13	0.001	0.008
14	0.034	0.08
15	0.031	
16	0.006	0.25
17	0.03	0.28
18	0.009	0.033
19	0.03	0.3
20	0.05	
21	0.015	0.61
22	0.03	
23	0.001	0.004
24	0.001	0.007
25	0.002	0.02
26	0.019	0.6
27	0.002	0.008
28	0.025	2
29	0.11	0.61
30	0.001	0.002
31	0.001	0.035
32	0.009	0.3
33	0.004	0.13
34	0.004	0.015
35	0.003	0.049

SEQ ID NO.	cAMP	INS1
36	0.002	0.046
37	0.001	0.026
38	0.001	0.025
39	0.001	0.007
40	0.002	0.011
41	0.002	0.01
42	0.005	0.08
43	0.005	0.4
44	0.007	0.35
45	0.017	4
46	0.08	
47	0.8	44
48	0.094	4.6
49	0.093	
50	0.029	
51	0.31	>80
52	0.001	0.004
53	0.001	0.008
54	0.005	0.054
55	0.002	0.027
56	0.28	
57	0.04	
58	0.54	
59	0.043	
60	0.19	
61	0.034	1.43
62	0.86	
63	0.001	0.26
64	0.001	0.01
65	0.015	3.5
66	0.005	0.6
67	0.015	0.06
68	0.002	0.021
69	0.003	0.16
70	0.002	0.025
71	0.01	0.27
72	0.001	0.006

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SEQ ID NO.	cAMP	INS1
73	0.004	0.7
74	0.073	>90
75	0.13	50
76	0.033	0.7
77	0.01	>24
78	0.012	
79	0.01	
80	0.039	
81	0.013	0.42
82	0.002	0.05
83	0.02	0.68
84	0.004	0.067
85	0.036	
86	0.001	0.02
87	0.002	0.082
88	0.013	0.22
89	0.2	8
90	0.76	21
91	0.001	0.01
92	0.001	0.028
93	0.003	0.25
94	0.002	0.17
95	0.001	0.027
96	0.006	0.16
97	0.001	0.022
98	0.001	0.029
99	0.001	0.03
100	0.002	0.18
101	0.001	0.02
102	0.001	0.017
103	0.006	
104	0.001	0.054
105	0.001	0.02
106	0.001	0.022
107	0.001	0.016
108	0.001	
109	0.003	0.28
110	0.2	
111	11	14

SEQ ID NO.	cAMP	INS1
112	0.001	>70
113	0.001	0.011
114	0.022	0.037
115	0.064	11
116	0.002	2.5
117	0.004	0.016
118	0.033	0.12
119	0.66	15
120	3.5	49
121	0.002	
122	0.029	0.017
123	0.012	0.6
124	0.005	0.4
125	0.14	0.082
126	0.08	
127	0.4	0.6
128	0.16	23
129	0.039	10
130	0.31	0.3
131	0.014	
132	0.17	0.25
133	0.001	0.03
134	0.001	0.069
135	0.003	0.1
136	0.014	
137	0.005	0.52
138	0.034	2.3
139	0.002	0.052
140	0.004	0.11
141	0.002	0.073
142	0.001	0.012
143	0.001	0.007
144	0.001	0.005
145	0.001	0.009
146	0.001	0.006
147	0.002	0.031
148	0.001	0.022
149	0.001	0.017

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SEQ ID NO.	cAMP	INS1
150	0.002	0.11
151	0.001	0.002
152	0.001	0.01
153	0.001	0.004
154	0.001	0.08
155	0.003	0.21
156	0.007	0.65
157	1.8	
158	0.005	0.2
159	0.001	0.065
160	0.001	0.038
161		
162		
163		
164		
165	0.005	0.11
166	0.006	0.11
167	0.001	0.072
168	0.001	0.1
169	0.006	0.16
170	0.27	
171	0.003	0.38
172	0.001	0.073
173	0.001	0.031
174	0.008	2
175	0.03	
176	0.001	0.002
177	0.001	0.016
178	0.002	0.058
179	0.001	0.026
180	0.001	0.002
181	0.001	0.005
182	0.002	0.059
183		
184		
185		
186		
187	0.003	0.23

SEQ ID NO.	cAMP	INS1
188	0.012	0.19
189	0.14	0.11
190	0.042	0.2
191	0.08	0.33
192	0.003	0.025
193	0.02	0.21
194	0.007	0.064
195	0.025	0.14
196	0.032	0.34
197	0.001	0.007
198	0.004	0.07
199	0.002	0.017
200	0.002	0.031
201	0.002	0.016
202	0.008	0.046
203	0.003	0.048

[00125] All documents, patents, journal articles and other materials cited in the present application are hereby incorporated by reference.

[00126] Although the present disclosure provides numerous embodiments including reference to the accompanying drawings, it is to be understood that various changes and modifications can be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present disclosure as defined by the appended claims, unless they depart there from.

