

CO-AGONISTS AT GLP-1 AND GIP RECEPTORS SUITABLE FOR ORAL DELIVERY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to novel compounds that are agonists of the glucagon-like peptide 1 (GLP-1) receptor and the glucose-dependent insulintropic polypeptide (GIP) receptor with a protracted profile of action, suitable for oral administration to humans.

INCORPORATION-BY-REFERENCE OF THE SEQUENCE LISTING

The present application is filed with a Sequence Listing in electronic form. The entire contents of the sequence listing are hereby incorporated by reference.

BACKGROUND

Glucagon-like peptide 1 (GLP-1) is a gut enteroendocrine cell-derived hormone and one of two prominent endogenous physiological incretins. GLP-1 improves glycemic control by stimulating glucose-dependent insulin secretion in response to nutrients (glucose), inhibits glucagon secretion from the pancreatic alpha-cells, slows gastric emptying, and induces body weight loss primarily by decreasing food consumption. Glucose-dependent insulintropic polypeptide (GIP), the other prominent incretin, improves glycemic control by stimulation of insulin secretion in response to nutrients (fat, glucose). Furthermore, GIP appears to improve plasma lipid profile and to stimulate calcium accumulation in bones. In contrast to GLP-1, the incretin effect of GIP is severely reduced in type 2 diabetes patients, though recent studies suggest that GIP efficiency can be regained in these patients after treatment to improve glucose control. Nonetheless, the role of GIP to regulate systemic metabolism beyond its direct effect at the endocrine pancreas remains controversial, particularly as it relates to GIP action to promote gain in fat mass in animal models. These results have fostered beliefs that GIPR antagonism can improve body weight. Thus, employment of compounds acting at GIP receptors, and specifically whether to agonize or antagonize, as a strategy to improve body weight remains a contentious subject of intense scientific investigation (Finan *et al*, TRENDS Mol Med, 2016, 22 (5): 359-376; Killian *et al*, Endo Rev, 2020, 41 (1): 1-21).

Protracted GIP analogs have been shown to lower body weight and improve glycemic control, though comparatively less potent than GLP-1 analogs to lower body weight in rodent models (Mroz *et al*, Mol Metab, 2019, 20: 51-62). Moreover, GIP analogs induce body weight loss by additive/synergistic action with GLP-1 analogs in dual administration

(Finan *et al*, Sci Transl Med, 2013, 5 (209): 209ra151; Nørregaard *et al*, Diabetes Obes Metab, 2018, 20 (1): 60-68), and as such represent suitable candidates for amplification of GLP-1-based pharmacology. GIPR agonism can be recruited as a non-redundant partner to GLP-1R agonism as a single molecule co-agonist to amplify GLP-1 metabolic benefits, as
5 has been shown in preclinical animal models, most notably body weight loss and glycemic control (Finan *et al*, Sci Transl Med, 2013, 5 (209): 209ra151; Coskun *et al*, Mol Metab, 2018, 18: 3-14). Two different peptides with high potency dual incretin receptor agonism have advanced to multi-dose clinical studies. The clinical results have demonstrated improvements in glycemic control and body weight that exceeds what is achieved with
10 comparable dosing of benchmark GLP-1 specific agonists (Frias *et al*, Cell Metab, 2017, 26 (2): 343-352; Frias *et al*, Lancet, 2018, 392 (10160): 2180-2193), demonstrating the translational aspects and therapeutic benefits of co-targeting GLP-1 and GIP receptors.

Oral delivery of GLP-1 derivatives has been investigated clinically in the form of a once-daily tablet of semaglutide and the permeation enhancer sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) (Hedrington & Davis, Exp. Opin. Pharmacother.
15 2019, 20 (2): 133-141) to improve the very low typical exposure and bioavailability of GLP-1 derivatives following oral administration.

GLP-1/GIP co-agonists and their potential medical uses are described in several
20 patent applications such as WO 2010/011439, WO 2013/164483, WO 2014/192284, WO 2015/067715, WO 2015/022420, WO 2015/086728, WO 2015/086729, WO 2016/111971, WO 2020/023386, US 9745360, US 2014/162945, and US 2014/0357552. Patent applications disclosing oral delivery of GLP-1 derivatives are described in e.g. WO 2011/080103, WO 2012/080471, WO 2013/189988, and WO 2019/149880.

25 However, no co-agonistic products have so far obtained market approval.

SUMMARY

The present invention relates to single molecule co-agonists comprising a peptide
and a substituent, which react with both the human GLP-1 and GIP receptors with high
30 potency and are suitable for daily oral administration to humans. This is achieved by the combination of certain peptide sequence variants with substituents via a single site acylation with a diacid based fatty acid.

An aspect of the invention relates to a peptide having the amino acid sequence

YX₂EGTX₆TS₆DYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈

X₃₉ (SEQ ID NO.: 36)

with an optional amide modification of the C-terminus;

wherein

- 5 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
 X₁₆ is K or E
 10 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
 X₂₄ is E, Q or N
 X₂₈ is A or R
 15 X₃₂ is E, S or absent
 X₃₃ is S, K or absent
 X₃₄ is G or absent
 X₃₅ is A or absent
 X₃₆ is P or absent
 20 X₃₇ is P or absent
 X₃₈ is P or absent
 X₃₉ is S or absent.

25 In another aspect, the invention relates to a peptide having the amino acid sequence

YX₂EGTX₆TS₆DYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈

X₃₉ (SEQ ID NO.: 36)

with an optional amide modification of the C-terminus, wherein

- 30 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 35 X₂₁ is A or E

X₂₃ is I or V

X₂₄ is E, Q or N

X₂₈ is A or R

X₃₂ is E, S or absent

5 X₃₃ is S, K or absent

X₃₄ is G or absent

X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

10 X₃₈ is P or absent

X₃₉ is S or absent;

and a substituent attached to the peptide via Lysine (K) at position 16 or 33;
or a pharmaceutically acceptable salt hereof.

15 A further aspect of the invention relates to a method for preparing the GLP-1/GIP co-agonists described herein.

In a further aspect the invention relates to a pharmaceutical composition comprising the GLP-1/GIP co-agonists compounds described herein.

20 A further aspect of the invention relates to medical use of the GLP-1/GIP co-agonists described herein.

In one aspect the invention relates to use of the GLP-1/GIP co-agonists described herein for prevention or treatment of diabetes, obesity, and/or liver diseases.

DESCRIPTION

25 In what follows, Greek letters may be represented by their symbol or the corresponding written name, for example: α = alpha; β = beta; ε = epsilon; γ = gamma; ω = omega; etc. Also, the Greek letter of μ may be represented by "u", e.g. in μ l=ul, or in μ M=uM.

GLP-1/GIP receptor co-agonist

30 The present invention relates to compounds that are GLP-1 receptor and the GIP receptor agonists, also referred to as GLP-1/GIP receptor co-agonists or simply co-agonists.

The term "compound" is used herein to refer to a molecular entity, and "compounds" may thus have different structural elements besides the minimum element defined for each compound or group of compounds. It follows that a compound may be a peptide or a

derivative thereof, as long as the compound comprises the defined structural and/or functional elements.

The term "compound" is also meant to cover pharmaceutically relevant forms hereof, i.e. a compound as defined herein or a pharmaceutically acceptable salt, amide, or ester thereof.

The term "analogue" generally refers to a peptide, the sequence of which has one or more amino acid changes when compared to a reference amino acid sequence. An "analogue" may also include amino acid elongations in the N-terminal and/or C-terminal positions and/or truncations in the N-terminal and/or C-terminal positions.

In general, amino acid residues may be identified by their full name, their one-letter code, and/or their three-letter code. These three ways are fully equivalent.

Amino acids are molecules containing an amino group and a carboxylic acid group, and, optionally, one or more additional groups, often referred to as a side chain.

The term "amino acid" includes proteinogenic (or natural) amino acids (amongst those the 20 standard amino acids), as well as non-proteinogenic (or non-natural) amino acids. Proteinogenic amino acids are those which are naturally incorporated into proteins. The standard amino acids are those encoded by the genetic code. Non-proteinogenic amino acids are either not found in proteins, or not produced by standard cellular machinery (e.g., they may have been subject to post-translational modification). Non-limiting examples of non-proteinogenic amino acids are Aib (α -aminoisobutyric acid, or 2-aminoisobutyric acid), norleucine, norvaline as well as the D-isomers of the proteinogenic amino acids.

In what follows, each amino acid of the peptides for which the optical isomer is not stated is to be understood to mean the L-isomer (unless otherwise specified).

The GLP-1/GIP receptor co-agonists described herein comprise or consist of a peptide and a substituent as defined below. In some embodiments, the peptide is a synthetic peptide created to optimize the activity at the GLP-1 and GIP receptors. Compounds having a suitable receptor binding activity towards both the GLP-1 receptor and the GIP receptor have been identified as demonstrated in the examples herein.

The compounds further display an extended half-life gained by the substituent comprising a fatty acid group.

In some embodiments, the carboxy terminus of a peptide holds a -COOH group. In some embodiments, the compounds may optionally include an amide group (C(=O)-NH₂) at the C-terminus, which is a naturally occurring modification substituting -OH with -NH₂, such as seen with native Exendin-4.

Peptide

The GLP-1/GIP receptor co-agonists described herein comprise a peptide and a substituent as defined below, in which the substituent is attached to the peptide backbone via an amino acid residue.

In some embodiments, the amino acid sequence of the peptide is
 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GX₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉
 (SEQ ID NO.: 47)

10 with an optional amide modification of the C-terminus; wherein

X₂ is Aib or A

X₆ is F or V

X₁₂ is I or Y

X₁₃ is Y, A, L or I

15 X₁₆ is K or E

X₂₀ is Q, R, E, H

X₂₁ is A or E

X₂₃ is I or V

X₂₄ is E, Q or N

20 X₂₈ is A or R

X₃₀ is G or absent

X₃₁ is P or absent

X₃₂ is E, S or absent

X₃₃ is S, K or absent

25 X₃₄ is G or absent

X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

X₃₈ is P or absent

30 X₃₉ is S or absent.

In some embodiments, the amino acid sequence of the peptide is
 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉ (SEQ
 ID NO.: 36) with an optional amide modification of the C-terminus;

35 wherein

X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
 5 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
 X₂₄ is E, Q or N
 10 X₂₈ is A or R
 X₃₂ is E, S or absent
 X₃₃ is S, K or absent
 X₃₄ is G or absent
 X₃₅ is A or absent
 15 X₃₆ is P or absent
 X₃₇ is P or absent
 X₃₈ is P or absent
 X₃₉ is S or absent.

20 In one embodiment, X₃₉ is absent. In one embodiment, X₃₈ and X₃₉ are absent. In one embodiment, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one
 25 embodiment, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In one embodiment, X₃₀, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent. In further such embodiments, X₃₂X₃₃X₃₄X₃₅ is SSGA. In further such embodiments, X₃₂X₃₃X₃₄X₃₅ is ESGA. In further such embodiments, X₃₂X₃₃X₃₄X₃₅ is SKGA. In a further embodiment thereof, the peptide has an amide modification of the C-terminus.

30

In one embodiment, the amino acid sequence of the peptide is
 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPSSGAPPPS (SEQ ID NO.: 37)
 wherein

35 X₂ is Aib or A
 X₆ is F or V

X₁₂ is I or Y
X₁₃ is Y, A, L or I
X₁₆ is K or E
X₂₀ is Q, R, E, H
5 X₂₁ is A or E
X₂₃ is I or V
X₂₄ is E, Q or N
X₂₈ is A or R.

10 In one embodiment, the amino acid sequence of the peptide is
YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPESGAPPPS (SEQ ID NO.: 38)

wherein

X₂ is Aib or A
X₆ is F or V
15 X₁₂ is I or Y
X₁₃ is Y, A, L or I
X₁₆ is K or E
X₂₀ is Q, R, E, H
X₂₁ is A or E
20 X₂₃ is I or V
X₂₄ is E, Q or N
X₂₈ is A or R.

In one embodiment, the amino acid sequence of the peptide is
25 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPSKGAPPPS (SEQ ID NO.: 39)

wherein

X₂ is Aib or A
X₆ is F or V
X₁₂ is I or Y
30 X₁₃ is Y, A, L or I
X₁₆ is K or E
X₂₀ is Q, R, E, H
X₂₁ is A or E
X₂₃ is I or V
35 X₂₄ is E, Q or N

X_{28} is A or R.

In one embodiment, X_2 is Aib. In one embodiment, X_2 is A. In one embodiment, X_6 is F. In one embodiment, X_6 is V. In one embodiment, X_{12} is I. In one embodiment, X_{12} is Y. In one embodiment, X_{13} is Y. In one embodiment, X_{13} is A. In one embodiment, X_{13} is L. In one embodiment, X_{13} is I. In one embodiment, X_{16} is K. In one embodiment, X_{16} is E. In one embodiment, X_{20} is Q. In one embodiment, X_{20} is R. In one embodiment, X_{20} is E. In one embodiment, X_{20} is H. In one embodiment, X_{21} is A. In one embodiment, X_{21} is E. In one embodiment, X_{23} is I. In one embodiment, X_{23} is V. In one embodiment, X_{24} is E. In one embodiment, X_{24} is Q. In one embodiment, X_{24} is N. In one embodiment, X_{28} is A. In one embodiment, X_{28} is R. In one embodiment, X_{30} is G. In one embodiment, X_{31} is P.

In one embodiment, $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is selected from the group consisting of: LLEKQAAREFIN, LLEKQAAREFIE, LLEKQAAQEFIE and LLEEQAAREFIE. In one embodiment, $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is LLEKQAAREFIN. In one embodiment, $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is LLEKQAAREFIE. In one embodiment, $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is LLEKQAAQEFIE. In one embodiment, $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is LLEEQAAREFIE.

In a further embodiment, the amino acid sequence of the peptide is any one of SEQ ID NO.: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 33, 34 and 35.

In one embodiment, the amino acid sequence of the peptide is any one of SEQ ID NO.: 10, 22 or 25.

In one embodiment, the amino acid sequence of the peptide is SEQ ID NO.: 25.

In one embodiment, the amino acid sequence of the peptide is any one of SEQ ID NO.: 18, 20, 23, 24.

In one embodiment, the amino acid sequence of the peptide is SEQ ID NO.: 33, 34 or 35.

In further such embodiments, the peptide has an amide modification of the C-terminus.

Derivatives

In some embodiments, the GLP-1 and GIP receptor agonists comprise or consist of a substituent as defined below covalently linked to a peptide.

Such compounds may be referred to as derivatives of the peptide, as they are obtained by covalently linking a substituent to a peptide backbone.

An aspect of the invention relates to a compound comprising a peptide and a
 5 substituent; wherein the amino acid sequence of the peptide is:
 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GX₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉
 (SEQ ID NO.: 47)

with an optional amide modification of the C-terminus, wherein

10 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 15 X₂₁ is A or E
 X₂₃ is I or V
 X₂₄ is E, Q or N
 X₂₈ is A or R
 X₃₀ is G or absent
 20 X₃₁ is P or absent
 X₃₂ is E, S or absent
 X₃₃ is S, K or absent
 X₃₄ is G or absent
 X₃₅ is A or absent
 25 X₃₆ is P or absent
 X₃₇ is P or absent
 X₃₈ is P or absent
 X₃₉ is S or absent;
 and wherein the substituent is attached to the peptide via Lysine (K) at position 16
 30 or 33;
 or a pharmaceutically acceptable salt hereof.

An embodiment of the invention relates to a compound comprising a peptide and a substituent; wherein the amino acid sequence of the peptide is:

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉ (SEQ ID NO.: 36)

with an optional amide modification of the C-terminus, wherein

X₂ is Aib or A

5 X₆ is F or V

X₁₂ is I or Y

X₁₃ is Y, A, L or I

X₁₆ is K or E

X₂₀ is Q, R, E, H

10 X₂₁ is A or E

X₂₃ is I or V

X₂₄ is E, Q or N

X₂₈ is A or R

X₃₂ is E, S or absent

15 X₃₃ is S, K or absent

X₃₄ is G or absent

X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

20 X₃₈ is P or absent

X₃₉ is S or absent;

and wherein the substituent is attached to the peptide via Lysine (K) at position 16

or 33;

or a pharmaceutically acceptable salt hereof.

25

In further embodiments, the peptide may be defined as described herein above.

Substituent

30 In one embodiment, the substituents described herein are attached to the peptide described herein via a lysine (K) residue in position 16 or 33.

In one embodiment, the substituent is attached to the peptide via the epsilon-amino group of a Lysine (K) when said Lysine is included at position 16 or 33.

35 In one embodiment, the substituent is a chemical structure covalently attached to the peptide that is capable of forming non-covalent complexes with plasma albumin, thereby

promoting the circulation of the co-agonist with the blood stream, and also having the effect of protracting the time of action of the co-agonist, due to the fact that the complex of the co-agonist and albumin is only slowly removed by renal clearance.

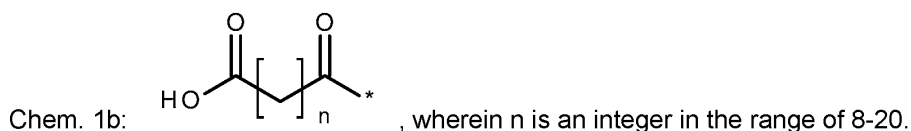
In one embodiment, the substituent comprises a fatty acid group. In such an embodiment, the fatty acid group comprises a carbon chain which contains at least 8 consecutive $-\text{CH}_2-$ groups. In one embodiment, the fatty acid group comprises at least 10 consecutive $-\text{CH}_2-$ groups, such as at least 12 consecutive $-\text{CH}_2-$ groups, at least 14 consecutive $-\text{CH}_2-$ groups, at least 16 consecutive $-\text{CH}_2-$ groups, or such as at least 18 consecutive $-\text{CH}_2-$ groups.

In one embodiment, the fatty acid group comprises 8-20 consecutive $-\text{CH}_2-$ groups. In one embodiment, the fatty acid group comprises 10-18 consecutive $-\text{CH}_2-$ groups. In one embodiment, the fatty acid group comprises 12-18 consecutive $-\text{CH}_2-$ groups. In one embodiment, the fatty acid group comprises 14-18 consecutive $-\text{CH}_2-$ groups.

In some embodiments, the substituent consists of several elements, such as a protractor element and one or more linker elements. In one embodiment, the term "protractor" is used to describe the fatty acid group which is the terminal part of the substituent responsible for extending half-life of the compound.

In one embodiment, the protractor (Prot) may be defined by:

Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which may also be referred to as a $\text{C}(n+2)$ diacid or as



In one embodiment, the substituent further comprises one or more linker elements. In some embodiments, the linker elements are linked to each other and the protractor by amide bonds and referred to as "Z" (see further below).

As further defined herein below the number of linker elements may be at most 3, referred to as $-\text{Z1-Z2-Z3}-$ where Z1 is connected with the protractor (Prot) and the last Z element is connected with the peptide, in which case the substituent may be referred to as $\text{Prot-Z1-Z2-Z3}-$. The symbol * above thus indicates the attachment point to Z1, which when bound via an amide bond is a nitrogen. In an embodiment, where Z1 is a bond (see below), the symbol * indicates the attachment point to the nitrogen of the neighbouring Z element.

In one embodiment, the substituent is defined by: $\text{Prot-Z1-Z2-Z3}-$ wherein Prot- is selected from Chem1, Chem 1b, and wherein n is an integer in the range of 16-20.

In a particular embodiment, n is 14, 15, 16, 17, 18, 19 or 20 in Chem. 1 or Chem. 1b.

In a particular embodiment, n is 14, 15, 16, 17, or 18 in Chem. 1 or Chem. 1b.

In a particular embodiment, n is 14, 16, or 18 in Chem. 1 or Chem. 1b.

5

In a particular embodiment, n is 16, 17, 18, 19 or 20 in Chem. 1 or Chem. 1b.

In a particular embodiment, n is 16, 18 or 20 in Chem. 1 or Chem. 1b.

In a particular embodiment, n is 18 or 20 in Chem. 1 or Chem. 1b.

In a particular embodiment, the protractor (Prot) is a C16 diacid or a C18 diacid.

10

In a particular embodiment, the protractor (Prot) is a C18 diacid or a C20 diacid.

In a particular embodiment, the protractor (Prot) is a C16, C18 diacid or a C20 diacid.

15

The term "bond" as used here means a covalent bond. When a linker element of Z1-Z3 is defined as a bond, it is equivalent to a situation wherein said linker element is absent. The indication herein below that any of Z1-Z3 is a bond may also be read as any of Z1-Z3 being absent, so that the previous Z element is covalently linked to the next Z element that is not "a bond" (or absent).

20

In some embodiments, the linker elements Z1-Z3 are individually selected from chemical moieties capable of forming amide bonds, including amino acid like moieties, such as Glu, γGlu (also termed gamma Glu or gGlu and defined by *-NH-CH-(COOH)-CH₂-CH₂-CO-*), ε-Lys (also termed epsilon Lys or eLys and defined by *-NH-(CH₂)₄-CH(NH₂)-CO-*), Ser, Ala, Thr, Ado, Aeep and Aeep and further moieties as described below.

25

In one embodiment, Z1 is selected from γGlu, Glu, or a bond.

In one embodiment, Z1 is γGlu.

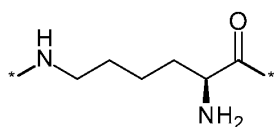
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In one embodiment, Z2 and Z3, are selected, independently of each other, from Glu, ε-Lys, γGlu, Gly, Ser, Ala, Thr, Ado, Aeep, Aeep and a bond.

Glu, Gly, Ser, Ala, Thr are amino acid residues well known in the art.

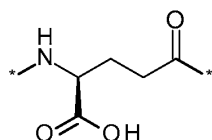
ε-Lys is defined by Chem. 2: *-NH-(CH₂)₄-CH(NH₂)-CO-* which may also be described by

Chem. 2b:



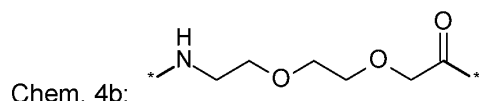
γ Glu is defined by Chem. 3: $^*\text{-NH-CH(COOH)-(CH}_2)_2\text{-CO-}^*$ which may also be described by

Chem. 3b:



5

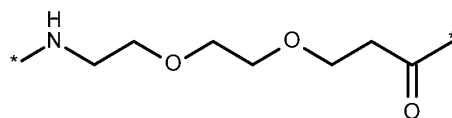
Ado is defined by Chem. 4: $^*\text{-NH-(CH}_2)_2\text{-O-(CH}_2)_2\text{-O-CH}_2\text{-CO-}^*$ may also be referred to as 8-amino-3,6-dioxaoctanoic acid and which may also be described by



10

Chem. 4b:

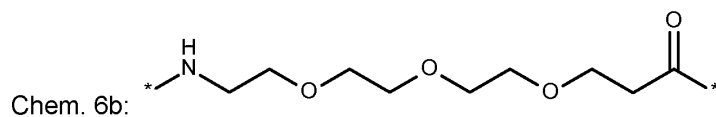
Aeep is defined by Chem. 5: $^*\text{-NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{CO-}^*$, which may also be described by



Chem. 5b:

15

Aeeep is defined of Chem. 6: $^*\text{-NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{CO-}^*$, which may also be described by



Chem. 6b:

20

In one embodiment, Z2 and Z3 are selected, independently of each other, from Glu, ϵ -Lys, γ Glu, Gly, Ala, Ado, Aeep, Aeeep and a bond.

