

LONG-ACTING DUAL GIP/GLP-1 PEPTIDE CONJUGATES AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/208,952 filed June 9, 2021, which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 2, 2022, is named 36271-714_601_SL.txt and is 46,765 bytes in size.

BACKGROUND OF THE INVENTION

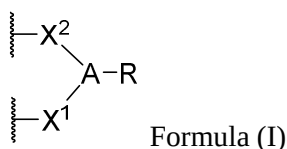
[0003] The development of therapeutic agents is often hampered by short half-lives. The biological half-life of an agent is the time it takes for the agent to lose half of its pharmacologic, physiologic, or radiologic activity. As a result, patients are often administered higher dosages of a therapeutic agent more frequently, which can lead to reduced compliance, higher costs and greater risk of side effects. Accordingly, there is a need for generation of therapeutic agents with extended half-lives.

SUMMARY

[0004] Disclosed herein is a peptide conjugate comprising:

- a) a peptide and
- b) a staple attached to the peptide at a first amino acid and a second amino acid;

wherein the staple is of Formula (I):



wherein

A is -N-;

X¹ and X² are a bond, -C(=O)-, -alkylene-C(=O)-, -C(=O)-alkylene-, -alkylene-C(=O)NR³-, -alkylene-NR³C(=O)-, -C(=O)NR³-alkylene-, -NR³C(=O)-alkylene-, -alkylene-C(=O)NR³-alkylene-, or -alkylene-NR³C(=O)-alkylene-;

wherein X¹ is attached to the first amino acid of the peptide, X² is attached to the second amino acid of the peptide, and X¹ and X² are identical;

R is hydrogen or -(L)_s-Y;

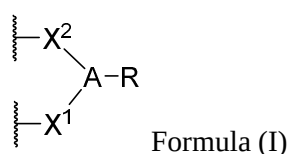
each L is independently -(CR¹R²)_v-, -alkylene-O-, -O-alkylene-, -C(=O)-alkylene-, -alkylene-C(=O)-, -NR³-alkylene-, -alkylene-NR³-, -S-alkylene-, -alkylene-S-, -S(=O)-alkylene-, -alkylene-S(=O)-, -S(=O)₂-alkylene-, -alkylene-S(=O)₂-, -C(=O)-, -C(=O)NR³-, -NR³C(=O)-, -NR³C(=O)NR³-, -NR³C(=O)NR³-alkylene-, -NR³C(=O)-alkylene-NR³-, -alkylene-C(=O)NR³-, -C(=O)NR³-alkylene-, -alkylene-NR³C(=O)-, or -NR³C(=O)-alkylene-;

[0020] In some embodiments, the method further comprises administering to the subject one or more additional therapeutic agents. In some embodiments, the one or more additional therapeutic agents comprises an incretin hormone or a derivative thereof. In some embodiments, the incretin hormone or derivative thereof is selected from GLP-1, exendin-4, glucagon (GCG), glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin, and combinations thereof.

[0021] In some embodiments, the peptide conjugate is administered about once every 7 days. In some embodiments, the peptide conjugate is administered about once every 14 days. In some embodiments, the peptide conjugate is administered about once a month. In some embodiments, the peptide conjugate is administered about once every two months. In some embodiments, the peptide conjugate is administered about once every three months.

[0021a] In one aspect, provided herein is a peptide conjugate comprising a peptide and a staple, wherein the peptide comprises: YAibEGTFTSDYSIYLDKXAAaibEFVXWLIAGGPSSGAPPPS (SEQ ID NO: 6), and a staple, wherein the staple is attached to the peptide at each X amino acid of the peptide; wherein each X is a sulfhydryl-containing amino acid, or each X is an amine-containing amino acid; and

wherein the staple is of Formula (I):



wherein

A is -N-;

X¹ and X² are a bond, -C(=O)-, -alkylene-C(=O)-, -C(=O)-alkylene-, -alkylene-C(=O)NR³-, -alkylene-NR³C(=O)-, -C(=O)NR³-alkylene-, -NR³C(=O)-alkylene-, -alkylene-C(=O)NR³-alkylene-, or -alkylene-NR³C(=O)-alkylene-;

wherein X¹ is attached to the first amino acid of the peptide, X² is attached to the second amino acid of the peptide, and X¹ and X² are identical;

