

TRI-AGONISTS OF THE GLP-1, GIP, AND AMYLIN RECEPTORS

TECHNICAL FIELD

GLP-1-/GIP-/amylin-receptor tri-agonists and compositions comprising such compound for use in medicine.

5 BACKGROUND

Overweight and obesity are the abnormal or excessive accumulation of body fat that present a risk to an individual's overall health. The WHO considers body mass index (BMI) to be the most convenient population-level measure of overweight and obesity. In adults, a body mass index (BMI) greater than or equal to 25 is considered overweight, and a BMI of greater
10 than or equal to 30 is considered obese. Obesity is further subclassified into class I (BMI 30-34.9), class II (BMI 35-39.9) and class III (BMI>40).

Obesity is a leading risk factor in a large number of serious conditions, including type 2 diabetes and its associated co-morbidities, and cardiovascular diseases such as heart disease and stroke, which are the leading causes of death worldwide. Obesity is now
15 recognised by the World Health Organization (WHO) as an issue that has grown to epidemic proportion, even in children: in 2016, 1.9 billion adults worldwide were reported to have obesity; in 2019, 38.3 million children under the age of 5 worldwide were reported to have obesity. According to the WHO, 422 million people worldwide have diabetes, and 1.6 million deaths are directly attributed to diabetes each year. There is, therefore, a huge incentive for
20 the individual, as well as society, to try to prevent and/or treat obesity.

When life-style modification such as diet and exercise alone do not suffice in reducing the body mass index (BMI) of an individual living with obesity to an acceptable level, treatment with pharmaceutical drugs such as liraglutide, orlistat and naltrexone–bupropion have been shown to cause some weight loss. Nonetheless, these weight losses are often not
25 sustained and are too small for individuals with class II and class III obesity. In these cases, bariatric surgery has proven necessary. While bariatric surgery is currently the most effective treatment in terms of obtaining long-term weight loss, it is an invasive procedure associated with high risk to the patient and high cost. Therefore, an efficacious and minimally invasive treatment would be a significant improvement in the treatment of obesity.

30 GLP-1 is a 30 or 31-amino acid polypeptide that is synthesised and secreted from enteroendocrine L-cells. GLP-1 is an incretin hormone, decreasing blood sugar levels in a glucose-dependent manner by enhancing the secretion of insulin. Endogenous GLP-1 is

rapidly degraded, primarily by dipeptidyl peptidase-4 (DPP-4), resulting in a half-life of <2 minutes.

Several marketed products containing a long-acting GLP-1 receptor agonist as the active pharmaceutical ingredient are approved for treatment of type 2 diabetes. These
5 include dulaglutide (Trulicity®), exenatide (Byetta®, Bydureon®), liraglutide (Victoza®), lixisenatide (Lyxumia®), and semaglutide (Ozempic®).

Two marketed products containing a GLP-1 receptor agonist as the active pharmaceutical ingredient are approved for use in individuals living with overweight and have at least one weight-related co-morbidity or in individuals living with obesity: liraglutide
10 (Saxenda®) and semaglutide (Wegovy®). The maximum efficacy that can be achieved with a GLP-1 receptor agonist is limited by tolerability. At increasing doses, side-effects such as nausea and vomiting become increasingly pronounced.

Native human GIP is a 42-amino acid polypeptide synthesized in and secreted by specialized enteroendocrine K-cells. These cells are concentrated primarily in the duodenum
15 and proximal jejunum, although they also can be found throughout the intestine. The main stimulant for GIP secretion is ingestion of carbohydrate- and lipid-rich meals. Following ingestion, circulating plasma GIP levels increase 10- to 20-fold. Like GLP-1, GIP is an incretin hormone and in healthy humans it actually appears to be a more powerful incretin than GLP-1. However, in individuals living with type-2-diabetes GIP has lost its incretin effect. The half-
20 life of intact GIP is estimated to be approximately 7 minutes in healthy subjects and approximately 5 minutes in people living with type-2 diabetes.

Long acting (or protracted) GIP analogues have been shown to lower body weight and improve glycaemic control. With regards to body weight reduction, this effect is comparatively less than long-acting GLP-1 analogues in rodent models (Mroz *et al*, Mol
25 Metab, 2019, 20: 51-62). Moreover, GIP analogues induce body weight loss by an additive/synergistic action with long-acting GLP-1 analogues 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 also be included as a partner to GLP-1 receptor agonism
30 as a single molecule co-agonist to amplify GLP-1 driven body weight loss and improvement in glycaemic control, as has been shown in preclinical animal models (Finan *et al*, Sci Transl Med, 2013, 5 (209): 209ra151; Coskun *et al*, Mol Metab, 2018, 18: 3-14). Two different peptides (MAR709 and LY3298176, the latter known as tirzepatide) with high potency on both GLP-1R and GIPR have been tested in multi-dose clinical trial studies. The clinical
35 results have demonstrated improvements in glycaemic control and body weight that exceed

that achieved with 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.