In one embodiment, Z2 and Z3 are selected, independently of each other, from Glu, ϵ -Lys, γ Glu, Gly, Ala, Ado and a bond.

In one embodiment, Z2 and Z3 are selected, independently of each other, from Glu, ϵ -Lys, γ Glu, Gly, Ado and a bond.

In one embodiment, Z2 and Z3 are selected, independently of each other, from ϵ -Lys, γ Glu, Gly, Ado and a bond.

5 In one embodiment, Z2 and Z3 are selected, independently of each other, from ϵ -Lys, γ Glu, Ado and a bond.

In one embodiment, Z2 and Z3 are ϵ -Lys or Ado.

In one embodiment, Z2 and Z3 are Ado.

In one embodiment, Z2 and Z3 are ϵ -Lys.

10 In one embodiment, the substituent is selected from substituents A, B, C, D, and E defined as below.

In one embodiment, the substituent is selected from substituents A, B and C defined as follows:

Substituent #	Prot	Z1	Z2	Z3
A	C18 diacid	γ Glu	Ado	Ado
B	C18 diacid	γ Glu	ϵ Lys	ϵ Lys
C	C20 diacid	γ Glu	ϵ Lys	ϵ Lys
D	C16 diacid	γ Glu	Ado	Ado
E	C16 diacid	γ Glu	ϵ Lys	ϵ Lys

15

In some embodiments, the substituent is covalently attached to a lysine residue of the co-agonist by acylation, i.e. via an amide bond formed between a carboxylic acid group of the substituent and the epsilon-amino group of the lysine residue.

20 In one embodiment, the substituent is covalently attached to a lysine residue in position 16 of the peptide backbone by acylation, i.e., via an amide bond formed between a carboxylic acid group of the substituent and the epsilon amino group of the lysine residue.

In one embodiment, the substituent is covalently attached to a lysine residue in position 33 of the peptide backbone by acylation, i.e., via an amide bond formed between a carboxylic acid group of the substituent and the epsilon amino group of the lysine residue.

25 The co-agonists may exist in different stereoisomeric forms having the same molecular formula and sequence of bonded atoms but differing only in the three-dimensional orientation of their atoms in space. The stereoisomerism of the exemplified co-agonists is

indicated in the experimental section, in the names as well as the structures, using standard nomenclature. Unless otherwise stated the invention relates to all stereoisomeric forms of the embodied derivative.

5 Functional receptor activation activity

The functional activity of the GLP-1/GIP receptor agonists as described herein can be tested *in vitro* as described herein in Example 2.

The term half maximal effective concentration (EC_{50}) generally refers to the concentration which induces a response halfway between the baseline and maximum, by
10 reference to the dose response curve. EC_{50} is used as a measure of the potency of a compound and represents the concentration where 50% of its maximal effect is observed.

The *in vitro* potency of compounds may thus be determined as described herein and the EC_{50} determined. The lower the EC_{50} value, the better the potency.

In order to characterize such compounds, it may further be relevant to consider the
15 *in vitro* potencies relative to the native hormones of each receptor.

The *in vitro* potency may, e.g., be determined in a medium containing membranes expressing the appropriate GLP-1 and/or GIP receptor, and/or in an assay with whole cells expressing the appropriate GLP-1 and/or GIP receptor.

For example, the functional response of the human GLP-1 and/or GIP receptor may
20 be measured in a reporter gene assay, e.g. in a stably transfected BHK cell line that expresses the human GLP-1 and/or GIP receptor and contains the DNA for the cAMP response element (CRE) coupled to a promoter and the gene for firefly luciferase (CRE luciferase). When cAMP is produced as a result of activation of the GLP-1 and/or GIP receptor, this in turn results in luciferase being expressed. Luciferase may be determined by
25 adding luciferin, which by the enzyme is converted to oxyluciferin and produces bioluminescence, which is measured as a reporter of the *in vitro* potency. One example of such an assay is described in Example 2 as described herein. Since the compounds may include a substituent designed to bind albumin, it is also important to note that the receptor activity may be affected by the presence or absence of human serum albumin (HSA) in the
30 assay medium. A decrease in potency of the compound in the presence of HSA, indicated by an increase in EC_{50} compared to the EC_{50} in the absence of HSA, indicates interaction of the compounds with HSA and predicts a protracted time of action *in vivo*.

In one embodiment, the compounds have potent *in vitro* effects to activate the human GLP-1 and GIP receptors.

In one embodiment, the compounds are capable of activating the human GLP-1 and GIP receptors *in vitro* with an EC₅₀ of less than 20 pM in CRE luciferase reporter assays as described in Example 2 herein, when performed without HSA.

5 In one embodiment, the compounds have an *in vitro* potency at the human GLP-1 and GIP receptors determined using the method of Example 2 corresponding to an EC₅₀ at or below 100 pM, more preferably below 50 pM, or most preferably below 20 pM.

In one embodiment, the EC₅₀ in human GLP-1 and GIP receptors assays are both 1-25 pM, such as 1-20 pM, such as 1-15 pM or such as 1-10 pM.

10 Pharmacokinetics properties

The pharmacokinetic properties of the co-agonistic compounds may further be determined *in vivo* via pharmacokinetic (PK) studies. Animal models such as the mouse, rat, monkey, dog, or pig may be used to perform this characterization.

15 In such studies, animals are typically administered with a single dose of the drug, either intravenously, subcutaneously (s.c.), or orally (p.o.) in a relevant formulation. Blood samples are drawn at predefined time points after dosing, and samples are analysed for concentration of drug with a relevant quantitative assay. Based on these measurements, time-plasma concentration profiles for the compound of study are plotted and a so-called non-compartmental pharmacokinetic analysis of the data is performed. An important
20 parameter is the terminal half-life as a long half-life indicates that less frequent administration of a compound may be possible. The terminal half-life (t_{1/2}) *in vivo* may be measured using a suitable model, such as after i.v. administration in minipigs described in Example 3; or after p.o administration in dogs described in Example 4.

25 In one embodiment, the terminal half-life is half-life (t_{1/2}) *in vivo* in minipigs after i.v. administration, e.g. as described in Example 3 herein.

In one embodiment, the terminal half-life in minipigs is at least 24 hours, such as at least 30 hours, or such as at least 40 hours.

In one embodiment, the terminal half-life is half-life (t_{1/2}) *in vivo* in dogs after p.o. administration, e.g. as described in Example 4 herein.

30 In one embodiment, the terminal half-life in dogs is at least 24 hours, such as at least 40 hours, or such as at least 50 hours.

Pharmaceutically acceptable salts

In some embodiments, the co-agonists as described herein are in the form of a pharmaceutically acceptable salt. Salts are e.g. formed by a chemical reaction between a base and an acid, e.g.: $2\text{NH}_3 + \text{H}_2\text{SO}_4 \rightarrow (\text{NH}_4)_2\text{SO}_4$. The salt may be a basic salt, an acid salt, or it may be neither (i.e. a neutral salt). Basic salts produce hydroxide ions and acid salts hydronium ions in water. The salts of the co-agonists may be formed with added cations or anions between anionic or cationic groups, respectively. These groups may be situated in the peptide and/or in the substituent of the derivatives. Non-limiting examples of anionic groups include any free carboxylic acid groups in the substituent, if any, as well as in the peptide. The peptide may include a free carboxylic acid group at the C-terminus, if present, as well as any free carboxylic acid group of internal acidic amino acid residues such as Asp and Glu.

Non-limiting examples of cationic groups include any free amino groups in the substituent, if any, as well as in the peptide. The peptide may include a free amino group at the N-terminus, if present, as well as any free imidazole or amino group of internal basic amino acid residues such as His, Arg, and Lys.

In a particular embodiment, the peptide or derivative is in the form of a pharmaceutically acceptable salt.

20 Production processes

The co-agonists may for instance be produced by classical peptide synthesis, e.g. solid phase peptide synthesis using t-Boc or Fmoc chemistry or other well established techniques, see e.g. Greene and Wuts, "Protective Groups in Organic Synthesis", John Wiley & Sons, 1999; Florencio Zaragoza Dörwald, "Organic Synthesis on Solid Phase", Wiley-VCH Verlag GmbH, 2000; and "Fmoc Solid Phase Peptide Synthesis", Edited by W.C. Chan and P.D. White, Oxford University Press, 2000.

Alternatively, the compounds may be produced by recombinant methods, e.g. by culturing a host cell containing a DNA sequence encoding the peptide sequence and capable of expressing the peptide, in a suitable nutrient medium under conditions permitting the expression of the peptide. Non-limiting examples of host cells suitable for expression of these peptides are: *Escherichia coli*, *Saccharomyces cerevisiae*, as well as mammalian BHK or CHO cell lines.

The co-agonists that include non-natural amino acids and/or covalently attached substituents may be produced as described in the experimental part.

Specific examples of methods of preparing a number of co-agonists are included in the experimental part.

A further aspect of the invention relates to a method for preparing the peptides described herein.

5 A further aspect of the invention relates to a method for preparing the GLP-1/GIP co-agonists described herein.

In one embodiment, the method for preparing a compound as described herein comprises a step of solid phase peptide synthesis. The substituent may be built sequentially as part of the solid phase peptide synthesis or produced separately and attached via the
10 lysine residue after peptide synthesis.

In one embodiment, the compounds are produced by a two-step process whereby two peptide fragments are ligated after attachment of the substituent to one of the peptide fragments.

15 **Pharmaceutical compositions**

In a further aspect the invention relates to a pharmaceutical composition comprising a GLP-1/GIP receptor co-agonist as described herein. Compositions comprising the compound or a pharmaceutically acceptable salt hereof, and optionally one or more a pharmaceutically acceptable excipients may be prepared as is known in the art.

20 The term "excipient" broadly refers to any component other than the active therapeutic ingredient(s). The excipient may be an inert substance, an inactive substance, and/or a not medicinally active substance. The excipient may serve various purposes, e.g. as a carrier, vehicle, filler, binder, lubricant, glidant, disintegrant, flow control agent, crystallization inhibitor, solubilizer, stabilizer, coloring agent, flavoring agent, surfactant,
25 emulsifier or combinations thereof and/or to improve administration, and/or to improve absorption of the active substance. The amount of each excipient used may vary within ranges conventional in the art. Techniques and excipients which may be used to formulate oral dosage forms are described in Handbook of Pharmaceutical Excipients (e.g. 8th edition, Sheskey et al., Eds., American Pharmaceuticals Association and Pharmaceutical Press,
30 publications department of the Royal Pharmaceutical Society of Great Britain (2017), and any later editions); and Remington: The Science and Practice of Pharmacy (e.g. 22nd edition, Remington and Allen, Eds., Pharmaceutical Press (2013), and any later editions).

In one embodiment, the pharmaceutical composition may be a solid formulation, e.g. a freeze-dried or spray-dried composition, which may be used as is, or whereto the physician

or the patient adds solvents, and/or diluents prior to use. In a further embodiment, the pharmaceutical composition may be a solid formulation consisting of the active ingredient, a salt of *N*-[8-(2-hydroxybenzoyl)amino]caprylate, and one or more further excipients as is known in the art, e.g. using any one or more of the formulations described in WO

5 2012/080471, WO 2013/189988, or WO 2019/149880.

Alternatively, the pharmaceutical composition is a liquid formulation, such as an aqueous formulation. Liquid compositions, suitable for injection, can be prepared using conventional techniques of the pharmaceutical industry which involve dissolving and mixing the ingredients as appropriate to give the desired end product. Thus, according to one
10 procedure, a GLP-1/GIP co-agonist as described herein is dissolved in a suitable buffer at a suitable pH. The composition may be sterilized, for example, by sterile filtration.

Pharmaceutical Indications

A further aspect of the invention relates to the use of GLP-1/GIP receptor co-agonist
15 compounds as described herein as a medicament.

In one embodiment, the compounds described herein are for use in the following medical treatments:

- (i) prevention and/or treatment of all forms of diabetes, such as hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent
20 diabetes, MODY (maturity onset diabetes of the young), gestational diabetes, and/or for reduction of HbA1C;
- (ii) delaying or preventing diabetic disease progression, such as progression in type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin requiring type 2 diabetes, delaying or preventing insulin resistance, and/or delaying
25 the progression of non-insulin requiring type 2 diabetes to insulin requiring type 2 diabetes;
- (iii) prevention and/or treatment of eating disorders, such as obesity, e.g. by decreasing food intake, reducing body weight, suppressing appetite, inducing satiety; treating or preventing binge eating disorder, bulimia nervosa, and/or obesity induced by
30 administration of an antipsychotic or a steroid; reduction of gastric motility; delaying gastric emptying; increasing physical mobility; and/or prevention and/or treatment of comorbidities to obesity, such as osteoarthritis and/or urine incontinence;
- (iv) weight maintenance after successful weight loss (either drug induced or by diet and exercise) – i.e. prevention of weight gain after successful weight loss.

- (v) prevention and/or treatment of liver disorders, such as hepatic steatosis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver inflammation or fatty liver.

In one embodiment, the compounds are for use in a method for prevention and/or treatment of diabetes and/or obesity.

In one embodiment, the compounds are for use in a method for treatment of diabetes and/or obesity.

In one embodiment, the compounds are for use in a method for treatment or prevention of type 2 diabetes.

In one embodiment, the compounds are for use in a method for treatment of type 2 diabetes.

In one embodiment, the compounds are for use in a method for treatment or prevention of obesity.

In one embodiment, the compounds are for use in a method for treatment of obesity.

In one embodiment, the compounds are for use in a method for weight management. In one embodiment, the compounds are for use in a method for reduction of appetite. In one embodiment, the compounds are for use in a method for reduction of food intake.

20

EMBODIMENTS

1. A compound comprising a peptide and a substituent; wherein the amino acid sequence of the peptide is
 $\text{YX}_2\text{EGTX}_6\text{TSDYSX}_{12}\text{X}_{13}\text{LEX}_{16}\text{QAAX}_{20}\text{X}_{21}\text{FX}_{23}\text{X}_{24}\text{WLLX}_{28}\text{GX}_{30}\text{X}_{31}\text{X}_{32}\text{X}_{33}\text{X}_{34}\text{X}_{35}\text{X}_{36}\text{X}_{37}\text{X}_{38}\text{X}_{39}$ (SEQ ID NO.: 47)
with an optional amide modification of the C-terminus, wherein
 X_2 is Aib or A
 X_6 is F or V
 X_{12} is I or Y
 X_{13} is Y, A, L or I
 X_{16} is K or E
 X_{20} is Q, R, E, H
 X_{21} is A or E

X₂₃ is I or V

X₂₄ is E, Q or N

X₂₈ is A or R

X₃₀ is G or absent

5 X₃₁ is P or absent

X₃₂ is E, S or absent

X₃₃ is S, K or absent

X₃₄ is G or absent

X₃₅ is A or absent

10 X₃₆ is P or absent

X₃₇ is P or absent

X₃₈ is P or absent

X₃₉ is S or absent;

15 and wherein the substituent is attached to the peptide via Lysine (K) at position 16 or 33;

or a pharmaceutically acceptable salt hereof.

2. The compound according to embodiment 1, comprising a peptide and a substituent; wherein the amino acid sequence of the peptide is

20 YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉ (SEQ ID NO.: 36)

with an optional amide modification of the C-terminus, wherein

X₂ is Aib or A

X₆ is F or V

25 X₁₂ is I or Y

X₁₃ is Y, A, L or I

X₁₆ is K or E

X₂₀ is Q, R, E, H

X₂₁ is A or E

30 X₂₃ is I or V

X₂₄ is E, Q or N

X₂₈ is A or R

X₃₂ is E, S or absent

X₃₃ is S, K or absent

35 X₃₄ is G or absent

X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

X₃₈ is P or absent

5 X₃₉ is S or absent;

and wherein the substituent is attached to the peptide via Lysine (K) at position 16 or 33;

or a pharmaceutically acceptable salt hereof.

- 10 3. The compound according to embodiment 1, wherein X₃₆, X₃₇, X₃₈ and X₃₉ are absent.
4. The compound according to embodiment 1, wherein X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent.
- 15 5. The compound according to embodiment 1, wherein X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent.
6. The compound according to embodiment 1, wherein X₃₀, X₃₁, X₃₂, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent.
- 20 7. The compound according to any one of embodiments 1-6, wherein the peptide has the amide modification of the C-terminus.
8. The compound according to any of the previous embodiments 1, 2 or 7, wherein
- 25 X₃₂X₃₃X₃₄X₃₅ is selected from the group consisting of SSGA, ESGA and SKGA.
9. The compound according to any of the previous embodiments 1, 2 or 7, wherein X₃₂X₃₃X₃₄X₃₅ SSGA.
- 30 10. The compound according to any of the previous embodiments 1, 2 or 7, wherein X₃₂X₃₃X₃₄X₃₅ is ESGA.
11. The compound according to any of the previous embodiments 1, 2 or 7, wherein
- 35 X₃₂X₃₃X₃₄X₃₅ is SKGA.

12. The compound according to embodiment 1, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPSSGAPPPS (SEQ ID NO.: 37)

5 wherein
 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
10 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
 X₂₄ is E, Q or N
15 X₂₈ is A or R.

13. The compound according to embodiment 1, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPESGAPPPS (SEQ ID NO.: 38)

20 wherein
 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
25 X₁₃ is Y, A, L or I
 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
30 X₂₄ is E, Q or N
 X₂₈ is A or R.

14. The compound according to embodiment 1, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPSKGAPPPS (SEQ ID NO.: 39)

wherein

X₂ is Aib or A

5 X₆ is F or V

X₁₂ is I or Y

X₁₃ is Y, A, L or I

X₁₆ is K or E

X₂₀ is Q, R, E, H

10 X₂₁ is A or E

X₂₃ is I or V

X₂₄ is E, Q or N

X₂₈ is A or R.

15 15. The compound according to any of the previous embodiments, wherein

X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄ is selected from the group consisting of:

LLEKQAAREFIN, LLEKQAAREFIE, LLEKQAAQEFIE and LLEEQAAREFIE.

20 16. The compound according to embodiment 1, wherein the amino acid sequence of the peptide is any one of SEQ ID NO.: 1-27 or 33-35.

17. The compound according to embodiment 16, wherein the peptide has the amide modification of the C-terminus.

25 18. The compound according to any of the previous embodiments, wherein the compound activates the human GLP-1 and GIP receptors *in vitro* with an EC₅₀ of less than 20 pM when measured without HSA in CRE luciferase reporter assays as described in Example 2.

30 19. The compound according to any of the previous embodiments, wherein the compound has a half-life in minipigs of at least 35 hours.

20. The compound according to any of the previous embodiments, wherein the substituent is attached via 16Lys.

35

21. The compound according to any of the previous embodiments, wherein the substituent is attached via 33Lys.
22. The compound according to any of the previous embodiments, wherein the substituent comprises at least one protractor.
23. The compound according to embodiment 22, wherein the protractor is a fatty acid group.
24. The compound according to embodiment 23, wherein the protractor is a diacid defined by Chem. 1: $\text{HOOC}-(\text{CH}_2)_n\text{-CO-}$ wherein n is an integer in the range of 8-20, such as n= 14, 16 or 18.
25. The compound according to embodiment 23, wherein the protractor is a diacid defined by Chem. 1: $\text{HOOC}-(\text{CH}_2)_n\text{-CO-}$ wherein n is an integer in the range of 8-20, such as n= 16 or 18.
26. The compound according to any of the previous embodiments, wherein the substituent comprises at least one linker element.
27. The compound according to embodiment 26, wherein the substituent comprises at most three linker elements.
28. The compound according to embodiment 27, wherein the substituent comprises at most three linker elements referred to as -Z1-Z2-Z3-, where -Z1- is connected with the protractor and -Z3- is connected to the peptide.
29. The compound according to any of the previous embodiments, wherein the substituent is :
- Prot-Z1-Z2-Z3-
- wherein
- Prot is a C16-C20 diacid
- Z1 is γGlu or a bond
- Z2 is ϵLys , γGlu or Ado and
- Z3 is ϵLys or Ado.

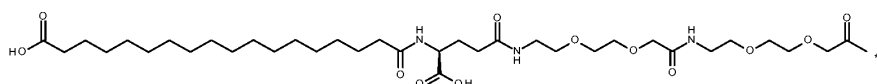
30. The compound according to embodiment 29, wherein -Z1- is -γGlu-.

31. The compound according to embodiments 29 or 30, wherein -Z2-Z3- and is -Ado-.

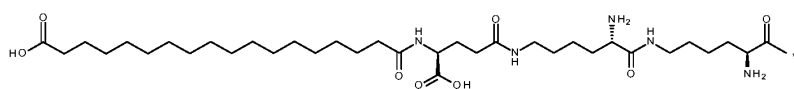
32. The compound according to embodiments 29, 30 or 31, wherein -Z2-Z3- and is -εLys-εLys-.

33. The compound according to any of the embodiments 1-17, wherein the substituent is selected from the group consisting of:

A:

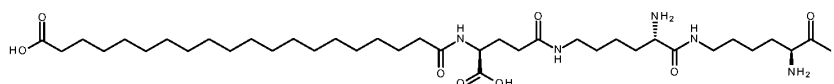


B:



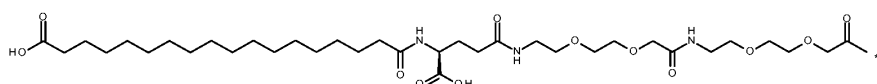
and

C:

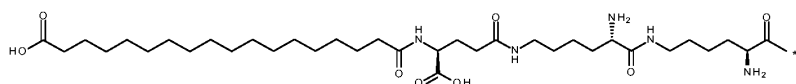


34. The compound according to any of the embodiments 1-17, wherein the substituent is selected from the group consisting of:

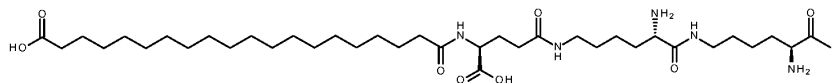
A:



B:

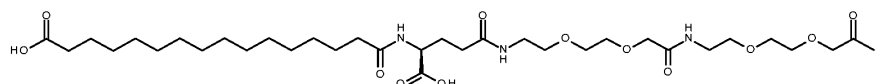


C:



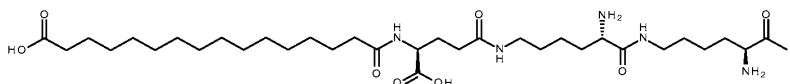
5

D:



and

E:



10

35. The compound according to any of the previous embodiments 1-17, wherein the substituent is attached to the peptide via the epsilon-amino group of a Lysine (K).