R is hydrogen or -(L)_s-Y;

each L is independently -(CR¹R²)_v-, -alkylene-O-, -O-alkylene-, -C(=O)-alkylene-, -alkylene-C(=O)-, -NR³-alkylene-, -alkylene-NR³-, -S-alkylene-, -alkylene-S-, -S(=O)-alkylene-, -alkylene-S(=O)-, -S(=O)₂-alkylene-, -alkylene-S(=O)₂-, -C(=O)-, -C(=O)NR³-, -NR³C(=O)-, -NR³C(=O)NR³-, -NR³C(=O)NR³-alkylene-, -NR³C(=O)-alkylene-NR³-, -alkylene-C(=O)NR³-, -C(=O)NR³-alkylene-, -alkylene-NR³C(=O)-, or -NR³C(=O)-alkylene-;

v is 2-20;

each R¹ or R² is independently hydrogen, halogen, -CN, -OR^a, -SR^a, -S(=O)R^b, -NO₂, -NR^cR^d, -S(=O)₂R^d, -NR^aS(=O)₂R^d, -S(=O)₂NR^cR^d, -C(=O)R^b, -OC(=O)R^b, -CO₂R^a, -OCO₂R^a, -C(=O)NR^cR^d, -OC(=O)NR^cR^d, -NR^aC(=O)NR^cR^d, -NR^aC(=O)R^b, -NR^aC(=O)OR^a, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, C₂-C₈ heterocycloalkyl, aryl, or heteroaryl; wherein the alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one, two, or three of halogen, -OR^a, or -NR^cR^d; and the cycloalkyl, heterocycloalkyl, aryl, and heteroaryl

is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OR^a, or -NR^cR^d;

or R¹ and R² are taken together to form a C₁-C₆ cycloalkyl or C₁-C₆ heterocycloalkyl;

each R³ is independently hydrogen, -S(=O)R^b, -S(=O)₂R^a, -S(=O)₂NR^cR^d, -C(=O)R^b, -CO₂R^a, -C(=O)NR^cR^d, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, C₂-C₈ heterocycloalkyl, aryl, or heteroaryl; wherein the alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one, two, or three of halogen, -OR^a, or -NR^cR^d; and the cycloalkyl, heterocycloalkyl, aryl, and heteroaryl is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OR^a, or -NR^cR^d;

Y is hydrogen, C₁-C₆ alkyl, -CO₂H, -CO₂(C₁-C₆ alkyl), -CO₂NH₂, -CO₂N(alkyl)₂, or -CO₂NH(alkyl);

s is 0-20;

R^a is hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, C₂-C₈ heterocycloalkyl, aryl, or heteroaryl; wherein the alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one, two, or three of halogen, -OH, -OMe, or -NH₂; and the cycloalkyl, heterocycloalkyl, aryl, and heteroaryl is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OH, -OMe, or -NH₂;

R^b is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, C₂-C₈ heterocycloalkyl, aryl, or heteroaryl; wherein the alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one, two, or three of halogen, -OH, -OMe, or -NH₂; and the cycloalkyl, heterocycloalkyl, aryl, and heteroaryl is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OH, -OMe, or -NH₂; and

each R^c and R^d is independently hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, C₂-C₈ heterocycloalkyl, aryl, or heteroaryl; wherein the alkyl, alkenyl, alkynyl, and heteroalkyl is optionally substituted with one, two, or three of halogen, -OH, -OMe, or -NH₂; and the cycloalkyl, heterocycloalkyl, aryl, and heteroaryl is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OH, -OMe, or -NH₂;

or R^c and R^d, together with the nitrogen atom to which they are attached, form a heterocycloalkyl or heteroaryl; wherein the heterocycloalkyl and heteroaryl is optionally substituted with one, two, or three of halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OH, -OMe, or -NH₂.

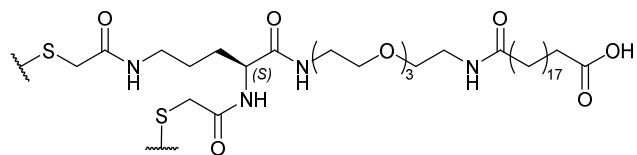
[0021b] In another aspect, provided herein is a peptide comprising

YAibEGTFTSDYSIYLDKXAAAibEFVXWLIAGGPSSGAPPPS (SEQ ID NO: 6), wherein each X is a sulfhydryl-containing amino acid or an amine-containing amino acid.