5 Lately this concept of co-targeting GLP-1 and GIP receptors with a GLP-1/GIP co-agonist has been proven, as the compound tirzepatide has been approved in 2022 for the treatment of diabetes. Furthermore, tirzepatide is also useful for the treatment of obesity, as it has shown in a phase 3 clinical trial that a high dose of tirzepatide (15 mg) helped patients to lose (mean) 20.9 % of their body weight after 72 weeks of treatment, including a 20-week
10 dose-escalation period (AM Jastreboff, LJ Aronne, NN Ahmad, *et al.*, N Engl J Med 2022; 387:205-216). Tirzepatide was recently approved for weight management in people with a BMI >30 or a BMI >27 and at least one weight related co-morbidity (tradename: Zepbound®).

Beside tirzepatide, which is described in WO 2016/111971 A1, GLP-1/GIP co-agonists and their potential medical uses are described in several patent applications such as
15 WO 2006/086769, 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 2014/162945, US 2014/357552, and WO 2022/018186.

Amylin is a 37-amino acid long polypeptide hormone produced in pancreatic beta (β)-cell from where it is co-secreted with insulin. Amylin has a half-life of 15-20 minutes. It
20 produces its effects in several different organ systems, primarily acting via amylin receptors 1-3 (AMYR1-3). Amylin is an important regulator of energy metabolism in health and disease, inhibiting glucagon secretion, delaying gastric emptying, signalling satiety, and suppressing appetite. Other amylin actions have also been reported, such as on the cardiovascular system and on bone.

25 Clinical studies have shown that amylin receptor agonists may be useful for the treatment of overweight, obesity, type 1 diabetes and/or type 2 diabetes. Currently, there is one product on the market (Symlin®) which contains an amylin receptor agonist (pramlintide acetate) as the active pharmaceutical ingredient. Symlin®, a liquid pharmaceutical composition for subcutaneous administration, is approved for use in patients with type 1 or
30 type 2 diabetes who use basal and mealtime insulin and have failed to achieve desired glycaemic control, despite optimal insulin therapy. Pramlintide for use in people living with overweight and obesity was also investigated. Pramlintide has a short half-life (less than 1 hour), necessitating administration thrice daily. Consequently, there is a large diurnal difference in the pramlintide plasma concentrations.

Amylin receptor agonist therapy is limited by tolerability in much the same way as GLP-1 receptor agonist therapy (and by similar side-effects such as nausea and vomiting). There has been a similar desire to be able to prolong the action of amylin and also the co-targeting of the amylin receptor and the GLP-1 receptor has been described. Amylin receptor agonists and their potential medical uses are described in several patent applications such as WO 2012/168432, WO 2016/034604, WO 2022/129254, WO 2022/063925, or US 2022/0288168

A fixed-dose combination of an amylin receptor agonist, cagrilintide, and a GLP-1 receptor agonist, semaglutide, is currently under investigation for the treatment of overweight and obesity (Lancet 2021; 397: 1736–48). The drug products being investigated are separate liquid pharmaceutical compositions for subcutaneous use. A clinical trial has demonstrated that a combination of cagrilintide and semaglutide induced greater weight loss in people living with obesity than the maximal approved dose of semaglutide monotherapy. Worsening of the side-effect profile was not observed. Co-agonists of GLP-1 and amylin receptors, and their potential medical uses are described in several patent applications such as WO 2022/129526 A1. Therein peptide co-agonists of the human GLP-1R and amylin receptor are disclosed, which are potent and are balanced, i.e., having a similar level of activation of both receptor systems, and which are showing oral bioavailability. Another example is WO 2007/022123, which describes hybrid polypeptides including exendin covalently linked to amylin. However, there is no co-agonist of GLP-1 and amylin receptors which has so far obtained market approval.

Finally, WO 2023/288313 and WO 2024/015922 disclose multi-agonist peptides useful as agents for the treatment and prevention of metabolic diseases and disorders, in particular diabetes and obesity. WO 2023/288313 discloses peptides including two or more component peptides including amylin, GIP, GLP-1, and/or calcitonin. The specifically disclosed peptides are tri-agonists of the GLP-1, GIP, and amylin receptors, showing activity on all three receptors and reduction of food intake and body weight in animals.

Whilst current therapy options and investigatory drugs provide promise, individuals living with overweight, obesity and/or associated co-morbidities can, at best, at current time hope to be treated with injectable pharmaceutical formulations or medications with some efficacy. There still remains a need in the art for a more efficacious medicament, which is potent *in vitro* and potent on weight loss, and which does not simultaneously result in a proportionally increased level of side-effects, and which has improved pharmacokinetic properties, has improved chemical stability, is suitable for once weekly dosing in humans, and/or is suitable for oral administration.