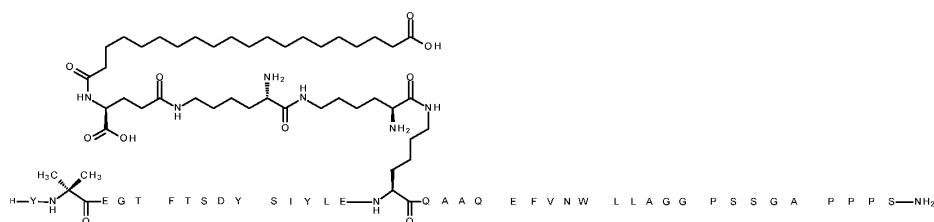
15

36. The compound according to embodiment 1, wherein the compound is selected from the group consisting of:

Compound No. 1

SEQ ID NO: 1

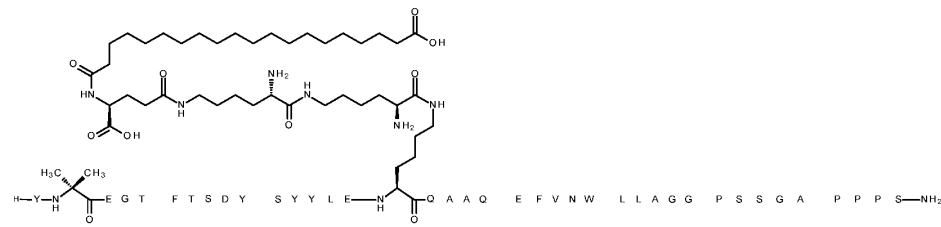
Substituent: C



Compound No. 2

SEQ ID NO: 2

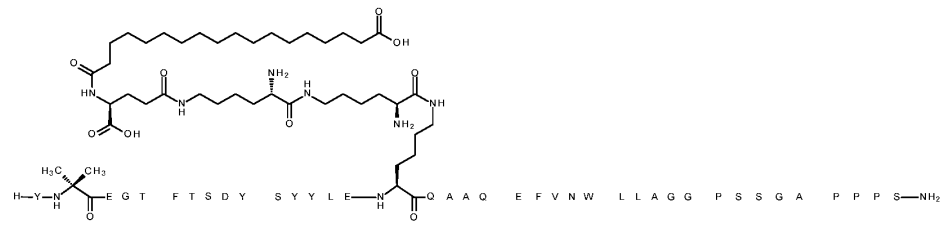
Substituent: C



Compound No. 3

SEQ ID NO: 2

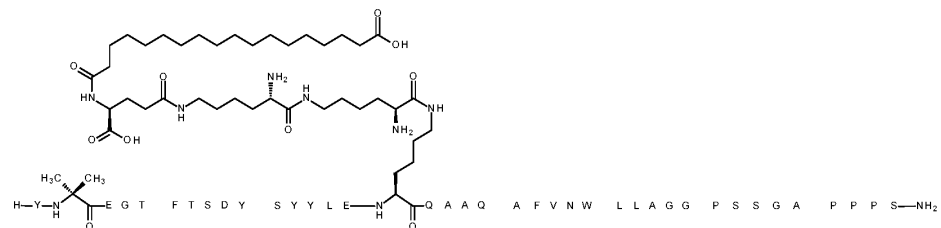
Substituent: B



Compound No. 4

SEQ ID NO: 3

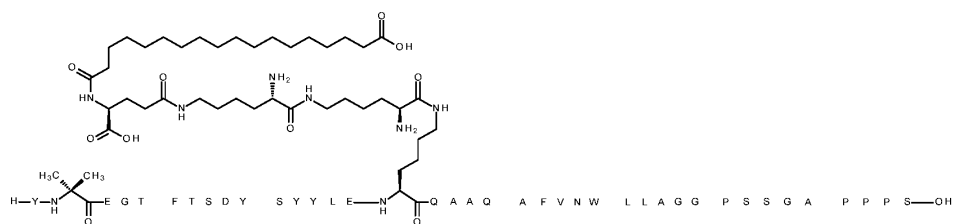
Substituent: B



Compound No. 5

SEQ ID NO: 3

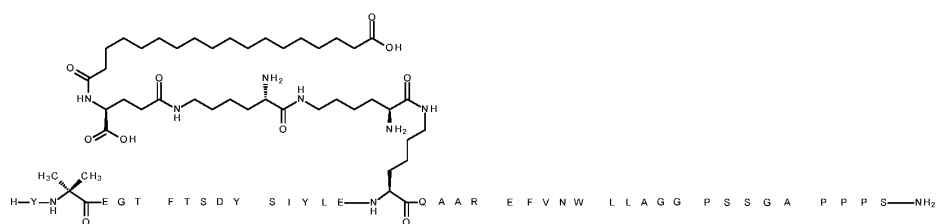
Substituent: B



Compound No. 6

SEQ ID NO: 4

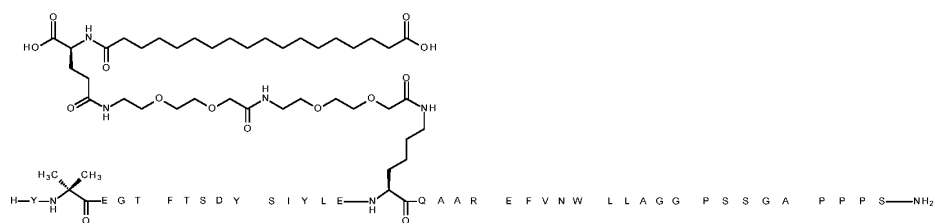
Substituent: B



Compound No. 7

SEQ ID NO: 4

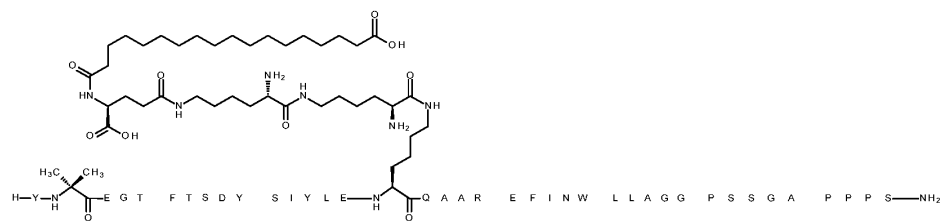
Substituent: A



Compound No. 8

SEQ ID NO: 5

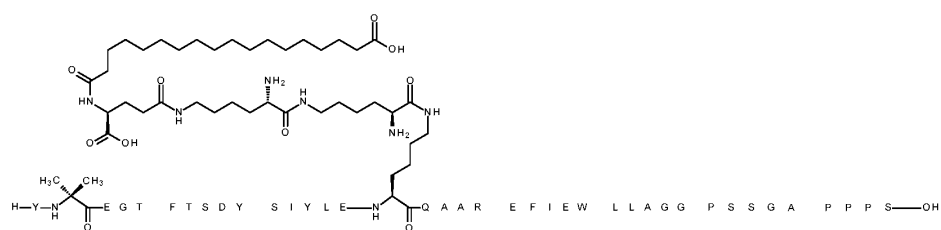
Substituent: B



Compound No. 9

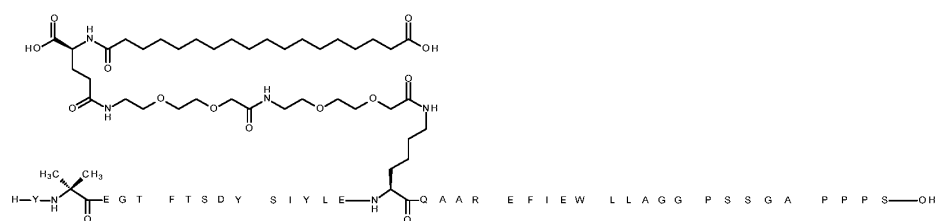
SEQ ID NO: 6

Substituent: B

**Compound No. 10**

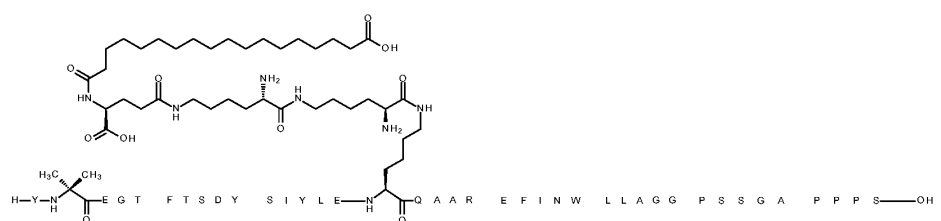
SEQ ID NO: 6

Substituent: A

**Compound No. 11**

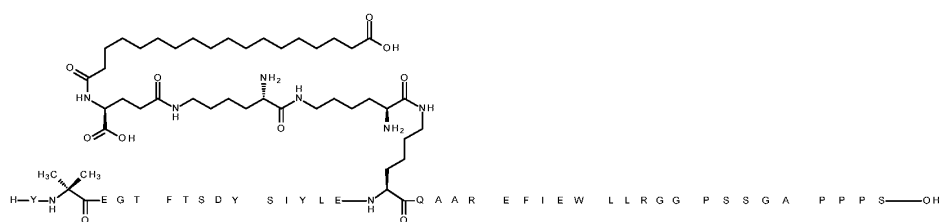
SEQ ID NO: 5

Substituent: B

**Compound No. 12**

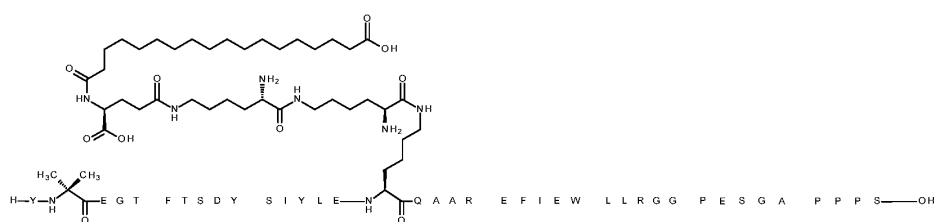
SEQ ID NO: 7

Substituent: B

**Compound No. 13**

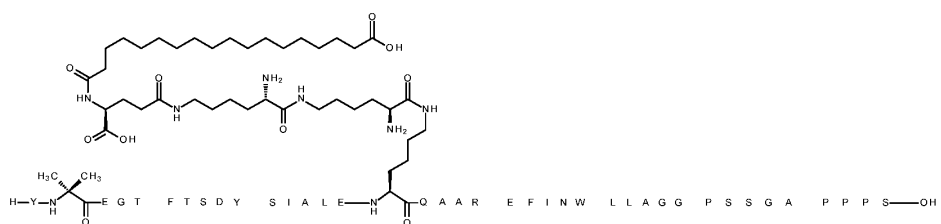
SEQ ID NO: 8

Substituent: B

**Compound No. 14**

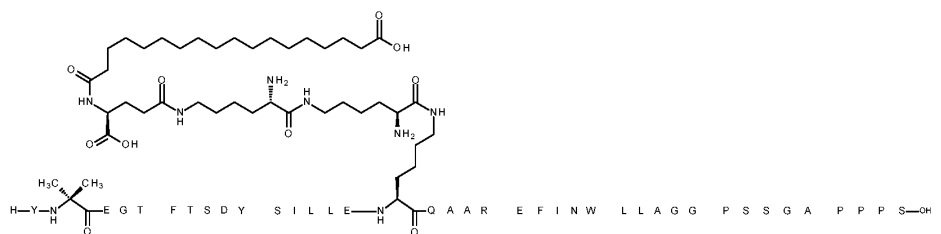
SEQ ID NO: 9

Substituent: B

**Compound No. 15**

SEQ ID NO: 10

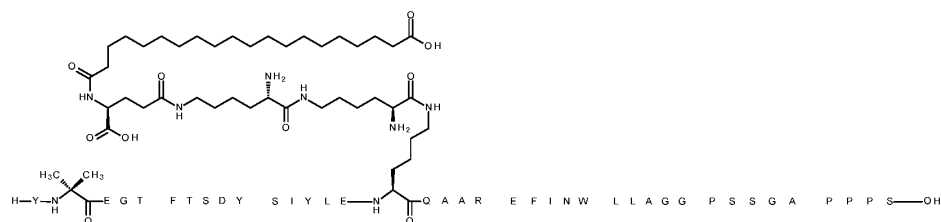
Substituent: B



Compound No. 16

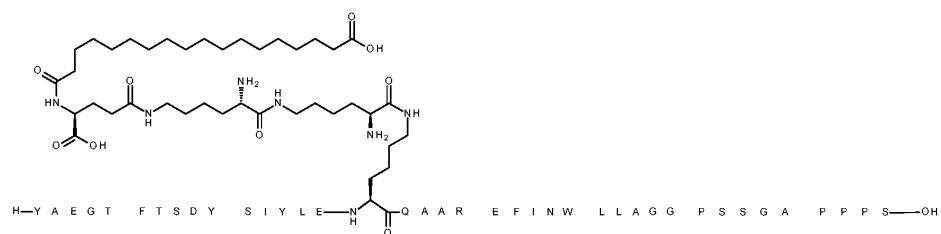
SEQ ID NO: 5

Substituent: C

**Compound No. 17**

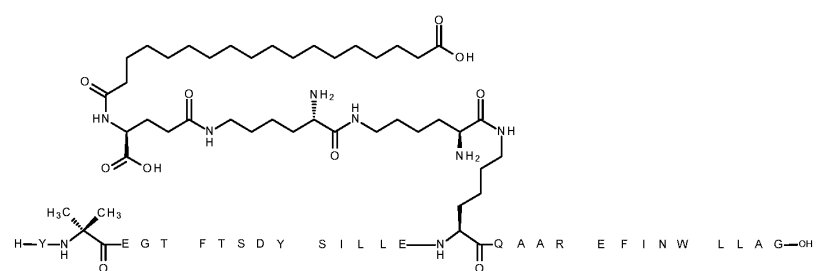
SEQ ID NO: 11

Substituent: B

**Compound No. 18**

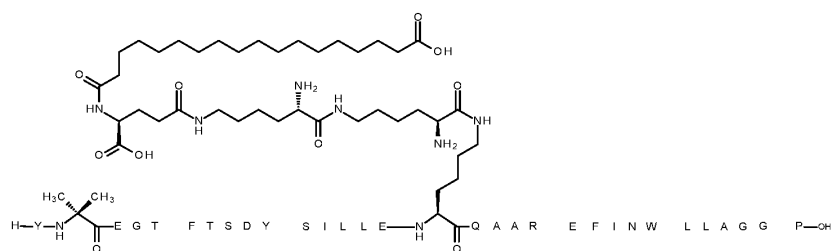
SEQ ID NO: 12

Substituent: B

**Compound No. 19**

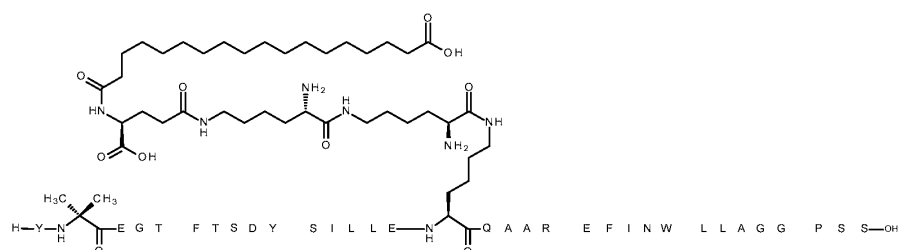
SEQ ID NO: 13

Substituent: B

**Compound No. 20**

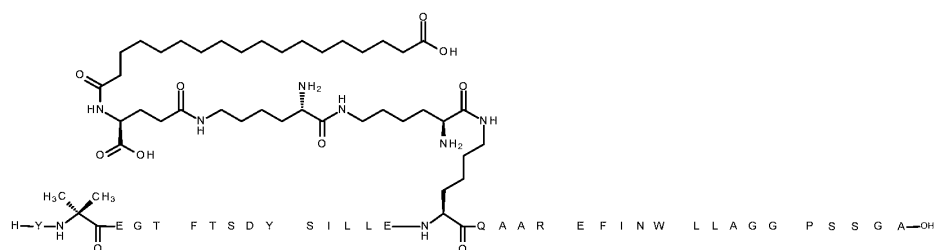
SEQ ID NO: 14

Substituent: B

**Compound No. 21**

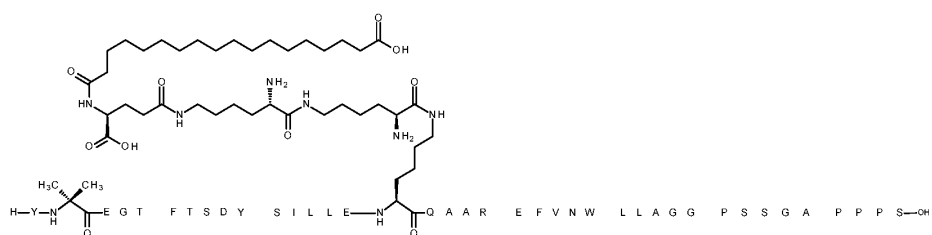
SEQ ID NO: 15

Substituent: B

**Compound No. 22**

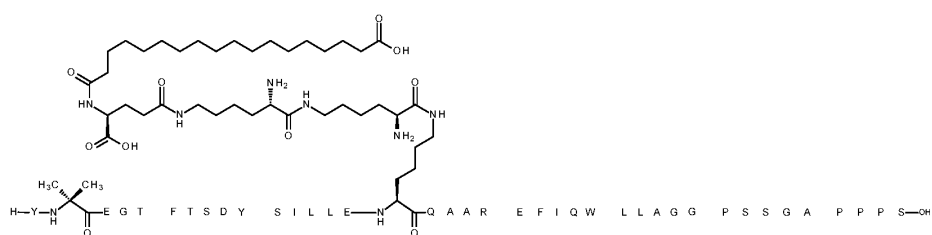
SEQ ID NO: 16

Substituent: B

**Compound No. 23**

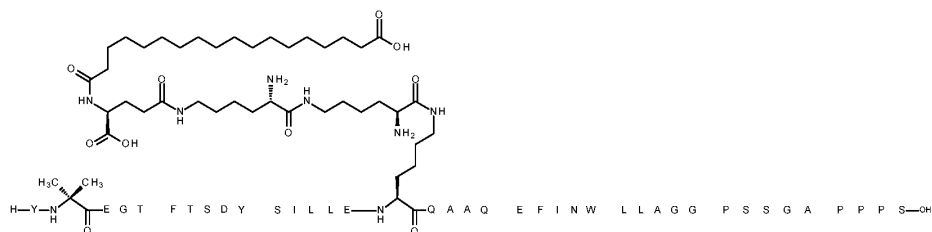
SEQ ID NO: 17

Substituent: B

**Compound No. 24**

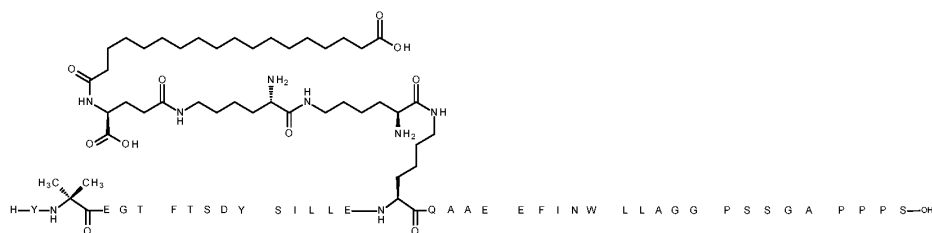
SEQ ID NO: 18

Substituent: B

**Compound No. 25**

SEQ ID NO: 19

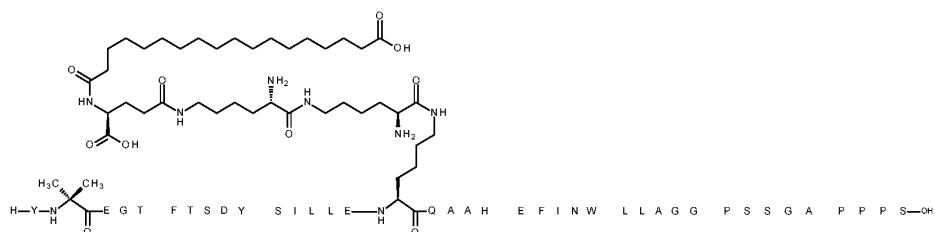
Substituent: B



Compound No. 26

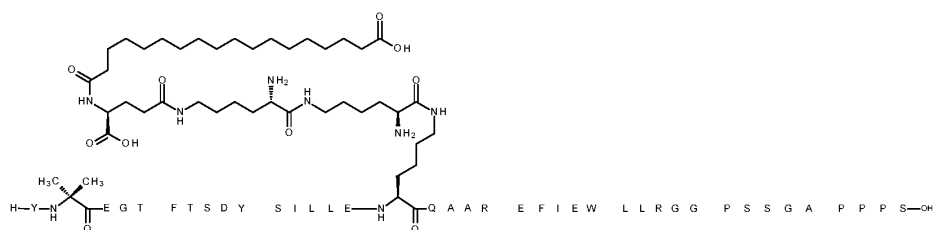
SEQ ID NO: 20

Substituent: B

**Compound No. 27**

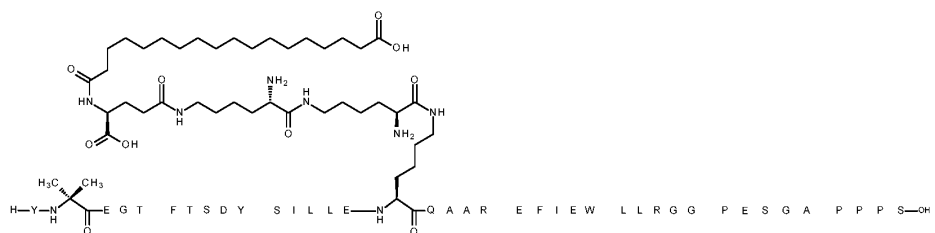
SEQ ID NO: 21

Substituent: B

**Compound No. 28**

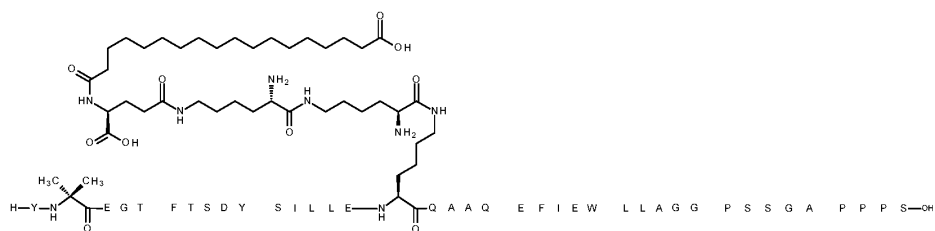
SEQ ID NO: 22

Substituent: B

**Compound No. 29**

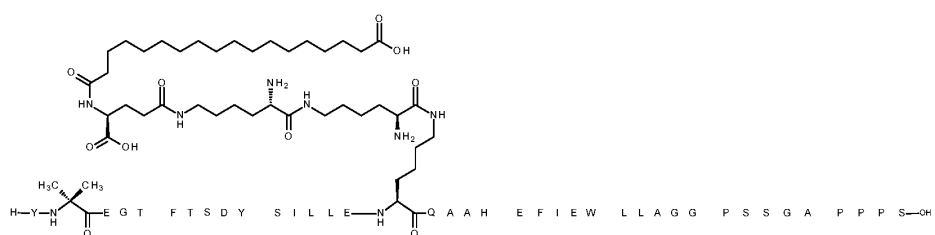
SEQ ID NO: 23

Substituent: B

**Compound No. 30**

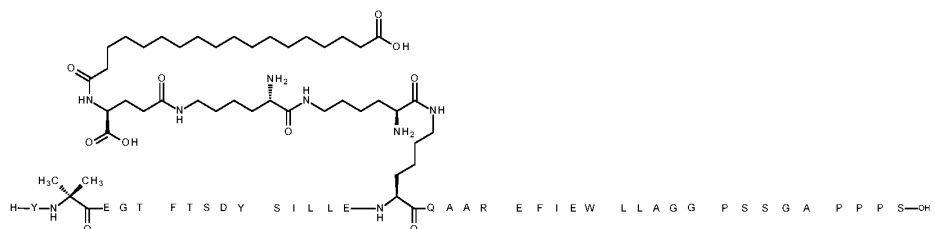
SEQ ID NO: 24

Substituent: B

**Compound No. 31**

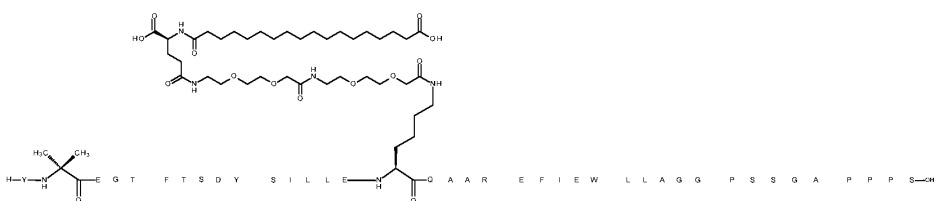
SEQ ID NO: 25

Substituent: B

**Compound No. 32**

SEQ ID NO: 25

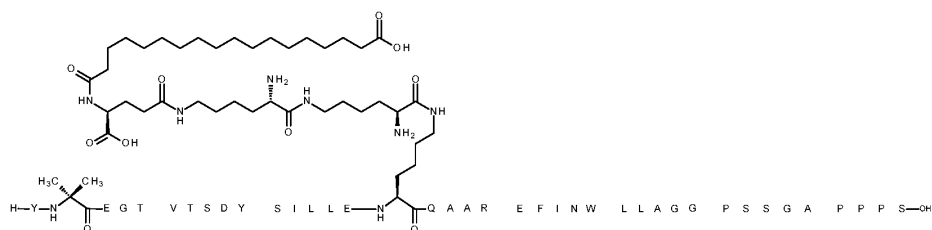
Substituent: A



Compound No. 33

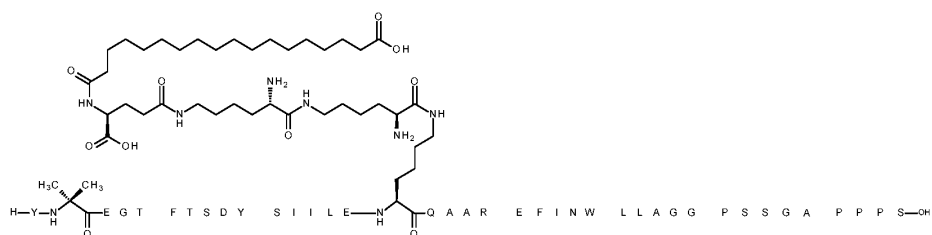
SEQ ID NO: 26

Substituent: B

**Compound No. 34**

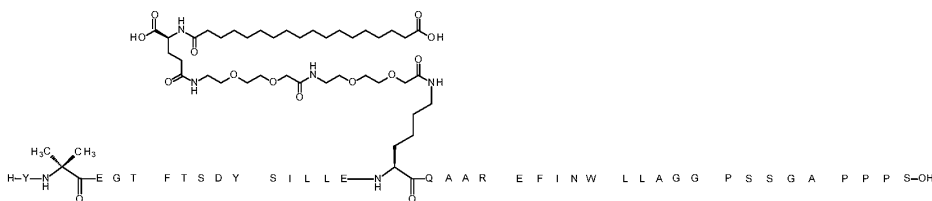
SEQ ID NO: 27

Substituent: B

**Compound No. 35**

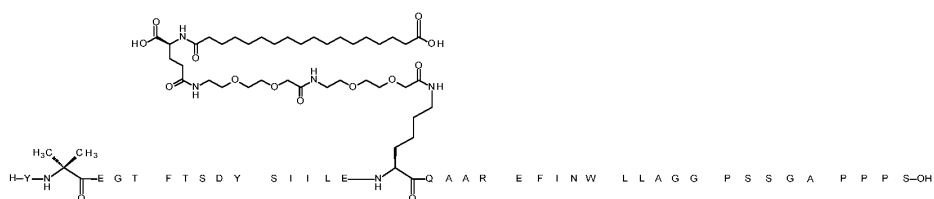