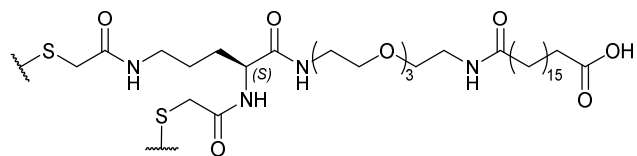
[0021c] In another aspect, provided herein is the use of the peptide conjugate described herein, or the peptide described herein, or the pharmaceutical composition described herein, in the manufacture of a medicament for treating diabetes, obesity, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), cardiovascular disease, short bowel syndrome (SBS), inflammatory bowel disease (IBD), inflammatory bowel syndrome (IBS), psoriasis, Crohn's disease, ulcerative colitis, Alzheimer's disease, Parkinson's disease, or Huntington's disease.



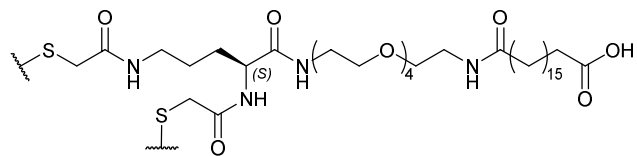




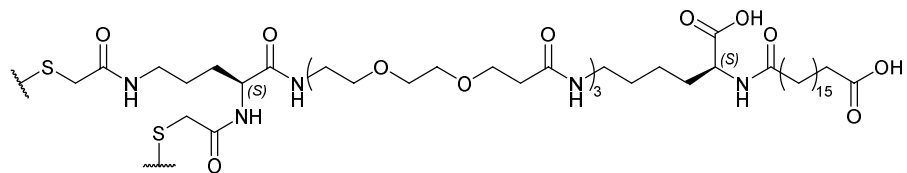
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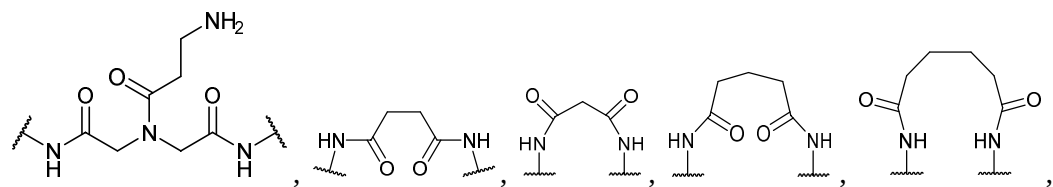
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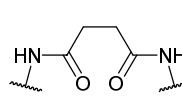
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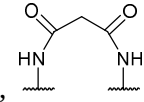
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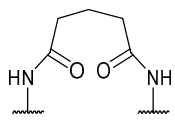
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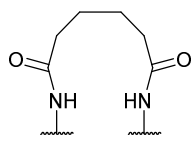