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WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising the amino acid sequence:

X0-H-X2-X3-GT-FTSD-X10-S-X12-X13-X14-X15-X16-X17-AA-X20-X21-X22-I-X24-X25-X26-X27-X28-X29-X30-X31-X32 (SEQ ID NO: 2);

wherein X0 is null, A, E, F, I, L, V or T

X2 is A, Aib or d-Ser,

X3 is E or I,

X10 is S or a lipid modified K,

X12 is S or a lipid modified K,

X13 is Y or a lipid modified K,

X14 is L or a lipid modified K,

X15 is E or a lipid modified K,

X16 is G or a lipid modified K,

X17 is E, Q, or a lipid modified K,

X20 is E, K, R, or a lipid modified K,

X21 is E or a lipid modified K,

X22 is F, Cha, 4Me-Phe, Bip, Dip, 2-CF₃-Phe, 4-CF₃-Phe, Nle or a lipid modified K,

X24 is A, E, Cha, Bip, Dip, Nle, or a lipid modified K,

X25 is W, Cha, Nle, NMe-Trp, αMe-Trp, or a lipid modified K

X26 is L or a lipid modified K,

X27 is V or a lipid modified K,

X28 is K, E, R or a lipid modified K,

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X29 is G or Aib

X30 is R, E, or G,

X31 is G, null, or a lipid modified K

X32 is null or a lipid modified K; and

wherein the polypeptide is lipidated on only one of X10, X12, X13, X14, X15, X16, X17, X20, X21, X22, X24, X25, X26, X27, X28, X31, or X32.

2. The polypeptide of claim 1, wherein the peptide comprises a C-terminal amide.
3. The polypeptide of claim 1, wherein the peptide comprising a C-terminal acid.
4. The poly peptide of claims 1 to 3, wherein the lipid modified K residue is selected from the group consisting of: (γ E-Palmitoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E-Palmitoyl), K(ϵ -(PEG)₂-(PEG)₂-Palmitoyl), K(ϵ -(PEG)₄- γ E-Palmitoyl), K(ϵ -(PEG)₄ - γ E-Stearoyl), K(ϵ -(PEG)₄ -Stearoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E-Stearoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E- γ E-Stearoyl), K(ϵ - γ E- γ E-Stearoyl)., and any combination thereof.
5. The polypeptide of any one of claims 1 to 4, wherein the polypeptide is substantially resistant to proteolytic degradation.
6. The polypeptide of claim 5, wherein the polypeptide is substantially resistant to DPP-IV, neprilysin, α -chymotrypsin, plasmin, thrombin, kallikrein, trypsin, elastase and/or pepsin degradation.
7. The polypeptide of any one of claims 1 to 6, wherein the polypeptide at least maintains substantially the same receptor potency as a corresponding non-lipidated polypeptide.

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8. The polypeptide of claim 7, wherein the polypeptide at least maintains substantially the same receptor selectivity as a corresponding non-lipidated polypeptide.

9. The polypeptide of claim 7 or 8, wherein the polypeptide exhibits increased receptor potency over a corresponding non-lipidated polypeptide.

10. An isolated polypeptide comprising the amino acid sequence:
X0-HGEGT-FTSD-X10-S-X12-Q-X14-EE-X17-AV-X20-L-X22-I-X24-
WLKNGGPSSGAPP-X39-X40 (SEQ ID NO: 204);

wherein X0 is F or A,

X10 is L or a lipid modified K,

X12 is K or lipid modified K,

X14 is M or a lipid modified K,

X17 is E or a lipid modified K,

X20 is R or a lipid modified K,

X22 is F, Cha, or Phe(4Me), 4Me-phenylalanine

X24 is L or a lipid modified K,

X39 is S or a lipid modified K,

X40 is null or a lipid modified K; and

wherein the polypeptide comprises one lipid modified K residue at X10, X12, X14, X17, X20, X24, X39, or X40.

11. The polypeptide of claim 10 to 11, wherein the peptide comprises a C-terminal amide.

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12. The polypeptide of any one of claims 10 to 11, wherein the lipid modified K residue is selected from the group consisting of: K(γ E-Palmitoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E-Palmitoyl), K(ϵ -(PEG)₂-(PEG)₂-Palmitoyl), K(ϵ -(PEG)₄- γ E-Palmitoyl), K(ϵ -(PEG)₄- γ E-Stearoyl), K(ϵ -(PEG)₄-Stearoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E-Stearoyl), K(ϵ -(PEG)₂-(PEG)₂- γ E- γ E-Stearoyl), K(ϵ - γ E- γ E-Stearoyl).
13. An isolated polynucleotide encoding the polypeptide of any one of claims 1 to 12.
14. A vector comprising the polynucleotide of claim 13.
15. A host cell comprising the polynucleotide of claim 13 or the vector of claim 14.
16. A method of making the polypeptide of any one of claims 1 to 11, comprising culturing the host cell of claim 15 under conditions allowing expression of the peptide, and recovering the peptide.
17. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 11, and a carrier.
18. A kit comprising the composition of claim 17.
19. A method of treating or preventing a disease or condition caused or characterized by hypoglycemia or impaired insulin release, comprising administering to a subject in need of treatment an effective amount of the polypeptide of any one of claims 1 to 16 or the pharmaceutical composition of claim 17.
20. The method of claim 19, wherein the disease or condition is diabetes.