SEQ ID NO: 10

Substituent: A

**Compound No. 36**

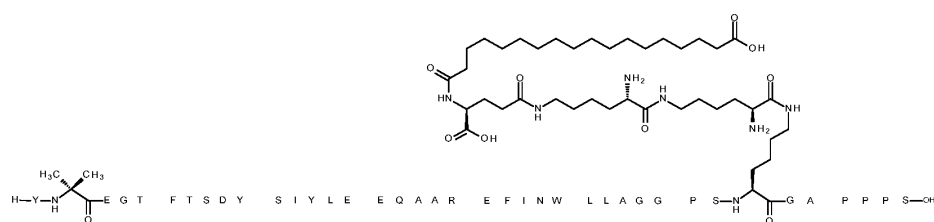
SEQ ID NO: 27

Substituent: A

**Compound No. 42**

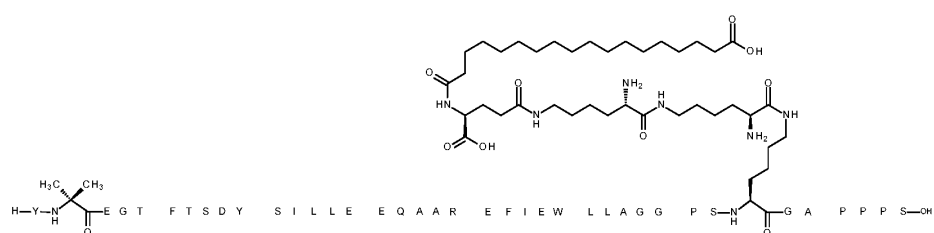
SEQ ID NO: 33

Substituent: B

**Compound No. 43**

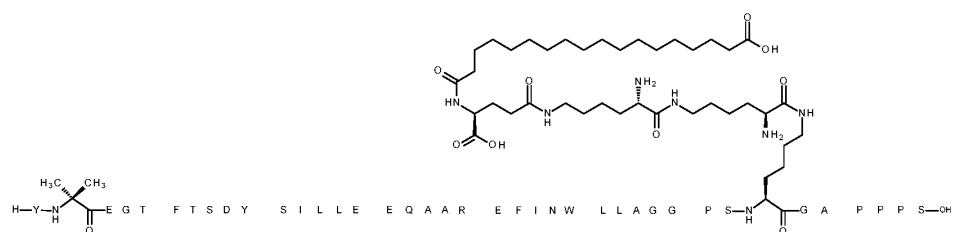
SEQ ID NO: 34

Substituent: B

**Compound No. 44**

SEQ ID NO: 35

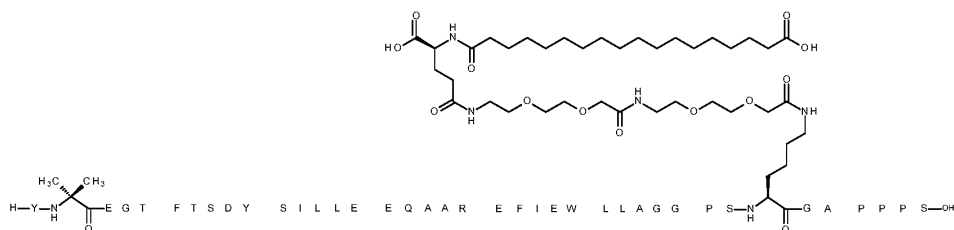
Substituent: B



Compound No. 45

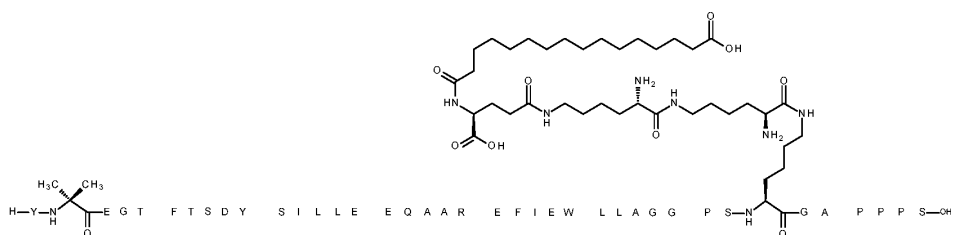
SEQ ID NO: 34

Substituent: A

**Compound No. 46**

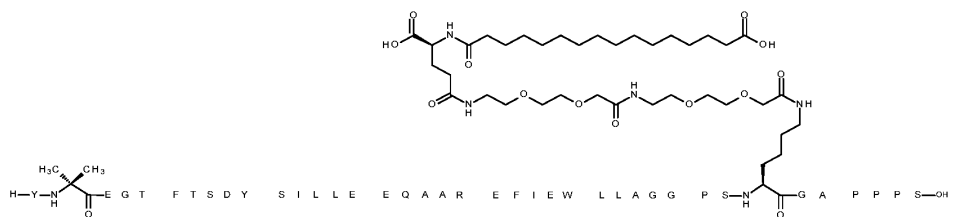
SEQ ID NO: 34

Substituent: E

**Compound No. 47**

SEQ ID NO: 34

Substituent: D

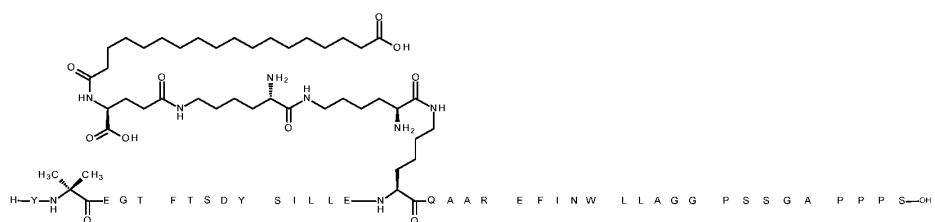


37. The compound according to embodiment 1, wherein the compound is selected from the group consisting of:

Compound No. 15

SEQ ID NO: 10

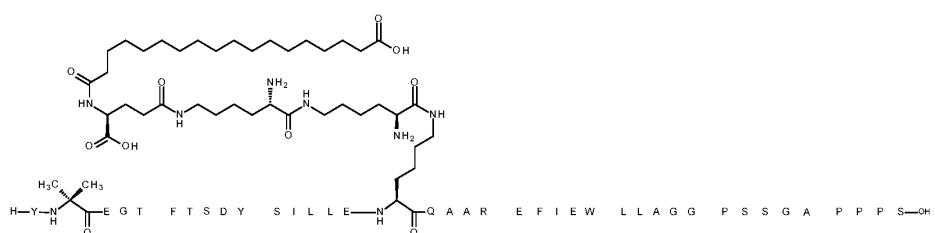
Substituent: B



Compound No. 31

SEQ ID NO: 25

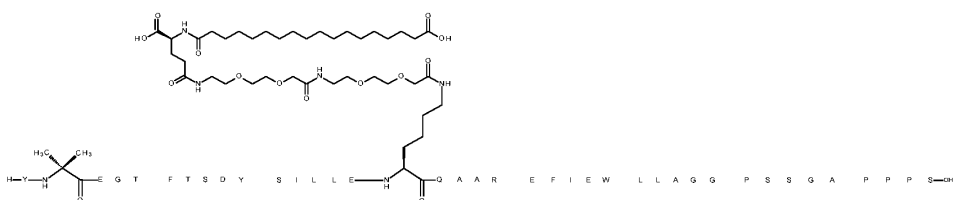
Substituent: B



Compound No. 32

SEQ ID NO: 25

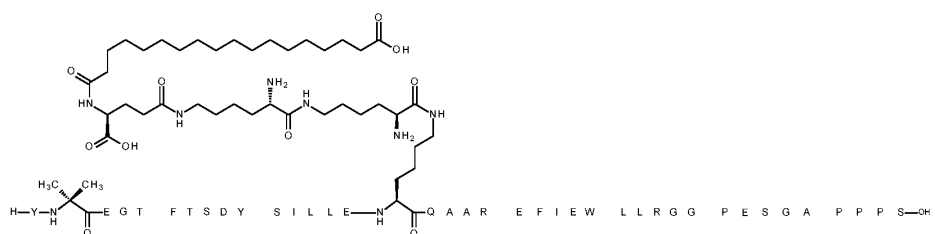
Substituent: A



Compound No. 28

SEQ ID NO: 22

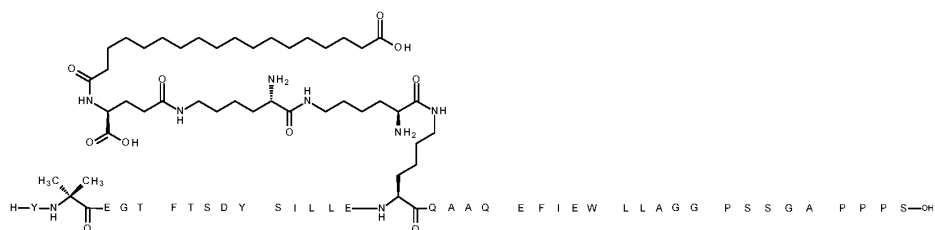
Substituent: B



Compound No. 29

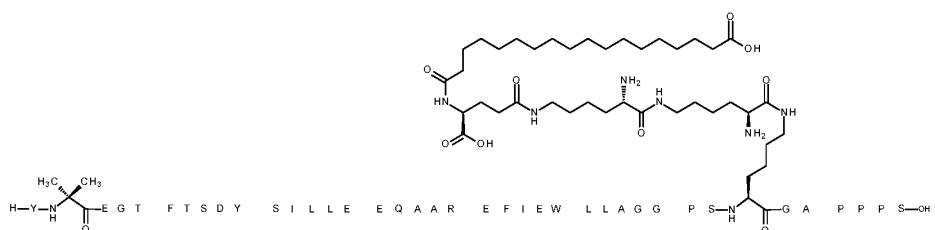
SEQ ID NO: 23

Substituent: B

**Compound No. 43**

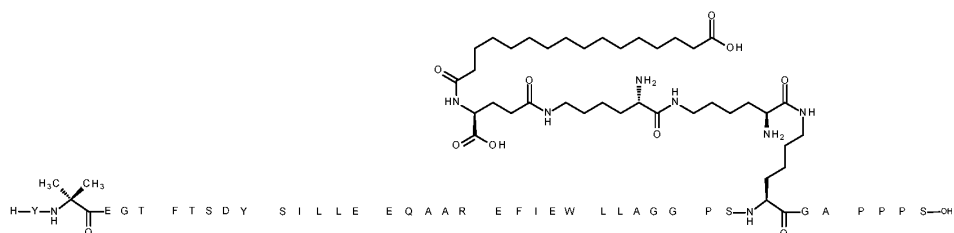
SEQ ID NO: 34

Substituent: B

**Compound No. 46**

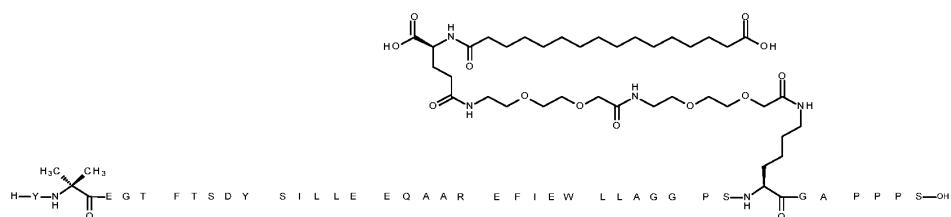
SEQ ID NO: 34

Substituent: E

**Compound No. 47**

SEQ ID NO: 34

Substituent: D

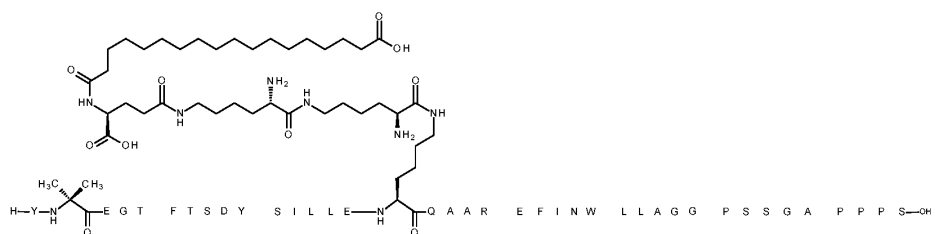


38. The compound according to embodiment 1, wherein the compound is selected from the group consisting of:

Compound No. 15

SEQ ID NO: 10

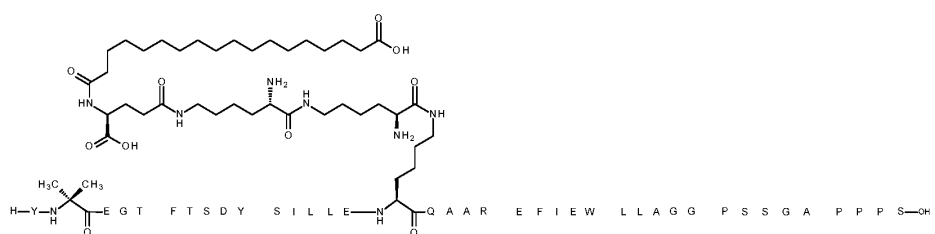
Substituent: B



Compound No. 31

SEQ ID NO: 25

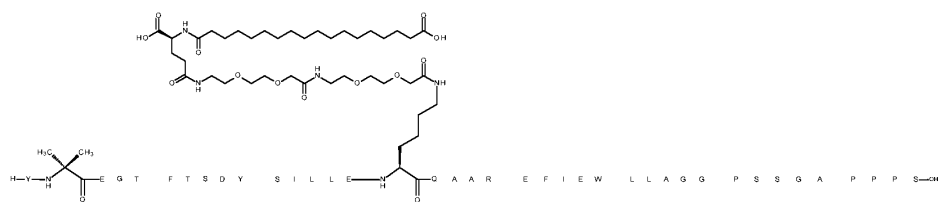
Substituent: B



Compound No. 32

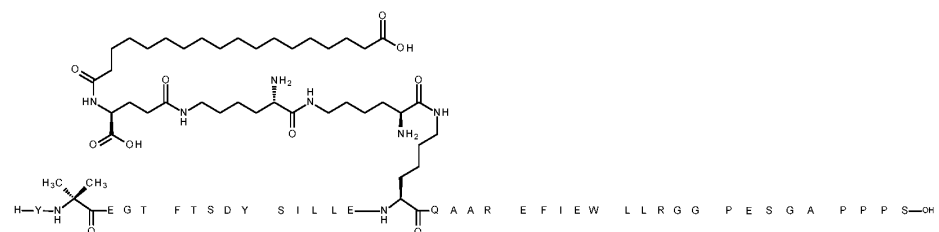
SEQ ID NO: 25

Substituent: A

**Compound No. 28**

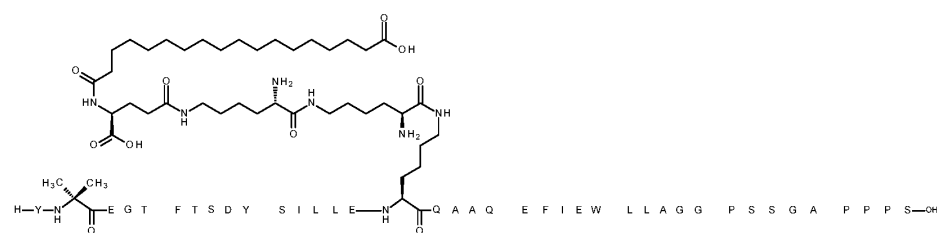
SEQ ID NO: 22

Substituent: B

**Compound No. 29**

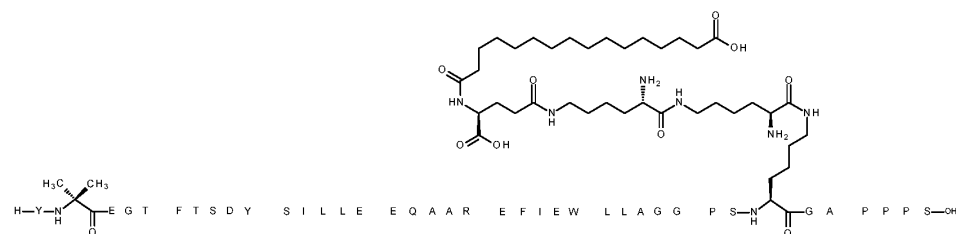
SEQ ID NO: 23

Substituent: B

**Compound No. 46**

SEQ ID NO: 34

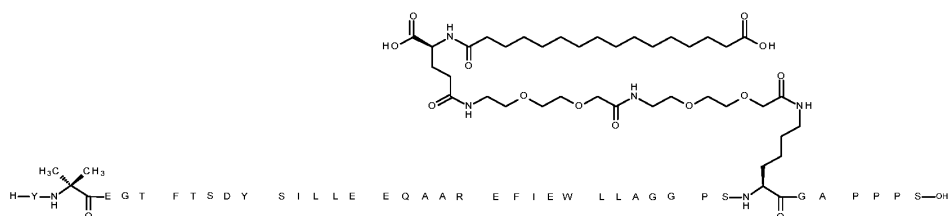
Substituent: E



Compound No. 47

SEQ ID NO: 34

Substituent: D

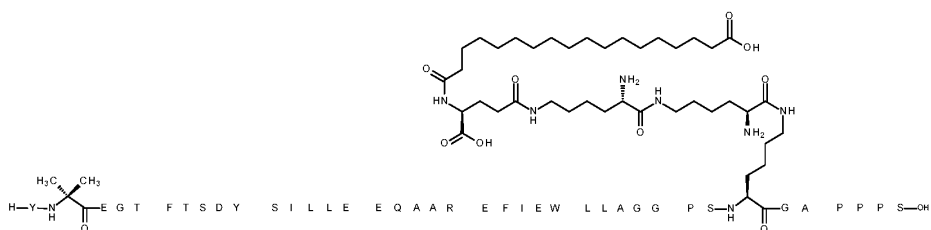


39. The compound according to embodiment 1, wherein the compound is:

Compound No. 43

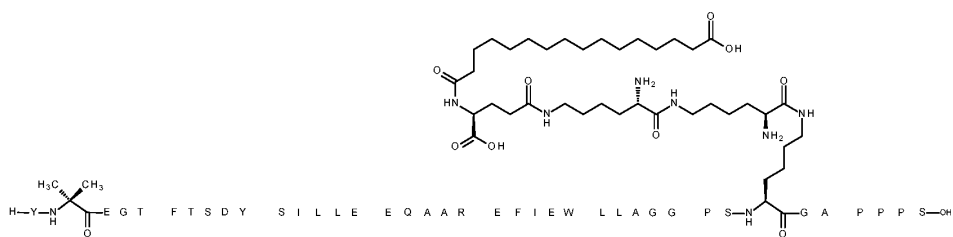
SEQ ID NO: 34

Substituent: B

**Compound No. 46**

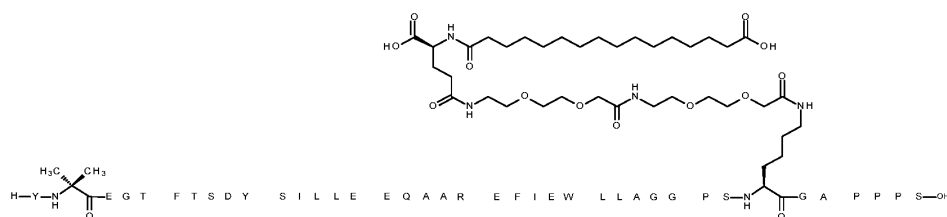
SEQ ID NO: 34

Substituent: E

**Compound No. 47**

SEQ ID NO: 34

Substituent: D

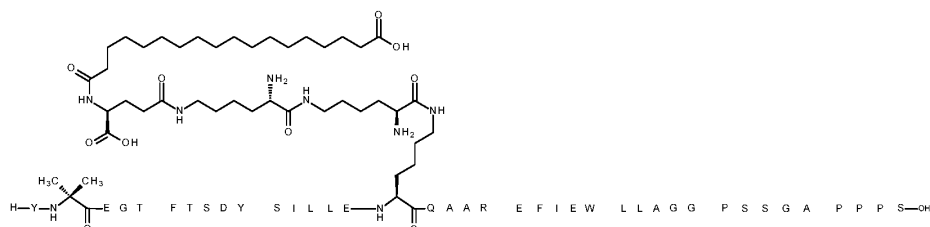


40. The compound according to embodiment 1, wherein the compound is:

Compound No. 31

SEQ ID NO: 25

Substituent: B



41. A compound according to any of the previous embodiments for use as a medicament.

5

42. A pharmaceutical composition comprising a compound according to any of the previous embodiments 1-40.

43. The composition according to embodiment 42, wherein said composition is an aqueous liquid.

10

44. The composition according to embodiment 42, wherein said composition is a solid composition.

45. A pharmaceutical composition according to any of the embodiments 42-44 for prevention and/or treatment of diabetes and/or obesity.