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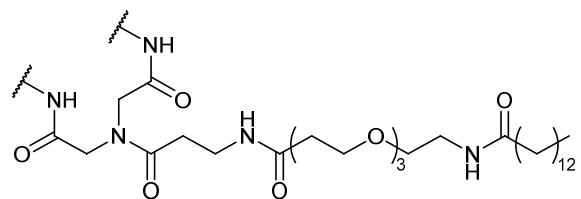
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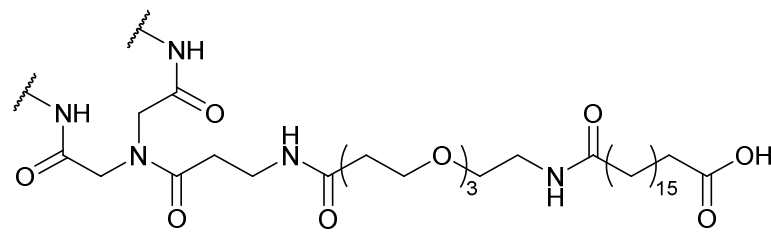
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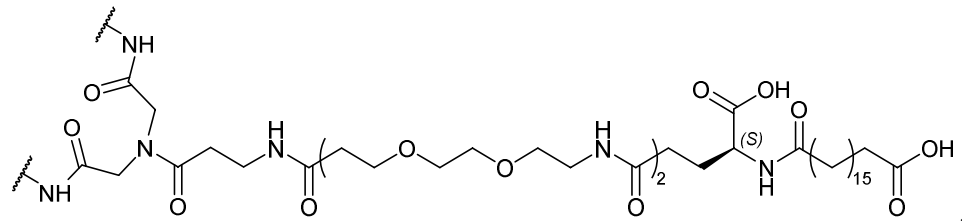
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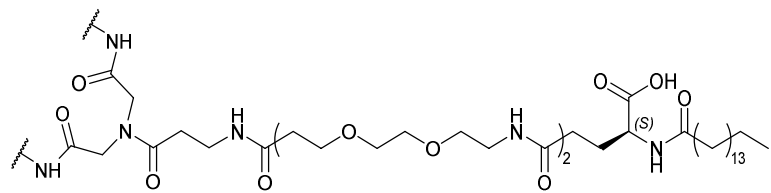
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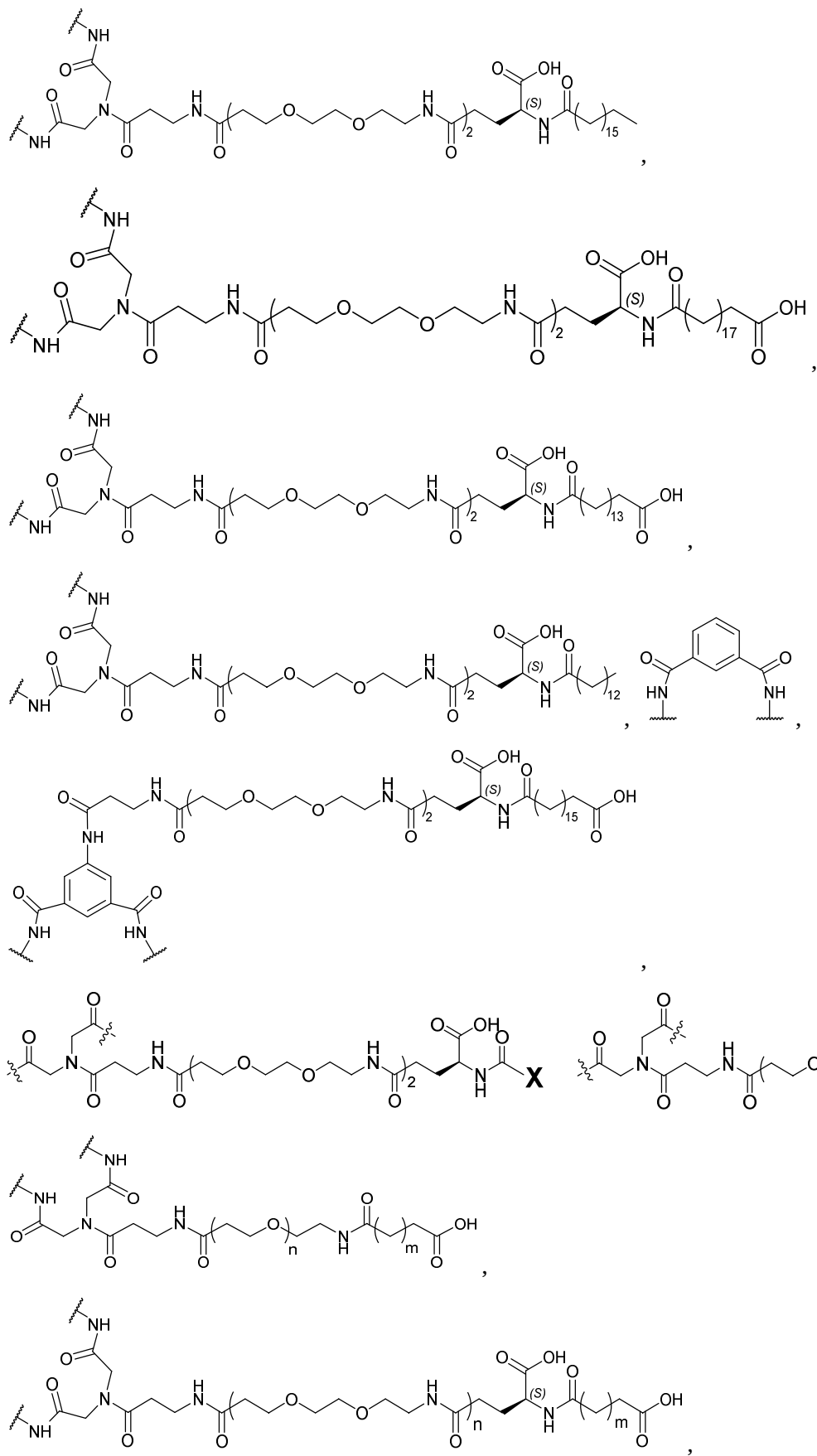
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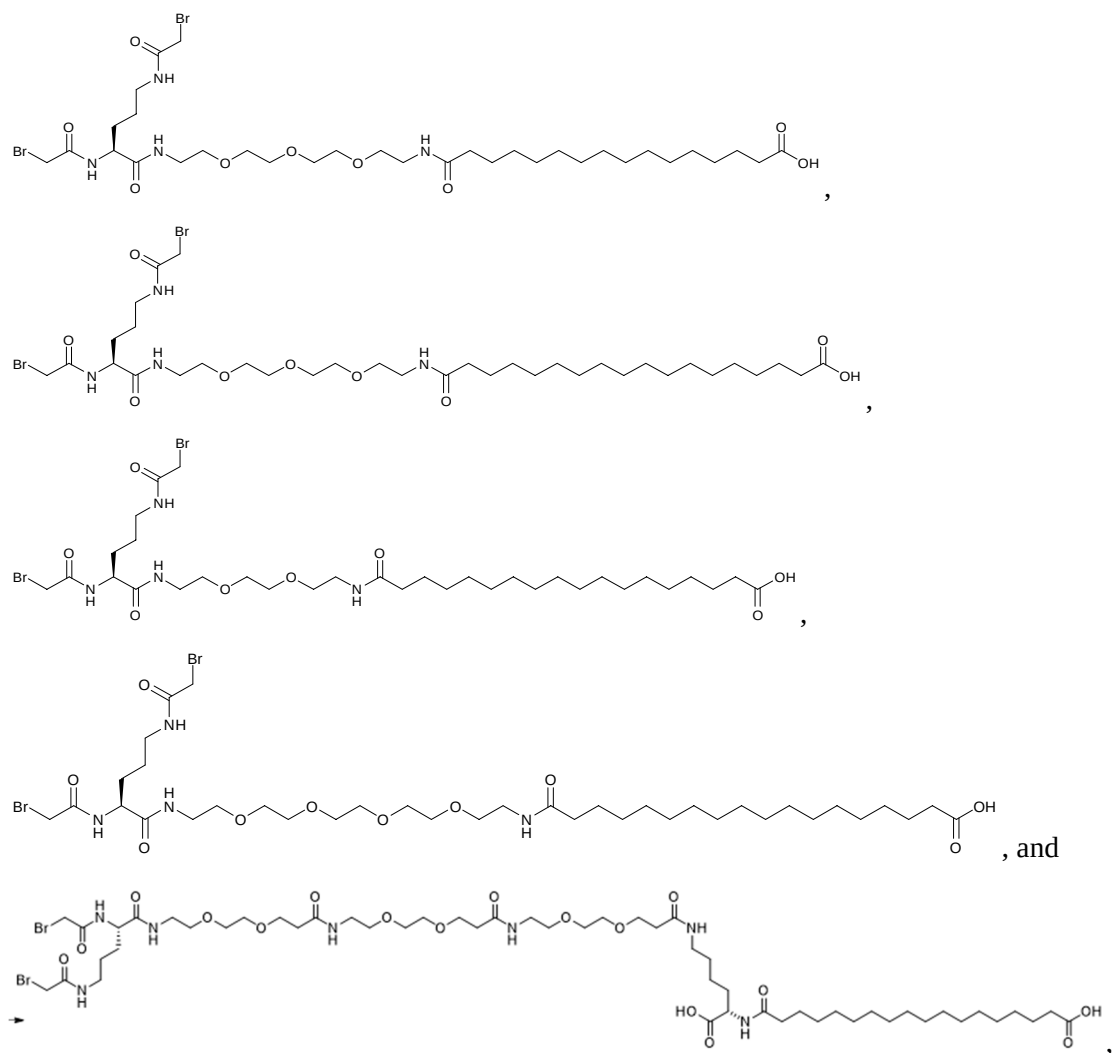
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wherein:

each “{S}” is a sulfur atom of a sulfhydryl-containing amino acid;

each “{NH}” is an amine of an amine-containing amino acid;

each “{C(=O)}” is a carbonyl of an amide bond to an amine-containing amino acid;

each X independently represents a C₁₄ alkyl, C₁₄ diacid, C₁₆ alkyl, C₁₆ diacid, C₁₈ alkyl, C₁₈ diacid, C₂₀ alkyl or C₂₀ diacid;

n is 1-4; and

m is 6-20.

[0021g] In another aspect, provided herein is the peptide conjugate described herein, or the peptide described herein, or the pharmaceutical composition described herein, for use in therapy.

BRIEF DESCRIPTION OF FIGURES

[0022] FIG. 1A and FIG. 1B display example components of a peptide, such as amino acids.

[0023] FIGS. 2A-2B are graphs showing plasma glucose excursion during oral glucose tolerance tests (OGTT) in normal mice after 2 h (FIG. 2A) and 96 h (FIG. 2B) post injection. C57B6 mice were s.c. injected with vehicle, L5A (mCMC759), K5 (mCLZ715), K4 (mCMD307), or semaglutide (at 10 nmol/kg each) 2 h prior to the glucose challenge. The bar graph shows the levels of glucose in mice obtained by measuring the area under curve [AUC] as well as the effects on fasted blood glucose. Data are means $\pm$ SE,

n = 6 per group. Statistics: AUC and fasted glucose data were compared by one-way ANOVA followed by Dunnett's multiple comparison tests vs. PBS-treated mice: ****P<.0001; ***P<.001.

[0024] FIG. 3 is a graph showing the pharmacokinetic profile of peptide mCMD307 (K4) after a single s.c. administration (1 mg kg⁻¹) or i.v. administration (0.3 mg kg⁻¹) in mice. Peptide dissolved in PBS (pH 8.2) was administered via i.v. or s.c. route into CD1 mice (n = 3 per group). Blood samples were collected at the indicated time points and analyzed by in vitro GLP-1R activity assay. The assay was performed in triplicate. Pharmacokinetic analyses were determined by noncompartmental analysis with WinNonLin.

[0025] FIGS. 4A-4B show in vivo efficacy of mCMD307 (K4) in DIO mice treated for 7 days. Effects on body weight change (**FIG. 4A**, top), crude body weight (**FIG. 4A**, middle), cumulative food intake (**FIG. 4A**, bottom), OGTT on day 7 (**FIG. 4B**, top and middle), and fasted blood glucose on day 7 (**FIG. 4B**, bottom). C57BL/6 mice (male, 37 weeks old) were treated with PBS (s.c., twice daily), mCMD307 (K4) (s.c. once daily; 10 nmol kg⁻¹) or semaglutide (s.c. once daily; 10 nmol kg⁻¹) for 7 days. The arrows indicate the day of administration. Data are means ± SE, n = 6 per group. Statistics: AUC and fasted glucose data were compared by one-way ANOVA followed by Dunnett's multiple comparison tests vs. PBS-treated mice: ****P<.0001; ***P<.001; **P<.01.

[0026] FIGS. 5A-5E show in vivo efficacy of mCMD307 (2050-K4) in high-fat-diet-induced obesity (DIO) mice treated for 24 days. Effects on body weight and food intake change (**FIG. 5A**), oral glucose tolerance test at day 21, fasted blood glucose prior to the OGTT (day 20) and fed glucose after the OGTT (day 21) (**FIG. 5B**). Upon termination at day 24, measurement of liver enzyme ALT, AST, ALP levels and plasma cholesterol and triglyceride levels (**FIG. 5C**) and liver weight, liver triglyceride, liver/body weight ratio and fat weight (**FIG. 5D**); and steatosis score and liver lipid accumulation measurement by Oil-red