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21. The method of claim 20, wherein the disease or condition is type-2 diabetes.
22. The method of any one of claims 19 to 21, wherein the administration further improves glycemic control, provides body weight control, improves β -cell function and mass, reduces the rate of gastric acid secretion and gastric emptying, or any combination thereof.
23. The method of any one of claims 19 to 22, wherein the polypeptide or the pharmaceutical composition is administered orally or by injection.
24. The method of claim 23, wherein the polypeptide or the pharmaceutical composition is administered orally.
25. The method of claim 23, wherein the injection is administered subcutaneously or intravenously.
26. The method of any one of claims 19 to 25, wherein the peptide or the pharmaceutical composition is administered once per day.
27. The method of any one of claims 19 to 26, further comprising administering one or more additional therapies.
28. The method of claim 27, wherein the additional therapy comprises blood sugar monitoring, diet modifications, exercise, insulin, a thiazolidinedione, a sulfonylurea, an incretin, metformin, a glyburide, a dipeptidyl peptidase 4 inhibitor, a bile acid sequestrant, or any combination thereof.
29. The method of any one of claims 19 to 28, wherein the subject is human.

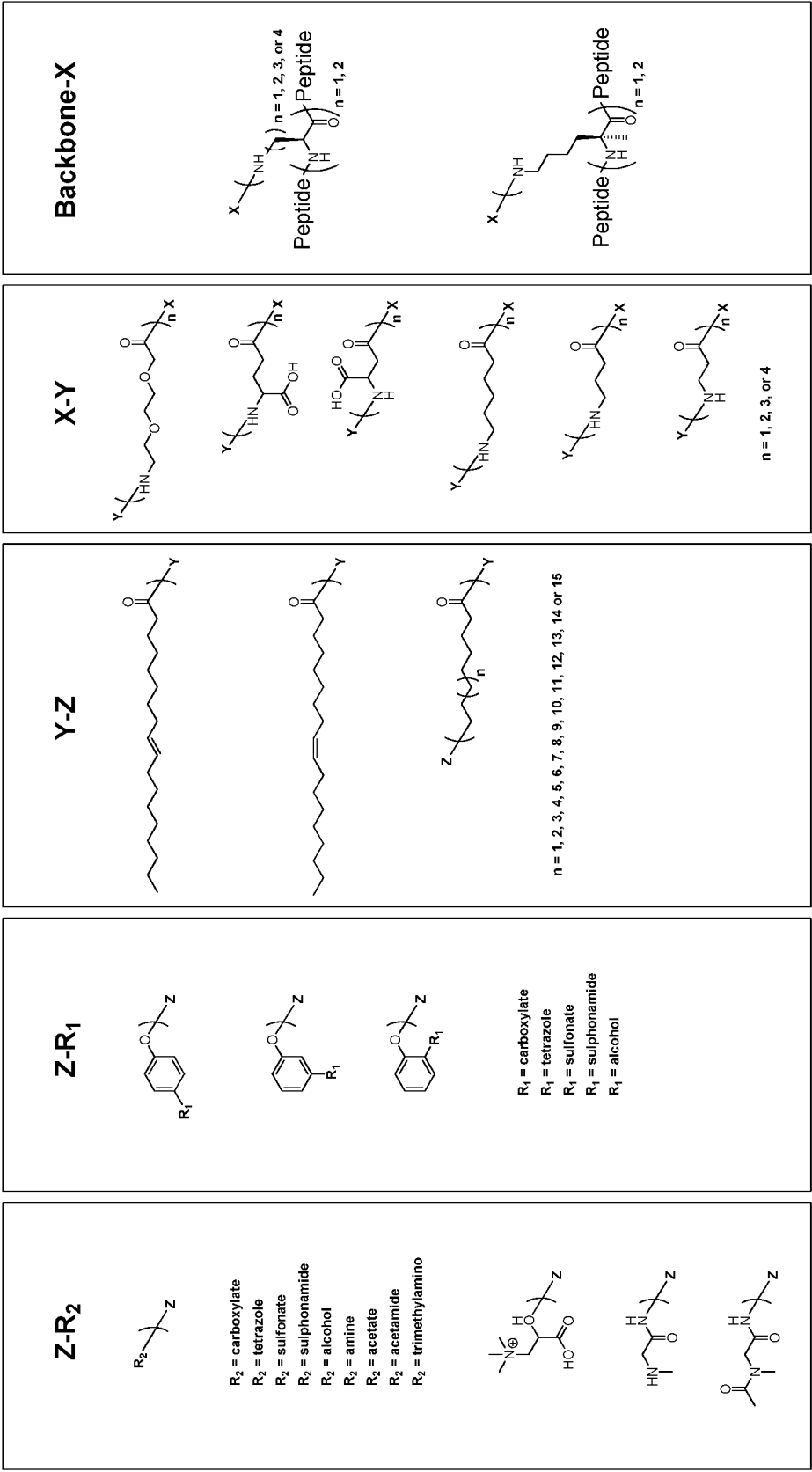


Figure 1