15

46. A pharmaceutical composition according to any of the embodiments 42-44 for prevention and/or treatment of liver disorders, such as hepatic steatosis, non-

alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) liver inflammation and/or fatty liver.

47. A method for prevention and/or treatment of diabetes and/or obesity comprising
5 administering to a patient a pharmaceutically active amount of the compound according to any one of embodiments 1-40.
48. A method for prevention and/or treatment of liver disorders, such as hepatic
10 steatosis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) liver inflammation and/or fatty liver comprising administering to a patient a pharmaceutically active amount of the compound according to any one of embodiments 1-40.
- 15 49. A peptide, wherein the amino acid sequence of the peptide is
YX₂EGTX₆TSDYX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GX₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇
X₃₈X₃₉ (SEQ ID NO.: 47)
with an optional amide modification of the C-terminus,
wherein
20 X₂ is Aib or A
X₆ is F or V
X₁₂ is I or Y
X₁₃ is Y, A, L or I
X₁₆ is K or E
25 X₂₀ is Q, R, E, H
X₂₁ is A or E
X₂₃ is I or V
X₂₄ is E, Q or N
X₂₈ is A or R
30 X₃₀ is G or absent
X₃₁ is P or absent
X₃₂ is E, S or absent
X₃₃ is S, K or absent
X₃₄ is G or absent
35 X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

X₃₈ is P or absent

X₃₉ is S or absent.

5

50. A peptide, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPX₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉ (SEQ ID NO.: 36)

10

with an optional amide modification of the C-terminus,

wherein

X₂ is Aib or A

X₆ is F or V

X₁₂ is I or Y

15

X₁₃ is Y, A, L or I

X₁₆ is K or E

X₂₀ is Q, R, E, H

X₂₁ is A or E

X₂₃ is I or V

20

X₂₄ is E, Q or N

X₂₈ is A or R

X₃₂ is E, S or absent

X₃₃ is S, K or absent

X₃₄ is G or absent

25

X₃₅ is A or absent

X₃₆ is P or absent

X₃₇ is P or absent

X₃₈ is P or absent

X₃₉ is S or absent.

30

51. The peptide according to embodiment 49, wherein X₃₆, X₃₇, X₃₈ and X₃₉ are absent.

52. The peptide according to embodiment 49, wherein X₃₄, X₃₅, X₃₆, X₃₇, X₃₈ and X₃₉ are absent.

35

53. The peptide according to embodiment 49, wherein X_{32} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} and X_{39} are absent.
54. The peptide according to embodiment 49, wherein X_{30} , X_{31} , X_{32} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} and X_{39} are absent.
55. The peptide according to embodiments 49 or 50, wherein $X_{32}X_{33}X_{34}X_{35}$ is selected from the group of: SSGA, ESGA and SKGA.
56. The peptide according to embodiment 55, wherein $X_{32}X_{33}X_{34}X_{35}$ SSGA.
57. The peptide according to embodiment 55, wherein $X_{32}X_{33}X_{34}X_{35}$ is ESGA.
58. The peptide according to embodiment 55, wherein $X_{32}X_{33}X_{34}X_{35}$ is SKGA.
59. The peptide according to any one of embodiments 47-58, wherein the peptide has the amide modification of the C-terminus.
60. The peptide according to embodiment 49, wherein the amino acid sequence of the peptide is
 $YX_2EGTX_6TSDYSX_{12}X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}WLLX_{28}GGPSSGAPPPS$ (SEQ ID NO. : 37)
wherein
 X_2 is Aib or A
 X_6 is F or V
 X_{12} is I or Y
 X_{13} is Y, A, L or I
 X_{16} is K or E
 X_{20} is Q, R, E, H
 X_{21} is A or E
 X_{23} is I or V
 X_{24} is E, Q or N
 X_{28} is A or R.

61. The peptide according to embodiment 48, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPESGAPPPS (SEQ ID NO.: 38)

5 wherein
 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
 X₁₃ is Y, A, L or I
10 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
 X₂₄ is E, Q or N
15 X₂₈ is A or R.

62. The peptide according to embodiment 49, wherein the amino acid sequence of the peptide is

YX₂EGTX₆TSDYSX₁₂X₁₃LEX₁₆QAAX₂₀X₂₁FX₂₃X₂₄WLLX₂₈GGPSKGAPPPS (SEQ ID NO.: 39)

20 wherein
 X₂ is Aib or A
 X₆ is F or V
 X₁₂ is I or Y
25 X₁₃ is Y, A, L or I
 X₁₆ is K or E
 X₂₀ is Q, R, E, H
 X₂₁ is A or E
 X₂₃ is I or V
30 X₂₄ is E, Q or N
 X₂₈ is A or R

63. The peptide according to embodiment 49 where the amino acid sequence of the peptide is any one of SEQ ID NO.: 1-27 or 33-35.

35

64. The peptide according to embodiment 63, wherein the peptide has the amide modification of the C-terminus.
- 5 65. The peptide according to any of the previous embodiments 49-64, wherein $X_{13}LEX_{16}QAAX_{20}X_{21}FX_{23}X_{24}$ is selected from the group consisting of: LLEKQAAREFIN, LLEKQAAREFIE, LLEKQAAQEFIE and LLEEQAAREFIE.
- 10 66. The peptide according to any of the previous embodiments 49-64, wherein the peptide activates the human GLP-1 and GIP receptors *in vitro* with an EC_{50} of less than 20 pM when measured without HSA in a CRE luciferase reporter assays as described in Example 2.
- 15 67. The peptide according to any of the previous embodiments 49-64 **Error! Reference source not found.**, wherein X_{16} is K.
68. The peptide according to any of the previous embodiments 49-64, wherein X_{33} is K.
- 20 69. The peptide according to embodiment 49, wherein the amino acid sequence of the peptide is any of SEQ ID NO.: 15, 28, 29, 31, 32 or 43.
70. The peptide according to embodiment 49, wherein the amino acid sequence of the peptide is SEQ ID NO.: 31.
- 25 71. A method for preparing a compound according to any of the previous embodiments 1-40.
72. A method for preparing a peptide according to any of the previous embodiments 49-64.
- 30 73. A method for preparing a peptide according to any of the previous embodiments 49-70.

METHODS AND EXAMPLES

List of Abbreviations

5 The following abbreviations are used in the following, in alphabetical order:

Ac: acetyl

Ado (also called OEG): 8-amino-3,6-dioxaoctanoic acid

Aib: α -aminoisobutyric acid

API: active pharmaceutical ingredient

10 AUC: area under the curve

BG: blood glucose

BHK: baby hamster kidney

Boc: *tert*-butoxycarbonyl

BW: body weight

15 CAS: Chemical Abstracts Service

Cl-HOBt: 6-chloro-1-hydroxybenzotriazole

DCM: dichloromethane

DIC: diisopropylcarbodiimide

DIPEA: *N,N*-diisopropylethylamine

20 DMEM: Dulbecco's Modified Eagle's Medium

DPBS: Dulbecco's phosphate buffered saline

EDTA: ethylenediaminetetraacetic acid

ELISA: enzyme linked immunosorbent assay

equiv: molar equivalent

25 FBS: fetal bovine serum

Fmoc: 9-fluorenylmethoxycarbonyl

GIP: glucose-dependent insulinotropic polypeptide

GIPR: glucose-dependent insulinotropic polypeptide receptor

GLP-1: glucagon-like peptide 1

30 GLP-1R: glucagon-like peptide 1 receptor

h: hours

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroisopropanol

- HPLC: high performance liquid chromatography
HSA: human serum albumin
i.p.: intraperitoneal
IPGTT: intraperitoneal glucose tolerance test
- 5 i.v. intravenously
LCMS: liquid chromatography mass spectroscopy
LYD: Landrace Yorkshire Duroc
MeCN: acetonitrile
MeOH: methanol
- 10 mM: millimolar
mmol: millimoles
min: minutes
Mtt: 4-methyltrityl
MW: molecular weight
- 15 nM: nanomolar
NMP: 1-methyl-pyrrolidin-2-one
OEG: 8-amino-3,6-dioxaoctanoic acid (also called Ado)
OtBu: *tert*-butyl ester
Oxyrna Pure®: cyano-hydroxyimino-acetic acid ethyl ester
- 20 Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PBS: phosphate buffered saline
PD: pharmacodynamic
PK: pharmacokinetic
pM: picomolar
- 25 RP: reverse phase
RP-HPLC: reverse phase high performance liquid chromatography
rpm: rounds per minute
RT: room temperature
Rt: retention time
- 30 s.c.: subcutaneous
SD: standard deviation
SEC-HPLC: size exclusion high performance liquid chromatography
SEM: standard error of the mean
SNAC: sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate
- 35 SPPS: solid phase peptide synthesis

tBu: *tert*-butyl

TFA: trifluoroacetic acid

TIS: triisopropylsilane

Trt: triphenylmethyl or trityl

5 Trx: tranexamic acid

General Methods of Preparation

Methods for solid phase peptide synthesis (SPPS methods, including methods for de-protection of amino acids, methods for cleaving the peptide from the resin, and for its
10 purification), as well as methods for detecting and characterising the resulting peptide (LCMS methods) are described here below.

Resins employed for the preparation of C-terminal peptide amides were H-Rink Amide-ChemMatrix resin (loading e.g. 0.5 mmol/g). The Fmoc-protected amino acid derivatives used, unless specifically stated otherwise, were the standard recommended:
15 Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Aib-OH, etc. supplied from e.g. AAPPTEC, Anaspec,
20 Bachem, ChemImpex, Iris Biotech, Midwest Biotech, Gyros Protein Technologies or Novabiochem. Where nothing else is specified, the natural L-form of the amino acids are used. The N-terminal amino acid was Boc protected at the alpha-amino group, either by using a reagent with the Boc group pre-installed (e.g. Boc-Tyr(tBu)-OH for peptides with Tyr at the N-terminus) or by exchanging the N-terminal Fmoc protective group for the Boc
25 protective group after installation of the amino acid at the peptide N-terminus.

In case of modular albumin binding moiety attachment using SPPS, the following suitably protected building blocks such as but not limited to Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-Ado-OH), Boc-Lys(Fmoc)-OH, Fmoc-Glu-OtBu, hexadecanedioic acid mono-*tert*-butyl ester, octadecanedioic acid mono-*tert*-butyl ester, nonadecanedioic acid mono-*tert*-
30 butyl ester, eicosanedioic acid mono-*tert*-butyl ester, tetradecanedioic acid mono-*tert*-butyl ester, or 4-(9-carboxynonyloxy) benzoic acid *tert*-butyl ester were used. All operations stated below were performed within a 0.1-0.2 mmol synthesis scale range.

1. Synthesis of Resin-Bound Protected Peptide Backbone:

Method: SPPS_A

SPPS was performed using Fmoc based chemistry on a Protein Technologies SymphonyX solid phase peptide synthesizer, using the manufacturer supplied protocols with minor modifications. Mixing was accomplished by occasional bubbling with nitrogen. The step-wise assembly was performed using the following steps: 1) pre-swelling of resin in DMF; 2) Fmoc-deprotection by the use of 20% (v/v) piperidine in DMF for two treatments of 10 min each; 3) washes with DMF to remove piperidine; 4) coupling of Fmoc-amino acid by the addition of Fmoc-amino acid (12 equiv) and Oxyma Pure® (12 equiv) as a 0.6 M solution each in DMF, followed by addition of DIC (12 equiv) as a 1.2 M solution in DMF, followed by the addition of DMF to reduce the final concentration of each component to 0.3 M, then mixing for 0.5-4 h; 4) washes with DMF to remove excess reagents; 5) final washes with DCM at the completion of the assembly. Some amino acids such as, but not limited to, those following a sterically hindered amino acid (e.g. Aib) were coupled with an extended reaction time (e.g. 4 h) to ensure reaction completion.

Method: SPPS_B

The protected peptidyl resin was synthesized according to the Fmoc strategy on an Applied Biosystems 431A solid-phase peptide synthesizer using the manufacturer supplied general Fmoc protocols. Mixing was accomplished by vortexing and occasional bubbling with nitrogen. The step-wise assembly was done using the following steps: 1) activation of Fmoc-amino acid by dissolution of solid Fmoc-amino acid (10 equiv) in Cl-HOBt (10 equiv) as a 1 M solution in NMP, then addition of DIC (10 equiv) as a 1 M solution in NMP, then mixing simultaneous to steps 2-3; 2) Fmoc-deprotection by the use of 20% (v/v) piperidine in NMP for one treatment of 3 min then a second treatment of 15 min; 3) washes with NMP to remove piperidine; 4) addition of activated Fmoc-amino acid solution to resin, then mixing for 45-90 min; 4) washes with NMP to remove excess reagents; 5) final washes with DCM at the completion of the assembly. The standard protected amino acid derivatives listed above were supplied in pre-weighed cartridges (from e.g. Midwest Biotech), and non-standard derivatives were weighed by hand. Some amino acids such as, but not limited to, those following a sterically hindered amino acid (e.g. Aib) were "double coupled" to ensure reaction completion, meaning that after the first coupling (e.g. 45 min) the resin is drained, more reagents are added (Fmoc-amino acid, DIC, Cl-HOBt), and the mixture allowed to react again (e.g. 45 min).

Method: SPPS_C

SPPS was performed using Fmoc based chemistry on a PreludeX solid phase peptide synthesizer, using the manufacturer supplied protocols with minor modifications. Mixing was accomplished by shaking at 350 rpm and occasional bubbling with nitrogen. The step-wise assembly was performed using the following steps: 1) pre-swelling of resin in DMF; 2) Fmoc-deprotection by the use of 20% (v/v) piperidine in DMF for one treatment of 3 min at 70°C; 3) washes with DMF to remove piperidine; 4) coupling of Fmoc-amino acid by the addition of Fmoc-amino acid (12 equiv) and Oxyma Pure® (12 equiv) as a 0.4 M solution each in DMF, followed by addition of DIC (12 equiv) as a 1.2 M solution in DMF, then mixing for 5 min at 70°C; 4) washes with DMF to remove excess reagents; 5) final washes with DCM at the completion of the assembly. Some amino acids such as, but not limited to, those following a sterically hindered amino acid (e.g. Aib) were coupled with an extended reaction time (e.g. 15 min) to ensure reaction completion.

2. Attachment of substituent to resin-bound protected peptide backbone

15 *Method: SC_A*

The N-epsilon-lysine protection Mtt protection group was removed by washing the resin with 30% HFIP in DCM for two treatments of 45 min each, following by washing with DCM and DMF. Acylation was performed on a Protein Technologies SymphonyX solid phase peptide synthesizer using the protocols described in method SPPS_A using stepwise addition of building blocks, such as, but not limited to, Boc-Lys(Fmoc)-OH, Fmoc-8-amino-3,6-dioxaoctanoic acid, Fmoc-Glu-OtBu, hexadecanedioic acid mono-*tert*-butyl ester, octadecanedioic acid mono-*tert*-butyl ester, and eicosanedioic acid mono-*tert*-butyl ester.

Method: SC_B

25 The N-epsilon-lysine protection Mtt protection group was removed by washing the resin with 30% HFIP in DCM for two treatments of 45 min each, following by washing with DCM and DMF. Acylation was performed on an Applied Biosystems 431A solid-phase peptide synthesizer using the protocols described in method SPPS_B using stepwise addition of building blocks, such as, but not limited to, Boc-Lys(Fmoc)-OH, Fmoc-8-amino-3,6-dioxaoctanoic acid, Fmoc-Glu-OtBu, hexadecanedioic acid mono-*tert*-butyl ester, octadecanedioic acid mono-*tert*-butyl ester, and eicosanedioic acid mono-*tert*-butyl ester.

Method: SC_C

35 The N-epsilon-lysine protection Mtt protection group was removed by washing the resin with 30% HFIP in DCM for two treatments of 45 min each, following by washing with

DCM and DMF. Acylation was performed on a Protein Technologies PreludeX solid phase peptide synthesizer using the protocols described in method SPPS_C using stepwise addition of building blocks, such as, but are not limited to, Boc-Lys(Fmoc)-OH, Fmoc-8-amino-3,6-dioxaoctanoic acid, Fmoc-Glu-OtBu, hexadecanedioic acid mono-*tert*-butyl ester, octadecanedioic acid mono-*tert*-butyl ester, and eicosanedioic acid mono-*tert*-butyl ester.

3. Cleavage of resin bound peptide and purification:

Method: CP_A

Following completion of the sidechain synthesis, the peptidyl resin was washed with DCM and dried, then treated with TFA/water/TIS (95:2.5:2.5 v/v/v) for approximately 2 h, followed by precipitation with diethyl ether. The precipitate was washed with diethyl ether, dissolved in a suitable solvent (e.g. 2:1 water/MeCN), and let stand until all labile adducts decomposed. Purification was performed by reversed-phase preparative HPLC (Waters 2545 binary gradient module, Waters 2489 UV/Visible detector, Waters fraction collector III) on a Phenomenex Luna C8(2) column (10 μ M particle size, 100 Å pore size, 250 x 21.2 mm dimensions). Separation of impurities and product elution was accomplished using an increasing gradient of MeCN in water containing 0.1% TFA. Relevant fractions were checked for identity and purity by analytical LCMS. Fractions containing the pure desired product were pooled and freeze-dried to afford the peptide TFA salt as a white solid.

4. Salt exchange from TFA to sodium salt:

Method: SX_A

The freeze-dried peptide isolated from method CP_A was dissolved to 5-20 mg/mL in an appropriate aqueous buffer (e.g. 4:1 water/MeCN, 0.2 M sodium acetate) and adjusted to pH 7-8 with 1 M NaOH if necessary to achieve full solubility. The buffered solutions containing the peptide were salt-exchanged using a Sep-Pak C18 cartridge (0.5-2 g): The cartridge was first equilibrated with 4 column volumes of isopropanol, then 4 column volumes of MeCN, then 8 column volumes of water. The peptide solution was applied to the cartridge, and the flow through was reapplied to ensure complete retention of peptide. The cartridge was washed with 4 column volumes of water, then 10 column volumes of a buffer solution (e.g. pH 7.5) containing such as, but not limited to, NaHCO₃, NaOAc, or Na₂HPO₄. The column was washed with 4 column volumes of water, and the peptide was eluted with 5-10 column volumes of 50-80% MeCN in water. The peptide-containing eluent was freeze-dried to afford the peptide sodium salt as a white solid, which was used as such.

General Methods of Detection and Characterisation

LCMS methods:

Method: LCMS_A

- LCMS_A was performed on a setup consisting of an Agilent 1260 Infinity series HPLC system and an Agilent Technologies 6120 Quadrupole MS. Eluents: A: 0.05% TFA in water; B: 0.05% TFA in 9:1 MeCN/water.

- The analysis was performed at RT (column temp 37C) by injecting an appropriate volume of the sample onto the column which was eluted with a gradient of A and B. Column: Phenomenex Kinetex C8, 2.6 μ m, 100 Å, 4.6 x 75 mm. Gradient run time: Linear 10-80% B over 10 min at a flow rate of 1.0 mL/min. Detection: diode array detector set to 214 nm. MS ionisation mode: API-ES, positive polarity. MS scan mass range: 500-2000 amu. The most abundant isotope of each m/z is reported.

Method: LCMS_B

- LCMS_B was performed on a setup consisting of an Agilent 1260 Infinity series HPLC system and an Agilent Technologies 6120 Quadrupole MS. Eluents: A: 0.05% TFA in water; B: 0.05% TFA in 9:1 MeCN/water.

- The analysis was performed at RT (column temp 37C) by injecting an appropriate volume of the sample onto the column which was eluted with a gradient of A and B. Column: Phenomenex Kinetex C8, 2.6 μ m, 100 Å, 4.6 x 75 mm. Gradient run time: Linear 20-100% B over 10 min at a flow rate of 1.0 mL/min. Detection: diode array detector set to 214 nm. MS ionisation mode: API-ES, positive polarity. MS scan mass range: 500-2000 amu. The most abundant isotope of each m/z is reported.

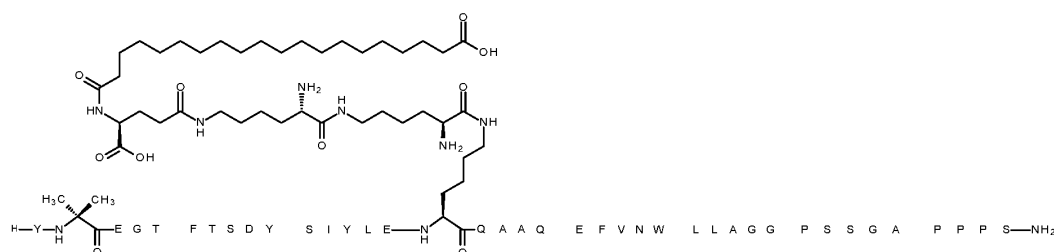
- While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended embodiments are intended to cover all such modifications and changes as fall within the true spirit of the invention.

Example 1: Synthesis of compounds

The compounds are in the following described using single letter amino acid codes, except for Aib. The substituent is included after the lysine (K) residue to which it is attached.

Compound No. 1

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAQEFVNWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 1 with C-terminal amide modification; Substituent: C; Substituent position: K16

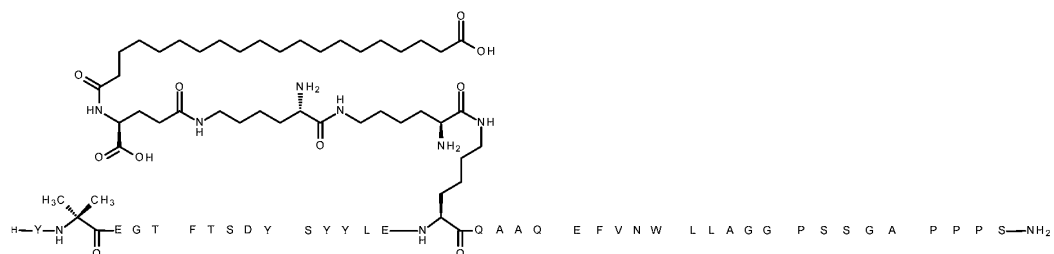
Synthesis methods: SPPS_A; SC_B; CP_A

Molecular weight (average) calculated: 4873.5 Da

LCMS_A: Rt = 6.0 min; found [M+3H]³⁺ 1625.4, [M+4H]⁴⁺ 1219.1.

Compound No. 2

Y-Aib-EGTFTSDYSYYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAQEFVNWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 2 with C-terminal amide modification; Substituent: C; Substituent position: K16

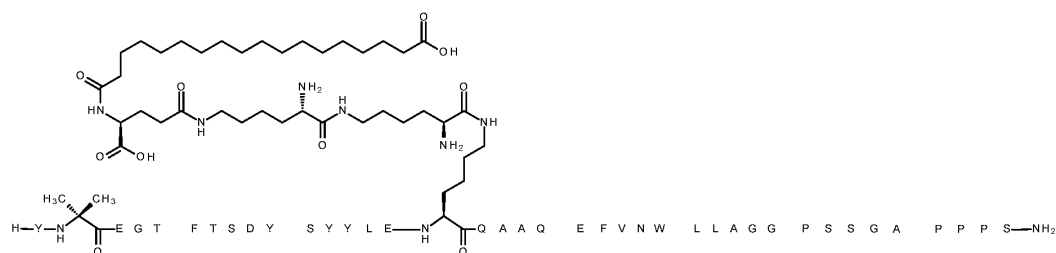
Synthesis methods: SPPS_A; SC_B; CP_A

Molecular weight (average) calculated: 4923.5 Da

LCMS_A: Rt = 6.0 min; found [M+3H]³⁺ 1641.8, [M+4H]⁴⁺ 1237.5

Compound No. 3

Y-Aib-EGTFTSDYSYYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAQEFVNWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 2 with C-terminal amide modification; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_B; CP_A

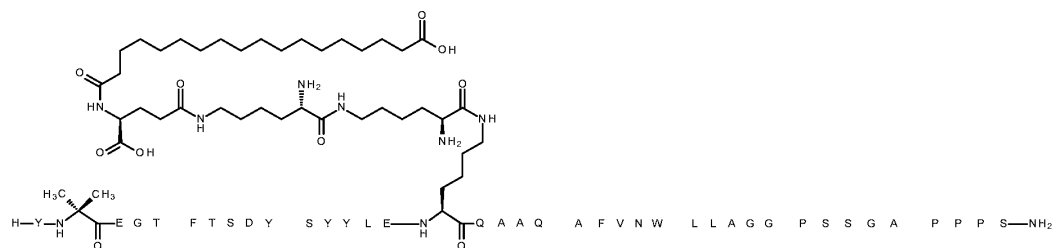
Molecular weight (average) calculated: 4895.4 Da

5 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1632.7, $[M+4H]^{4+}$ 1224.6

Compound No. 4

Y-Aib-EGTFTSDYSYYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAQAFVNWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 3 with C-terminal amide modification; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_B; CP_A

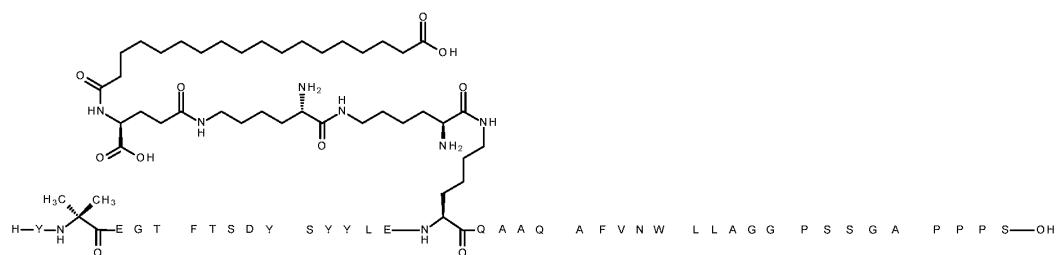
Molecular weight (average) calculated: 4837.4 Da

15 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1613.3, $[M+4H]^{4+}$ 1210.1

Compound No. 5

Y-Aib-EGTFTSDYSYYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAQAFVNWLLAGGPSSGAPPPS-OH



SEQ ID NO: 3; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_B; SC_B; CP_A

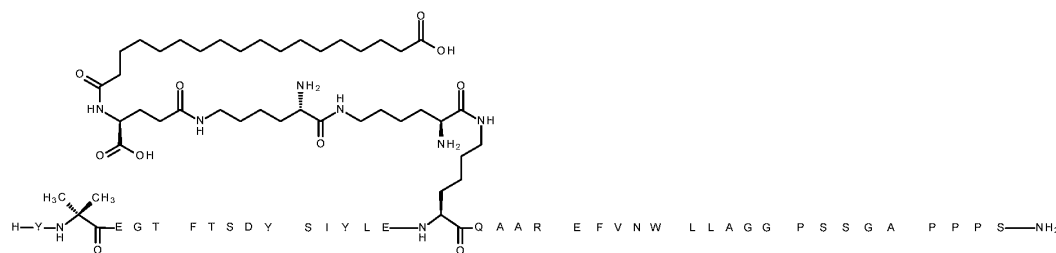
Molecular weight (average) calculated: 4838.4 Da

5 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1613.6, $[M+4H]^{4+}$ 1210.4

Compound No. 6

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFVNWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 4 with C-terminal amide modification; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_B; SC_B; CP_A

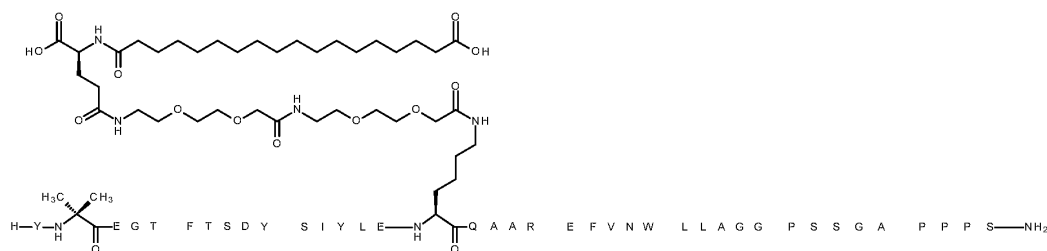
Molecular weight (average) calculated: 4873.5 Da

15 LCMS_A: Rt = 5.6 min; found $[M+3H]^{3+}$ 1625.3, $[M+4H]^{4+}$ 1219.1

Compound No. 7

Y-Aib-EGTFTSDYSIYLE-K[2-[2-[2-[[2-[2-[2-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-QAAREFVNWLLAGGPSSGAPPPS-NH₂

20



SEQ ID NO: 4 with C-terminal amide modification; Substituent: A; Substituent position: K16

Synthesis methods: SPPS_B; SC_B; CP_A

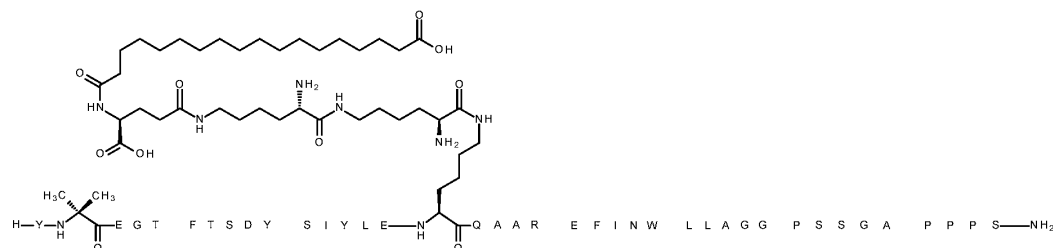
Molecular weight (average) calculated: 4907.4 Da

5 LCMS_A: Rt = 6.3 min; found $[M+3H]^{3+}$ 1636.5, $[M+4H]^{4+}$ 1227.9

Compound No. 8

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFINWLLAGGPSSGAPPPS-NH₂



SEQ ID NO: 5 with C-terminal amide modification; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

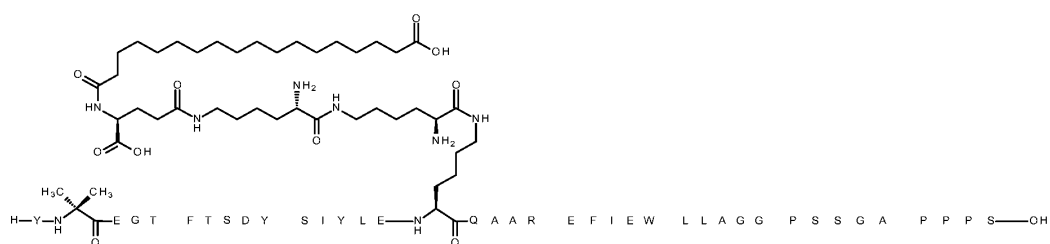
Molecular weight (average) calculated: 4887.5 Da

15 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1629.8, $[M+4H]^{4+}$ 1222.8

Compound No. 9

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 6; Substituent: B; Substituent position: K16

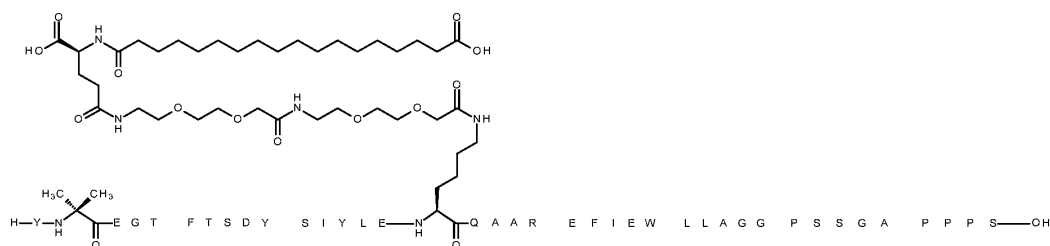
Synthesis methods: SPPS_A; SC_B; CP_A

Molecular weight (average) calculated: 4903.5 Da

5 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1635.2, $[M+4H]^{4+}$ 1226.8

Compound No. 10

Y-Aib-EGTFTSDYSIYLE-K[2-[2-[2-[[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-QAAREFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 6; Substituent: A; Substituent position: K16

Synthesis methods: SPPS_A; SC_B; CP_A

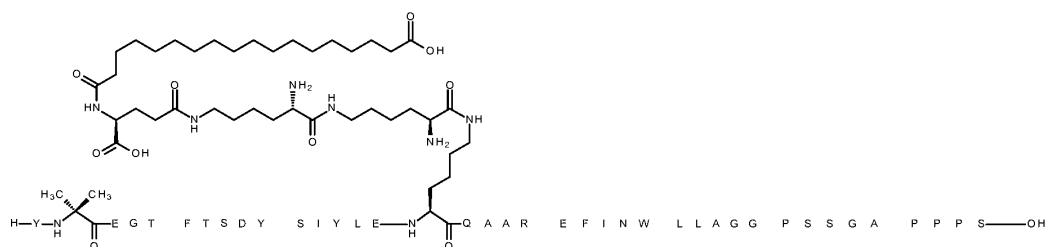
Molecular weight (average) calculated: 4937.5 Da

15 LCMS_A: Rt = 6.5 min; found $[M+3H]^{3+}$ 1646.6, $[M+4H]^{4+}$ 1235.1

Compound No. 11

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAREFINWLLAGGPSSGAPPPS-OH

20



SEQ ID NO: 5; Substituent: B; Substituent position: K16

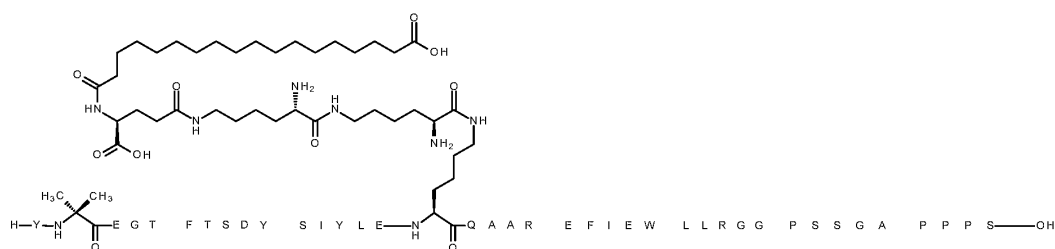
Synthesis methods: SPPS A; SC C; CP A

Molecular weight (average) calculated: 4888.5 Da

5 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1630.2, $[M+4H]^{4+}$ 1222.9

Compound No. 12

Y-Aib-EGFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAREFIEWLLRGGPSSGAPPPS-OH



Synthesis methods: SPPS_A; SC_C; CP_A

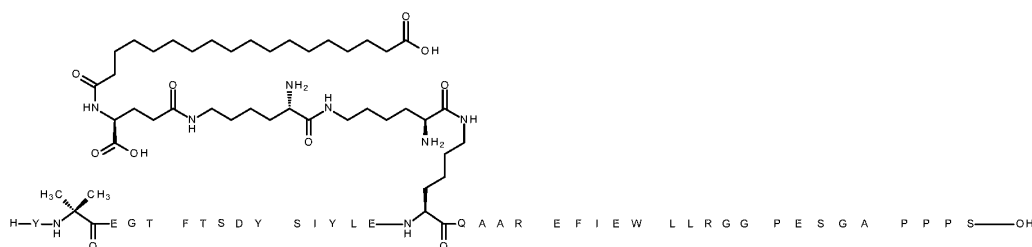
SEQ ID NO: 7; Substituent: B; Substituent position: K16

15 Molecular weight (average) calculated: 4988.6 Da

LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1663.6, $[M+4H]^{4+}$ 1248.0

Compound No. 13

Y-Aib-EGFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAREFIEWLLRGGPESGAPPPS-OH



SEQ ID NO: 8; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

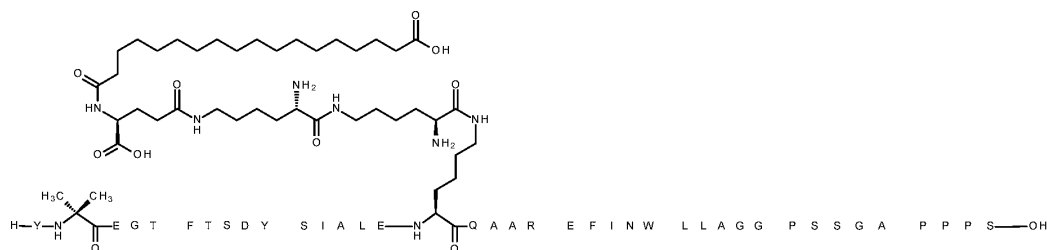
Molecular weight (average) calculated: 5030.6 Da

5 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1677.7, $[M+4H]^{4+}$ 1258.4

Compound No. 14

Y-Aib-EGTFTSDYSIALE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 9; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

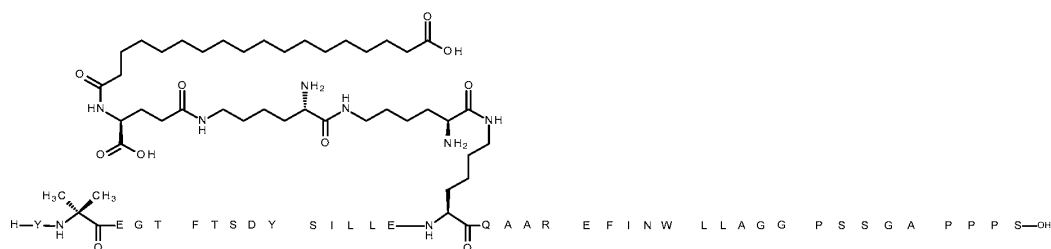
Molecular weight (average) calculated: 4796.4 Da

15 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1599.6, $[M+4H]^{4+}$ 1199.8

Compound No. 15

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 10; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A; SX_A

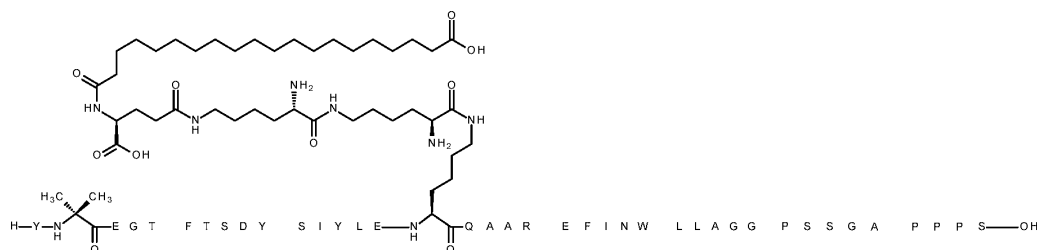
Molecular weight (average) calculated: 4838.5 Da

5 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1613.4, $[M+4H]^{4+}$ 1210.3

Compound No. 16

Y-Aib-EGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 5; Substituent: C; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

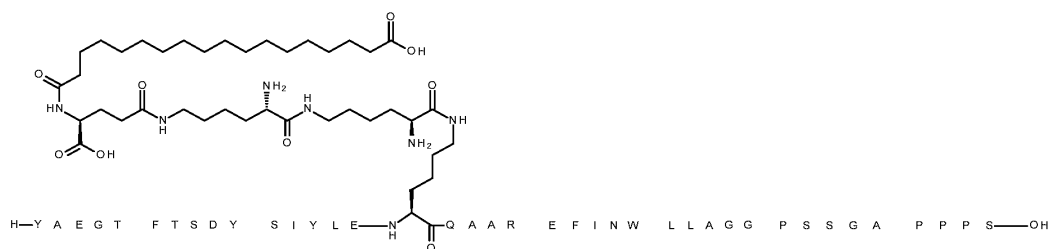
Molecular weight (average) calculated: 4916.5 Da

15 LCMS_A: Rt = 6.0 min; found $[M+3H]^{3+}$ 1639.6, $[M+4H]^{4+}$ 1229.9

Compound No. 17

YAEGTFTSDYSIYLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 11; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_C; SC_A; CP_A

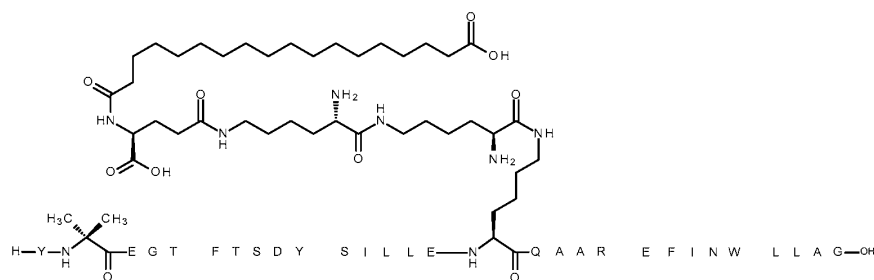
Molecular weight (average) calculated: 4874.5 Da

5 LCMS_A: Rt = 5.7 min; found [M+3H]³⁺ 1625.4, [M+4H]⁴⁺ 1219.4

Compound No. 18

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAREFINWLLAG-

10 OH



SEQ ID NO: 12; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

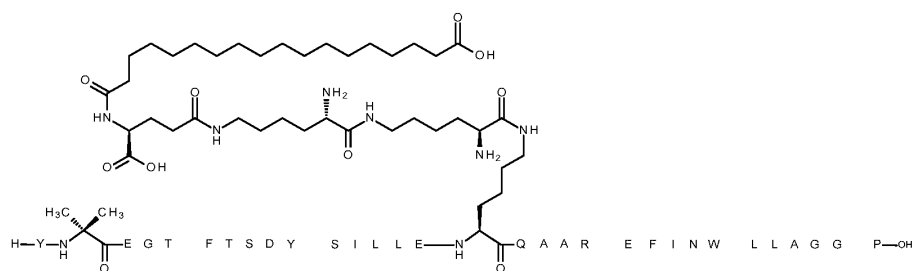
Molecular weight (average) calculated: 4003.6 Da

15 LCMS_A: Rt = 6.2 min; found [M+3H]³⁺ 1335.3, [M+4H]⁴⁺ 1001.7

Compound No. 19

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFINWLLAGGP-OH



SEQ ID NO: 13; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

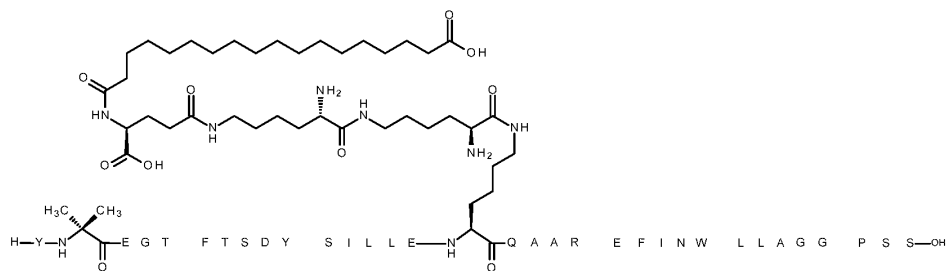
Molecular weight (average) calculated: 4157.8 Da

5 LCMS_A: Rt = 6.1 min; found $[M+3H]^3+$ 1386.6, $[M+4H]^4+$ 1040.3

Compound No. 20

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-butanoyl]amino

10 QAAREFINWLLAGGPSS-OH



SEQ ID NO: 14; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

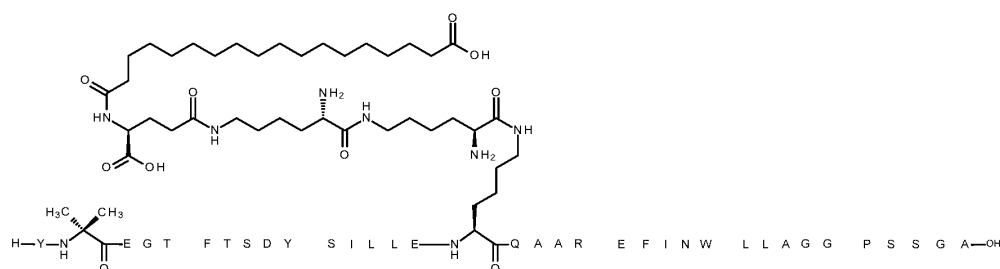
Molecular weight (average) calculated: 4331.9 Da

15 LCMS_A: Rt = 5.9 min; found $[M+3H]^3+$ 1444.7, $[M+4H]^4+$ 1083.7

Compound No. 21

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAR-

20 EFINWLLAGGPSSGA-OH



SEQ ID NO: 15; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

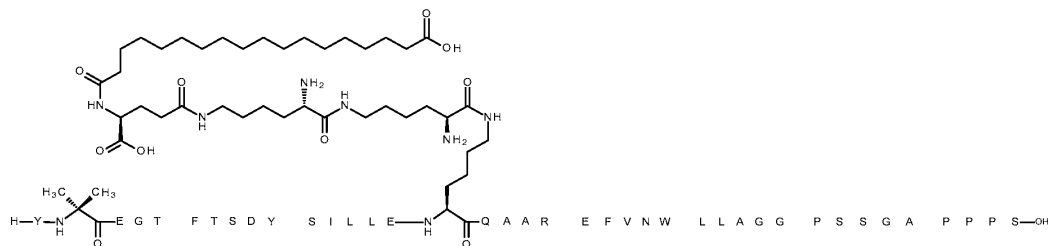
Molecular weight (average) calculated: 4460.0 Da

5 LCMS_A: Rt = 5.9 min; found $[M+3H]^3+$ 1487.5, $[M+4H]^4+$ 1116.1

Compound No. 22

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFVNWLLAGGPSSGAPPPS-OH



SEQ ID NO: 16; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

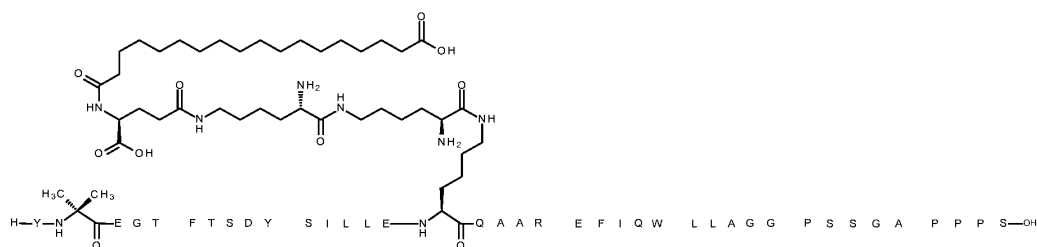
Molecular weight (average) calculated: 4824.4 Da

15 LCMS_A: Rt = 5.7 min; found $[M+3H]^3+$ 1608.9, $[M+4H]^4+$ 1206.9

Compound No. 23

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFIQWLLAGGPSSGAPPPS-OH



SEQ ID NO: 17; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

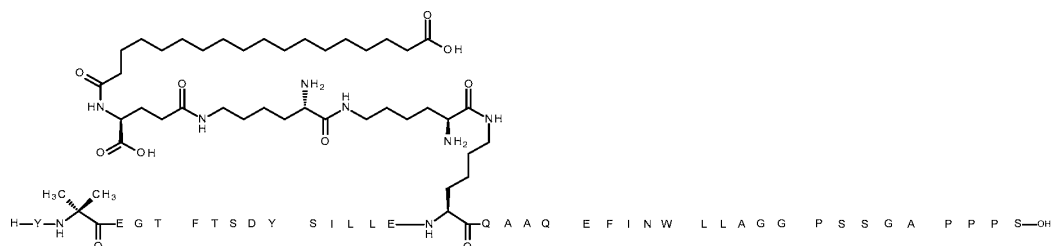
Molecular weight (average) calculated: 4852.5 Da

5 LCMS_A: Rt = 5.9 min; found [M+3H]³⁺ 1618.4, [M+4H]⁴⁺ 1214.1

Compound No. 24

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAQEFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 18; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

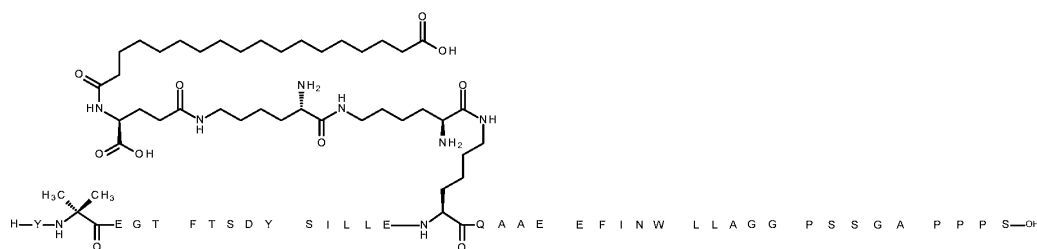
Molecular weight (average) calculated: 4810.4 Da

15 LCMS_A: Rt = 6.0 min; found [M+3H]³⁺ 1604.0, [M+4H]⁴⁺ 1203.4

Compound No. 25

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAEEFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 19; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

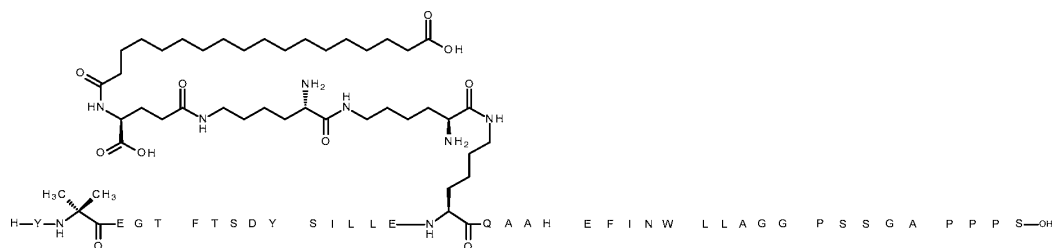
Molecular weight (average) calculated: 4811.4 Da

5 LCMS_A: Rt = 6.1 min; found $[M+3H]^{3+}$ 1604.4, $[M+4H]^{4+}$ 1203.6

Compound No. 26

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAHEFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 20; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

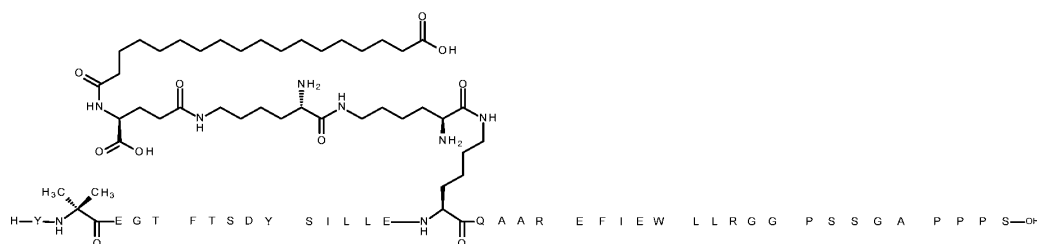
Molecular weight (average) calculated: 4819.4 Da

15 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1607.1, $[M+4H]^{4+}$ 1205.5

Compound No. 27

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFIEWLLRGGPSSGAPPPS-OH



SEQ ID NO: 21; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_C; CP_A

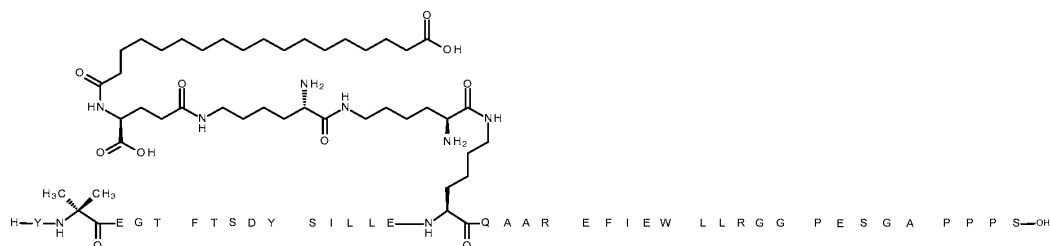
Molecular weight (average) calculated: 4938.6 Da

5 LCMS_A: Rt = 5.7 min; found $[M+3H]^{3+}$ 1646.9, $[M+4H]^{4+}$ 1235.4

Compound No. 28

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-(((2S)-2-amino-6-(((4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl)amino)hexanoyl)amino)hexanoyl]-

10 QAAREFIEWLLRGGPESGAPPPS-OH



SEQ ID NO: 22; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A; SX_A

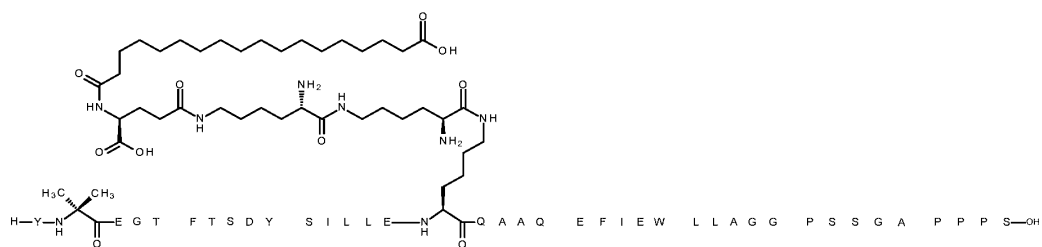
Molecular weight (average) calculated: 4980.6 Da

15 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1660.7, $[M+4H]^{4+}$ 1246.0

Compound No. 29

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-(((2S)-2-amino-6-(((4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl)amino)hexanoyl)amino)hexanoyl]-

20 QAAQEFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 23; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A; SX_A

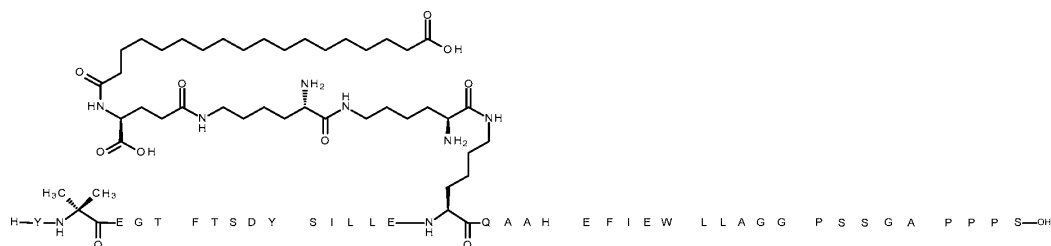
Molecular weight (average) calculated: 4825.4 Da

5 LCMS_A: Rt = 6.1 min; found $[M+3H]^{3+}$ 1609.3, $[M+4H]^{4+}$ 1207.0

Compound No. 30

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAHEFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 24; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

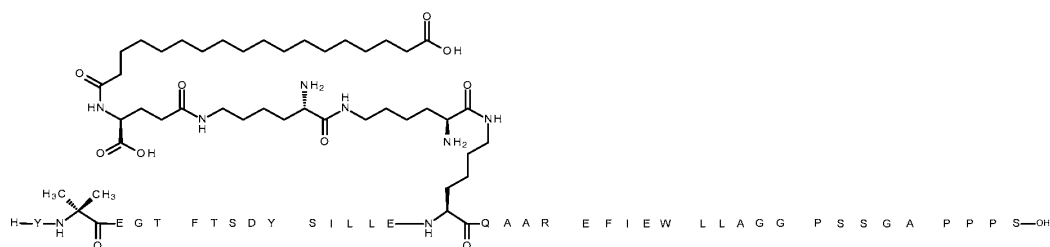
Molecular weight (average) calculated: 4834.4 Da

15 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1612.1, $[M+4H]^{4+}$ 1209.3

Compound No. 31

Y-Aib-EGTFTSDYSILLE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 QAAREFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 25; Substituent: B; Substituent position: K16

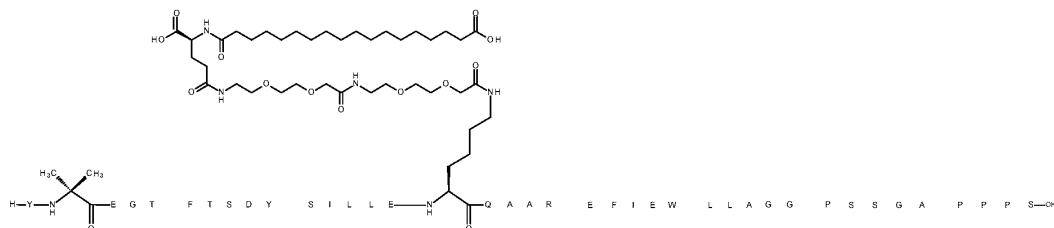
Synthesis methods: SPPS_A; SC_A; CP_A; SX_A

Molecular weight (average) calculated: 4853.5 Da

5 LCMS_A: Rt = 5.9 min; found $[M+3H]^{3+}$ 1618.5, $[M+4H]^{4+}$ 1214.2

Compound No. 32

Y-Aib-EGTFTSDYSILLE-K[2-[2-[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-QAAREFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 25; Substituent: A; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

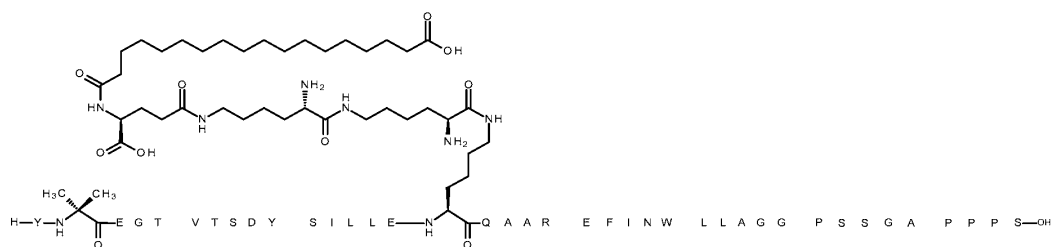
Molecular weight (average) calculated: 4887.4 Da

15 LCMS_A: Rt = 6.6 min; found $[M+3H]^{3+}$ 1629.8, $[M+4H]^{4+}$ 1222.8

Compound No. 33

Y-Aib-EGTVTSDYSILLE-K[(2S)-2-amino-6-[(2S)-2-amino-6-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-QAAREFINWLLAGGPSSGAPPPS-OH

20



SEQ ID NO: 26; Substituent: B; Substituent position: K16

Synthesis methods: SPPS A; SC A; CP A

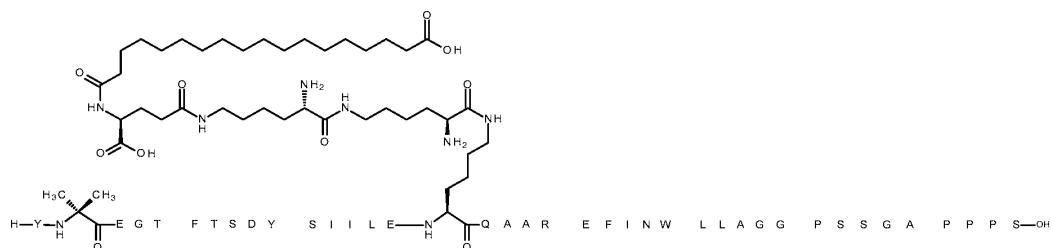
Molecular weight (average) calculated: 4790.4 Da

5 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1597.4, $[M+4H]^{4+}$ 1198.3

Compound No. 34

Y-Aib-EGTFTSDYSIIIE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 27; Substituent: B; Substituent position: K16

Synthesis methods: SPPS_A; SC_A; CP_A

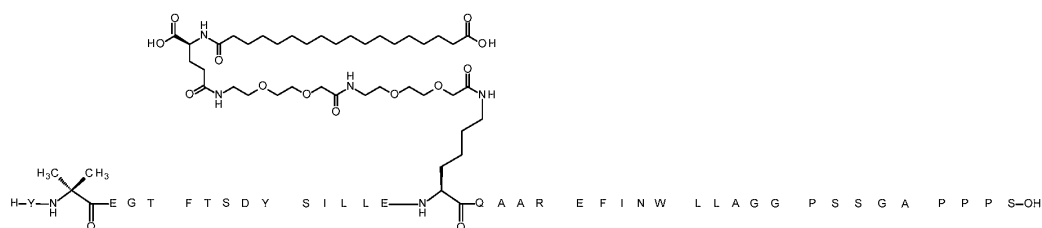
Molecular weight (average) calculated: 4838.5 Da

15 LCMS_A: Rt = 5.8 min; found $[M+3H]^{3+}$ 1613.4, $[M+4H]^{4+}$ 1210.3

Compound No. 35

Y-Aib-EGTFTSDYSILLE-K[2-[2-[2-[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]

20 acetyl]-QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 10; Substituent: A; Substituent position: K16

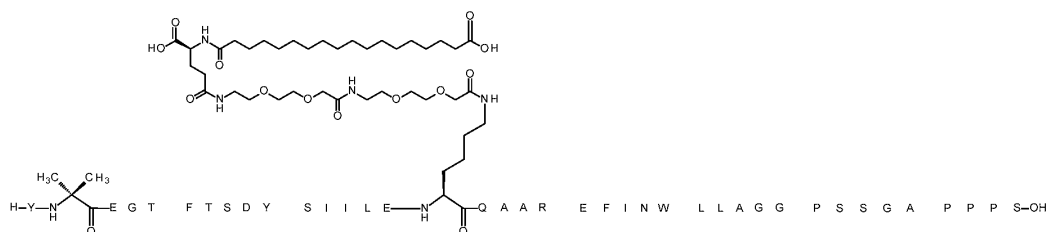
Synthesis methods: SPPS_A; SC_A; CP_A

Molecular weight (average) calculated: 4872.4 Da

5 LCMS_A: Rt = 6.6 min; found $[M+3H]^{3+}$ 1624.9, $[M+4H]^{4+}$ 1218.9

Compound No. 36

Y-Aib-EGTFTSDYSIILE-K[2-[2-[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]-QAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 27; Substituent: A; Substituent position: K16

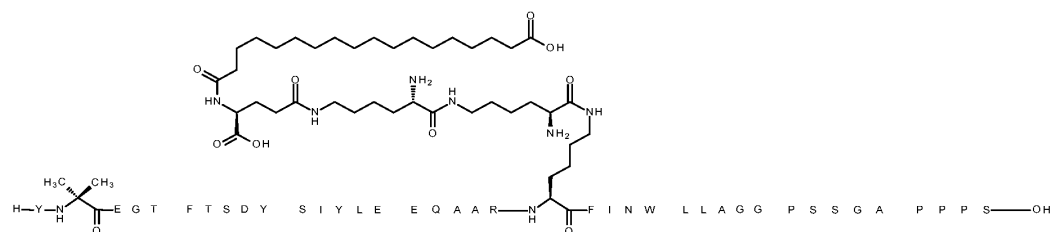
Synthesis methods: SPPS_A; SC_A; CP_A

Molecular weight (average) calculated: 4872.4 Da

15 LCMS_A: Rt = 6.5 min; found $[M+3H]^{3+}$ 1624.8, $[M+4H]^{4+}$ 1218.9

Compound No. 37

Y-Aib-EGTFTSDYSIYLEEQAAR-K[(2S)-2-amino-6-[(2S)-2-amino-6-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-FINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 28; Substituent: B; Substituent position: K21

Synthesis methods: SPPS_A; SC_C; CP_A

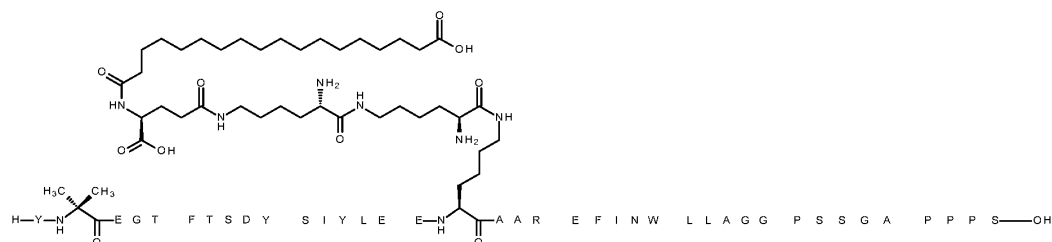
Molecular weight (average) calculated: 4888.5 Da

LCMS_A: Rt = 6.0 min; found $[M+3H]^{3+}$ 1630.3, $[M+4H]^{4+}$ 1223.0

5

Compound No. 38

Y-Aib-EGTFTSDYSIYLEE-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-AAREFINWLLAGGPSSGAPPPS-OH



10

SEQ ID NO: 29; Substituent: B; Substituent position: K17

Synthesis methods: SPPS_C; SC_C; CP_A

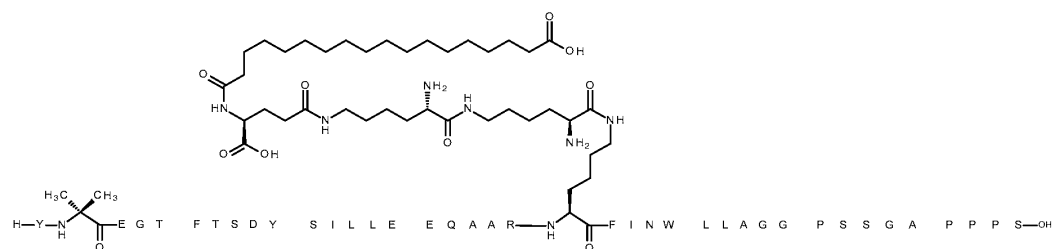
Molecular weight (average) calculated: 4889.5 Da

LCMS_A: Rt = 6.0 min; found $[M+3H]^{3+}$ 1630.6, $[M+4H]^{4+}$ 1223.2

15

Compound No. 39

Y-Aib-EGTFTSDYSILLEEQAAAR-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-FINWLLAGGPSSGAPPPS-OH



20

SEQ ID NO: 30; Substituent: B; Substituent position: K21

Synthesis methods: SPPS_A; SC_C; CP_A

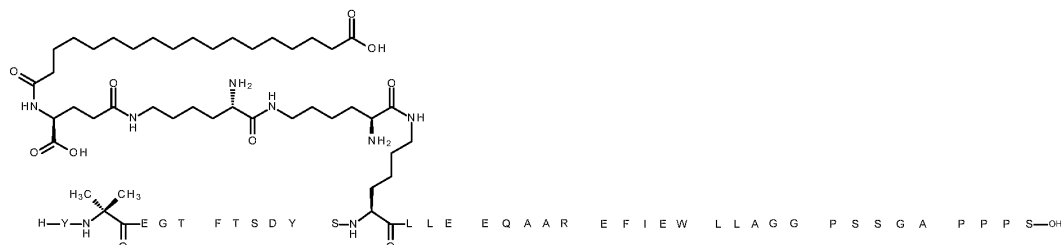
Molecular weight (average) calculated: 4838.5 Da

LCMS_A: Rt = 6.2 min; found $[M+3H]^{3+}$ 1613.5, $[M+4H]^{4+}$ 1210.4

25

Compound No. 40

Y-Aib-EGTFTSDYS-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-LLEEQAAREFIEWLLAGGPSSGAPPPS-OH



SEQ ID NO: 31; Substituent: B; Substituent position: K12

Synthesis methods: SPPS_A; SC_A; CP_A

Molecular weight (average) calculated: 4869.4 Da

LCMS_A: Rt = 5.7 min; found [M+3H]³⁺ 1623.8, [M+4H]⁴⁺ 1218.2

Compound No. 41

Y-Aib-EGTFTSDYS-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-YLEEQAAREFINWLLAGGPSSGAPPPS-OH



SEQ ID NO: 32; Substituent: B; Substituent position: K12

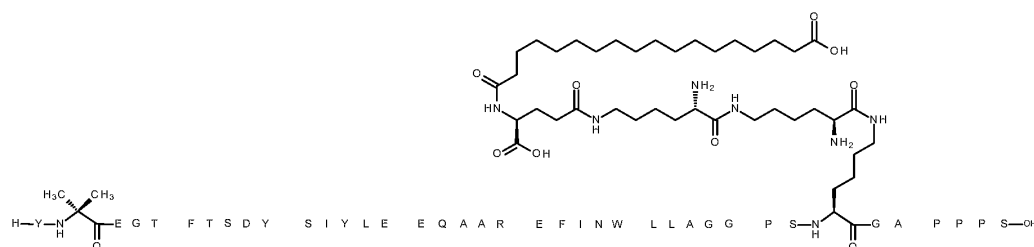
Synthesis methods: SPPS_A; SC_A; CP_A

Molecular weight (average) calculated: 4904.4 Da

LCMS_A: Rt = 5.7 min; found [M+3H]³⁺ 1635.4, [M+4H]⁴⁺ 1226.7

Compound No. 42

Y-Aib-EGTFTSDYSIYLEEQAAREFINWLLAGGPS-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-GAPPPS-OH



SEQ ID NO: 33; Substituent: B; Substituent position: K33

Synthesis methods: SPPS_A; SC_A; CP_A

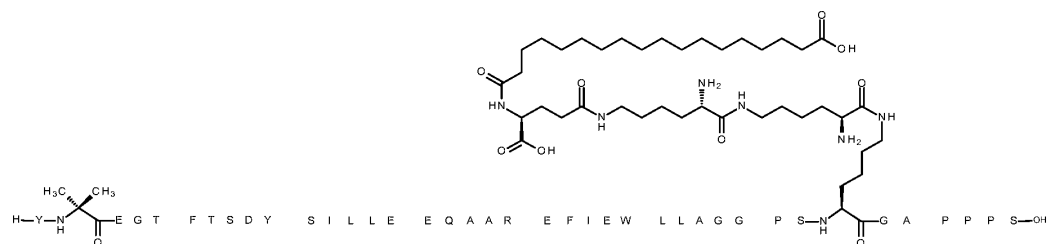
Molecular weight (average) calculated: 4930.5 Da

5 LCMS_A: Rt = 6.2 min; found $[M+3H]^{3+}$ 1644.2, $[M+4H]^{4+}$ 1233.4

Compound No. 43

Y-Aib-EGTFTSDYSILLEEQAAAREFIEWLLAGGPS-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

10 GAPPPS-OH



SEQ ID NO: 34; Substituent: B; Substituent position: K33

Synthesis methods: SPPS_A; SC_A; CP_A

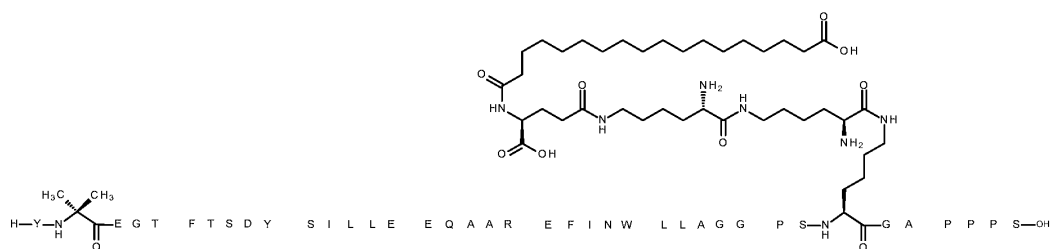
Molecular weight (average) calculated: 4895.5 Da

15 LCMS_A: Rt = 6.3 min; found $[M+3H]^{3+}$ 1632.4, $[M+4H]^{4+}$ 1224.6

Compound No. 44

Y-Aib-EGTFTSDYSILLEEQAAAREFINWLLAGGPS-K[(2S)-2-amino-6-[[[(2S)-2-amino-6-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 GAPPPS-OH



SEQ ID NO: 35; Substituent: B; Substituent position: K33

Synthesis methods: SPPS_A; SC_A; CP_A

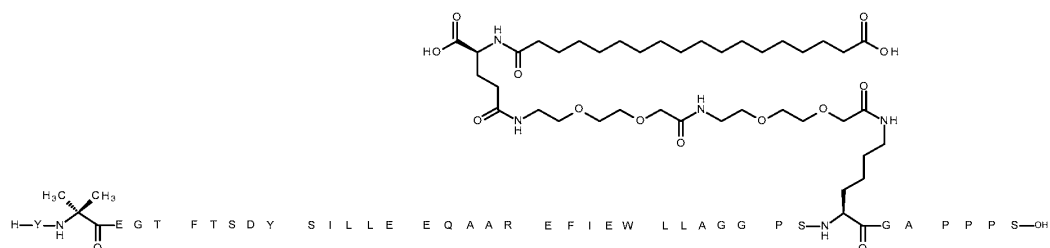
Molecular weight (average) calculated: 4880.5 Da

5 LCMS_A: Rt = 6.2 min; found $[M+3H]^{3+}$ 1627.5, $[M+4H]^{4+}$ 1220.9

Compound No. 45

Y-Aib-EGTFTSDYSILLEEQAAREFIEWLLAGGPS-K[2-[2-[2-[2-[2-[2-[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]

10 acetyl]-GAPPPS-OH



SEQ ID NO: 34; Substituent: A; Substituent position: K33

Synthesis methods: SPPS_A; SC_A; CP_A

Molecular weight (average) calculated: 4929.5 Da

15 LCMS_A: Rt = 6.6 min; found $[M+3H]^{3+}$ 1643.8, $[M+4H]^{4+}$ 1233.1

Compound No. 46

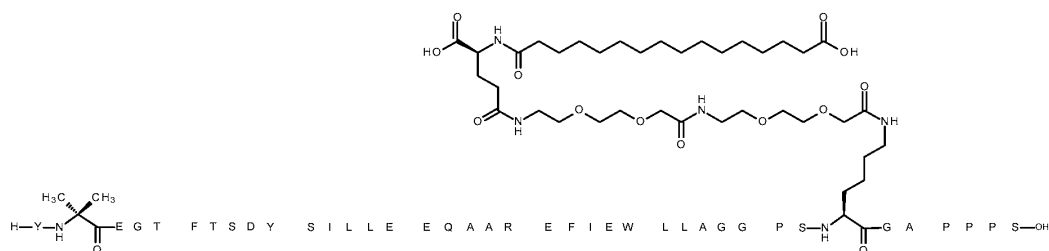
Y-Aib-EGTFTSDYSILLEEQAAREFIEWLLAGGPS-K[(2S)-2-amino-6-[(2S)-2-amino-6-[(4S)-4-carboxy-4-(15-carboxypentadecanoylamino)butanoyl]amino]hexanoyl]amino]hexanoyl]-

20 GAPPPS-OH

Molecular weight (average) calculated: 4867.5 Da

Compound No. 47

10 acetyl]-GAPPPS-OH



Molecular weight (average) calculated: 4901.4 Da

15 LCMS_A: Rt = 6.3 min; found $[M+3H]^{3+}$ 1634.6, $[M+4H]^{4+}$ 1226.1

The purpose of this example is to test the functional activity, or potency, of the compounds *in vitro* at the human GLP-1 and GIP receptors. The *in vitro* functional potency is the measure of target receptor activation in a whole cell assay. The potencies of derivatives of Example 1 were determined as described below. Human GLP-1(7-37) and human GIP were included in appropriate assays for comparison.

Principle

In vitro functional potency was determined by measuring the response of the target receptor in a reporter gene assay in individual cell lines. The assay was performed in stably transfected BHK cell lines that express one of the following G-protein coupled receptors: human GLP-1 receptor or human GIP receptor; and where each cell line contains the DNA for the cAMP response element (CRE) coupled to a promoter and the gene for firefly luciferase (CRE luciferase). When the respective receptor is activated, it results in the production of cAMP, which in turn results in expression of the luciferase protein. When assay incubation is completed, luciferase substrate (luciferin) is added resulting in the enzymatic conversion of luciferin to oxyluciferin and producing bioluminescence. The luminescence is measured as the readout for the assay.

Cell culture and preparation

The cell lines used in these assays were BHK cells with BHKTS13 as a parent cell line. The cell lines were derived from a clone containing the CRE luciferase element and were established by further transfection with the respective human receptor to obtain the relevant cell line: BHK CRE luc2P hGLP-1R or BHK CRE luc2P hGIPR. The cells were cultured at 37 °C with 5% CO₂ in Cell Culture Medium. They were aliquoted and stored in liquid nitrogen. The cells were kept in continuous culture and were seeded out the day before each assay.

Materials

The following chemicals were used in the assay: Pluronic F-68 10% (Gibco 2404), human serum albumin (HSA; Sigma A9511), 10% fetal bovine serum (FBS; Invitrogen 16140-071), chicken egg white ovalbumin (Sigma A5503), DMEM w/o phenol red (Gibco 21063-029), DMEM (Gibco 12430-054), 1 M Hepes (Gibco 15630), Glutamax 100x (Gibco 35050), G418 (Invitrogen 10131-027), hygromycin (Invitrogen 10687-010), and steadylite plus (PerkinElmer 6016757).

Buffers

GLP-1R Cell Culture Medium consisted of DMEM medium with 10% FBS, 500 µg/mL G418, and 300 µg/mL hygromycin. GIPR Cell Culture Medium consisted of DMEM medium with 10% FBS, 400 µg/mL G418, and 300 µg/mL hygromycin. Assay Buffer consisted of DMEM w/o phenol red, 10 mM Hepes, 1x Glutamax, 1% ovalbumin, and 0.1% Pluronic F-68 with the addition of HSA at twice the final assay concentration. The Assay

Buffer was mixed 1:1 with an equal volume of the test compound in Assay Buffer to give the final assay concentration of HSA.

Procedure

- 5 1) Cells were plated at 5000 cells/well and incubated overnight in the assay plate.
- 2) Cells were washed once in DPBS.
- 3) Stocks of the test compounds and reference compounds in concentrations ranging from 100-300 μ M were diluted 1:150 in Assay Buffer. Compounds were then diluted 1:10 in column 1 of a 96 deep well dilution plate and then carried across the row creating a 3.5 fold,
- 10 12 point dilution curve.
- 4) Assay Buffer (50 μ l aliquot) with or without HSA was added to each well in the assay plate.
- 5) A 50 μ l aliquot of compound or blank was transferred from the dilution plate to the assay plate containing the Assay Buffer with or without HSA.
- 6) The assay plate was incubated for 3 h in a 5% CO₂ incubator at 37 °C.
- 15 7) The cells were washed once with DPBS.
- 8) A 100 μ l aliquot of DPBS was added to each well of the assay plate.
- 9) A 100 μ l aliquot of steadylite plus reagent (light sensitive) was added to each well of the assay plate.
- 10) Each assay plate was covered with aluminum foil to protect it from light and shaken at
- 20 250 RPM for 30 min at room temperature.
- 11) Each assay plate was read in a microtiter plate reader.

Calculations and Results

- The data from the microtiter plate reader was first regressed in an Excel in order to
- 25 calculate the x-axis, log scale concentrations based on the individual test compound's stock concentration and the dilutions of the assay. This data was then transferred to GraphPad Prism software for graphing and statistical analysis. The software performs a non-linear regression (log(agonist) vs response). EC₅₀ values which were calculated by the software and reported in pM are shown in Tables 1-3 below. A minimum of two replicates was
 - 30 measured for each sample. The reported values are averages of the replicates.

Table 1: Functional potencies at human GLP-1R and GIPR in the presence of 0% and 1% HSA.

Compound No.	hGLP-1R, CRE Luc 0% HSA EC ₅₀ (pM)	hGLP-1R, CRE Luc 1% HSA EC ₅₀ (pM)	hGIPR, CRE Luc 0% HSA EC ₅₀ (pM)	hGIPR, CRE Luc 1% HSA EC ₅₀ (pM)
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hGLP-1(7-37)	8.4	6.7	nd	nd
hGIP	nd	nd	11.3	6.4
1	7.8	1659.0	2.8	172.5
2	6.0	899.7	3.8	275.4
3	8.5	626.3	6.9	229.2
4	6.6	484.3	8.5	293.8
5	9.4	624.8	14.2	362.4
6	3.4	242.7	18.5	434.0
7	4.2	513.2	28.7	686.8
8	2.3	82.1	6.7	191.1
9	3.8	278.8	10.8	508.2
10	3.9	939.9	11.3	1745.1
11	3.3	132.3	9.9	450.2
37	2.7	94.6	11.2	297.8
12	4.0	54.4	9.0	71.5
13	7.4	204.1	13.9	257.2
14	5.4	306.2	11.6	580.1
15	5.0	129.7	7.1	137.5
38	3.1	258.9	12.4	815.1
16	3.1	321.1	13.4	803.8
17	5.2	256.6	34.9	1319.0
18	19.7	226.2	20.9	285.1
19	8.6	364.8	6.5	275.4
20	4.2	256.0	7.1	247.4
21	5.7	118.2	5.4	130.9
22	2.8	170.5	6.2	168.9
23	4.1	117.8	6.7	134.3
24	12.1	490.2	3.9	115.1
25	15.5	740.2	7.2	263.0
26	7.4	594.6	4.9	153.0
39	8.2	135.4	28.4	365.2
27	4.6	127.0	6.3	131.8
28	6.2	139.1	6.6	86.5
29	16.8	378.5	3.8	101.7

30	7.3	267.5	3.2	185.0
41	1.2	31.4	4.9	79.3
42	2.3	83.5	5.9	305.7
40	6.4	333.6	9.5	500.6
31	4.2	202.0	4.7	188.5
32	5.0	981.9	7.1	678.7
43	4.0	359.5	5.6	444.5
44	4.2	240.9	5.7	365.3
33	14.8	360.7	2.9	102.8
34	6.0	186.4	8.3	212.8
35	3.0	412.6	5.2	303.0
36	4.7	413.8	5.5	356.5
45	3.9	1580.0	3.8	1220.0
46	1.5	110.8	1.8	97.0
47	2.1	154.5	3.1	155.7

nd=not determined.

The compounds of the present invention display potent functional activation of the human GLP-1R and human GIPR receptors under the given conditions.

5

Example 3: Pharmacokinetic study in minipigs

The purpose of this example is to determine the half-life *in vivo* of the derivatives of the present invention after i.v. administration to minipigs, i.e. the prolongation of their time in the body and thereby their time of action. This is done in a pharmacokinetic (PK) study, where the terminal half-life of the derivative in question is determined. By terminal half-life is generally meant the period of time it takes to halve a certain plasma concentration, measured after the initial distribution phase.

10

Study

Female Göttingen minipigs were obtained from Ellegaard Göttingen Minipigs (Dalmose, Denmark) approximately 7-14 months of age and weighing from approximately 16-35 kg were used in the studies. The minipigs were housed individually and fed restrictedly once daily with SDS minipig diet (Special Diets Services, Essex, UK).

15

After at 3 weeks of acclimatisation two permanent central venous catheters were implanted in vena cava caudalis in each animal. The animals were allowed 1 week recovery after the surgery, and were then used for repeated pharmacokinetic studies with a suitable wash-out period between successive derivative dosing.

5 The animals were fasted for approximately 18 hours before dosing and from 0 to 4 hours after dosing but had *ad libitum* access to water during the whole period.

The sodium salts of compounds of Examples 1 were dissolved to a concentration of 20-40 nmol/mL in a buffer containing 0.007% polysorbate 20, 50 mM sodium phosphate, 70 mM sodium chloride, pH 7.4. Intravenous injections (the volume corresponding to usually 10 1.5-2 nmol/kg, for example 0.1 mL/kg) of the compounds were given through one catheter, and blood was sampled at predefined time points for up to 14 days post dosing (preferably through the other catheter). Blood samples (for example 0.8 mL) were collected in 8 mM EDTA buffer and then centrifuged at 4 °C and 1942g for 10 minutes.

15 Sampling and analysis

Plasma was pipetted into Micronic tubes on dry ice and kept at -20 °C until analysed for plasma concentration of the compounds using ELISA, or a similar antibody-based assay, or LCMS. Individual plasma concentration-time profiles were analysed by a non-compartmental model in Phoenix WinNonLin ver. 6.4. (Pharsight Inc., Mountain View, CA, 20 USA), and the resulting terminal half-lives (harmonic mean) determined.

Results

Table 2: Terminal half-life as measured after i.v. administration to minipigs

Compound No.	t _{1/2} (h)
15	36
28	35
29	68
31	49

25 The tested compounds of the present invention have very long half-lives as compared to the half-lives of hGLP-1 and hGIP measured in man to be approximately 2 – 4 min and 5 – 7 min, respectively. The measured half-lives in minipigs predict half-lives in humans sufficient for at least once-weekly administration via liquid injection or at least once-daily administration via oral tablet.

30

Example 5: Pharmacokinetic study in dogs

The purpose of this example is to determine the half-life and plasma exposure *in vivo* of the compounds of the present invention after p.o. administration to beagle dogs, i.e. the terminal half-life and concentration of test substance that reaches circulation with time. This is done in a pharmacokinetic (PK) study, where these parameters of the compound in question are determined. By terminal half-life is generally meant the period of time it takes to halve a certain plasma concentration, measured after the initial distribution phase.

Preparation of Tablet Compositions

Tablet compositions comprising the test substance and SNAC (sodium *N*-(8-(2-hydroxybenzoyl)amino)caprylate) were prepared according to methods known to the person skilled in the art by mixing test substance with roller compacted SNAC and magnesium stearate as e.g. described in WO 2019/149880. The amount of SNAC in the tablet composition was 100-300 mg, the amount of magnesium stearate in the tablet composition was 7.7 mg, and the target amount of each test substance in the tablet composition was 3-4 mg.

Animals, dosing, and sampling

Male beagle dogs, 1-7 years of age and weighing 9-17 kg during the study period, were included in the study. The dogs were dosed in a fasting state. The dogs were group housed in pens (12 hours light: 12 hours dark), and fed individually and restrictedly once daily with Royal Canin Medium Adult dog food (Royal Canin Products, China Branch, or Brogaarden A/S, Denmark). The dogs were used for repeated PK studies with a suitable wash-out period between successive dosing. An appropriate acclimatization period was given prior to initiation of the first PK study. All handling, dosing, and blood sampling of the animals were performed by trained and skilled staff. Before the studies, the dogs were fasted overnight and from 0 to 4 hours after dosing. The dogs were restricted to water 1 hour before dosing until 4 hours after dosing, but otherwise had *ad libitum* access to water during the whole period.

The compositions were administered by a single oral dosing to the dogs in groups of 6-8 dogs. The tablets were administered in the following manner: 10 min prior to tablet administration, the dogs may be dosed subcutaneously with approximately 3 nmol/kg of SEQ ID NO: 48, then tablets were placed in the back of the mouth of the dog to prevent chewing.

The mouth was then closed, and 10 mL of tap water was given by syringe or gavage to facilitate swallowing of the tablet.

One blood sample was drawn before dosing, and additional samples were drawn at predefined time points after dosing, such as after 5, 10, 15, 20, 30, and 45 minutes and after 1, 1.5, 2, 4, 7, 10, 24, 48, 72, 120, 144, 168, 192, 216 hours for up to 240 hours, to adequately cover the full plasma concentration-time absorption profile of the test substance. For each blood sample time point, approximately 0.8 mL of whole blood was collected in a 1.5 mL EDTA-coated tube, which was gently turned to mix the sample with EDTA. Blood samples were collected in EDTA buffer (8 mM) and then centrifuged at 4°C and 2000g for 10 minutes. Plasma was pipetted into Micronic tubes on dry ice, and kept at -20°C or lower until analysis. Blood samples were taken as appropriate, for example from a venflon in the cephalic vein in the front leg for the first 2 hours and then with syringe from the jugular vein for the rest of the time points. The first few drops were allowed to drain from the venflon to avoid heparin saline from the venflon in the sample.

All blood samples were collected into test tubes containing EDTA for stabilisation and kept on ice until centrifugation. Plasma was separated from whole blood by centrifugation and the plasma was stored at -20°C or lower until analysis.

Analysis and calculations

The plasma was analysed for test substance using LC-MS (Liquid Chromatography-Mass Spectrometry) as known to the person skilled in the art. The system consisted of either: a Thermo Fisher QExactive mass spectrometer equipped with a 10-valve interface module TurboFlow system, CTC HTS PAL autosampler, Accela 1250 pumps, and Hot Pocket column oven; or a Thermo Fisher QExactive Plus mass spectrometer equipped with a valve interface module TurboFlow system, TriPlus RSI autosampler, Dionex UltiMate 3000 pumps, and Hot Pocket column oven. RP-HPLC separation was achieved using a linear gradient of 1:1 acetonitrile/methanol in 1% aqueous formic acid using either: a Phenomenex Onyx Monolithic C18 column (50 x 2.0 mm) and a flow rate of 0.8 mL/min at 30°C; or an Agilent Poroshell 120 SB-C18 column (50 x 2.1 mm, 2.7 µm) at a flow rate of 0.4 mL/min at 60°C. The mass spectrometer was operated in either positive ionization SIM mode or positive ionization PRM mode.

For each individual animal, a plasma concentration-time profile was analysed by a non-compartmental model in Pharsight Phoenix WinNonLin ver. 6.4 software or other relevant software for PK analysis, and the resulting terminal half-life ($t_{1/2}$), maximum plasma concentration per dose (C_{max}/D), time for maximum plasma concentration (t_{max}), and area

under the curve to infinity per dose (AUC/D) were determined. Summary statistics of pharmacokinetic results were presented as median (for $t_{\max}$), harmonic mean ($t_{1/2}$), or arithmetic mean ($C_{\max}$, AUC).

5 Results

Table 3: Pharmacokinetic parameters following oral dosing of tablet compositions of test substance and SNAC to Beagle dogs.

Compound No.	Amount of SNAC (mg)	Target amount of test substance (mg)	$T_{\max}$ (h)	$t_{1/2}$ (h)	$C_{\max}$ /Dose (kg/L)	AUC/Dose (kg*h/L)
9	100	4	2.0	50	0.24	12.1
10	100	4	1.0	36	0.20	8.4
11	100	4	1.4	34	0.19	7.1
13	100	3	1.5	60	0.29	14.8
15	100	3	1.5	59	0.24	11.9
15	300	3	2.0	46	0.28	11.6
16	100	3	1.3	47	0.21	8.6
20	100	3	2.0	56	0.21	10.8
24	300	3	1.8	74	0.29	16.4
25	300	3	1.8	43	0.30	15.2
26	300	3	1.2*	70*	0.49*	27.4*
28	300	3	1.3**	67**	0.36**	18.6**
29	300	3	1.2**	69**	0.26**	16.6**
30	300	3	0.8	81	0.24	6.5
40	300	3	2.0	61	0.48	27.0
31	300	3	1.3**	56**	0.35**	17.9**
32	300	3	1.8*	80*	0.45*	25.3*
43	300	3	1.5	131	0.22	21.4

* = averaged data from two experiments under same formulation conditions; ** = averaged data from three experiments under same formulation conditions.

10

The tested compounds of the present invention demonstrate oral bioavailability in this model, as concentrations of the compound in plasma were detected ($C_{\max}/D > 0$ and $AUC/D > 0$) following oral administration. Furthermore, the tested compounds of the present

invention further have very long half-lives as compared to the half-lives of hGLP-1 and hGIP measured in man to be approximately 2 – 4 min and 5 – 7 min, respectively.

5 While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended embodiments are intended to cover all such modifications and changes as fall within the true spirit of the invention.

10