SUMMARY

The present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



5 comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide comprising a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib;

10 • L1 is a peptide linker; and

• Z2 is a peptide comprising a C-terminal amide and a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



15 In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

20 • Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib,

and wherein

Z1 comprises an amino acid sequence according to Formula X (SEQ ID NO: 161):



wherein

X₂₁ represents His (H) or Tyr (Y),

X₂₂ represents Aib,

X₂₃ represents Glu (E) or His (H),

30 X₂₄ represents Ile (I) or Lys (K),

X₂₅ represents Gln (Q) or Ile (I),

X₂₆ represents Arg (R) or Gln (Q),

X₂₇ represents Ala (A), Glu (E) or Gln (Q),

X₂₈ represents Leu (L) or I (Ile),

35 X₂₉ represents Ala (A) or Gln (Q),

X₃₀ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₃₁ represents Gly (G), Glu (E) or Lys (K);

• L1 is a peptide linker; and

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10

5 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula XII (SEQ ID NO: 164):

AX₃₂X₃₃LSTAX₃₄X₃₅X₃₆RLSAX₃₇LHX₃₈LX₃₉X₄₀X₄₁PX₄₂TETGSGX₄₃P (XII),

10 wherein

X₃₂ represents Gly (G) or Ser (S),

X₃₃ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₃₄ represents Ala (A) or Gln (Q),

X₃₅ represents Gln (Q), Leu (L) or Thr (T),

15 X₃₆ represents Ala (A), Gly (G) or Gln (Q),

X₃₇ represents Glu (E) or Lys (K),

X₃₈ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₃₉ represents Ala (A) or Lys (K),

X₄₀ represents Asp (D) or Thr (T),

20 X₄₁ represents Leu (L) or Glu (E),

X₄₂ represents Arg (R) or Lys (K),

X₄₃ represents Ala (A) or Ser (S).

In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist
25 comprising a peptide according to Formula I:

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula
II (SEQ ID NO: 1):

30 YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

and wherein

Z1 comprises an amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

35 wherein

X₁ represents His (H) or Tyr (Y),

X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G), Lys (K) or Ser (S),

5 X₅ represents Gly (G) or Lys (K);

• L1 is a peptide linker; and

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

10 and

Z2 comprises an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

15 X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

20 X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

25 In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

and wherein

Z1 comprises an amino acid sequence according to Formula Xa (SEQ ID NO: 162):

35 YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K),

X₅₃ represents Arg (R) or Gln (Q),

5 X₅₄ represents Ala (A), Glu (E) or Gln (Q),

X₅₅ represents Leu (L) or I (Ile),

X₅₆ represents Ala (A) or Gln (Q),

X₅₇ represents Gly (G) or Glu (E);

- L1 is a peptide linker; and

10 • Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula XIIa (SEQ ID NO:

15 165):

AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

wherein

X₅₈ represents Gly (G) or Ser (S),

X₅₉ represents Gln (Q), Glu (E), or His (H),

20 X₆₀ represents Ala (A) or Gln (Q),

X₆₁ represents Leu (L) or Thr (T),

X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E).

25

In some embodiments, the GLP-1-/GIP-/amylin-receptor tri-agonist, has an amino acid sequence which comprises or consists of

YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQR
LSAELHKLATLPRTETGSGSP (SEQ ID NO: 62), or

30 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQR
LSAKLHRLATLPRTETGSGSP (SEQ ID NO: 65), or

YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRL
SAELHKLATLPRTETGSGSP (SEQ ID NO: 68), or

35 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSPRGEASHLSTAQTQRLSAELHKLATL
PRTETGSGSP (SEQ ID NO: 78), or

YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRL
SAELHKLATLPRTETGSGSP (SEQ ID NO: 87), or

HXHGFTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSGGGEASHLSTAQTARLS
AELHQLATLPRTETGSGSP (SEQ ID NO: 111); or

5 YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
SAELHKLATLPRTETGSGSP (SEQ ID NO: 170); wherein in each amino acid sequence X
represents Aib.

The GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide linker L1, which
comprises or consists of the amino acid sequence according to Formula IV:

10 $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (IV),

wherein

X_1 represents Ala (A), Glu (E), Gly (G),

X_2 represents Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P) or is absent,

X_3 represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or is absent,

15 X_4 represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or is absent,

X_5 represents Glu (E), Gly (G), Pro (P), Ser (S), Thr (T) or is absent,

X_6 represents Glu (E), Gly (G), Leu (L), Gln (Q) or is absent,

X_7 represents Ala (A), Gln (Q), Glu (E), Gly (G), Phe (F) or is absent,

X_8 represents Ala (A), Gln (Q), Glu (E), Gly (G), Thr (T), Pro (P), Val (V) or is absent,

20 X_9 represents Glu (E), Asn (N), Pro (P), Thr (T) or is absent,

X_{10} represents Ala (A), Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P), Ser (S), Val (V) or
is absent,

X_{11} represents Ala (A) or is absent,

X_{12} represents Gln (Q) or is absent,

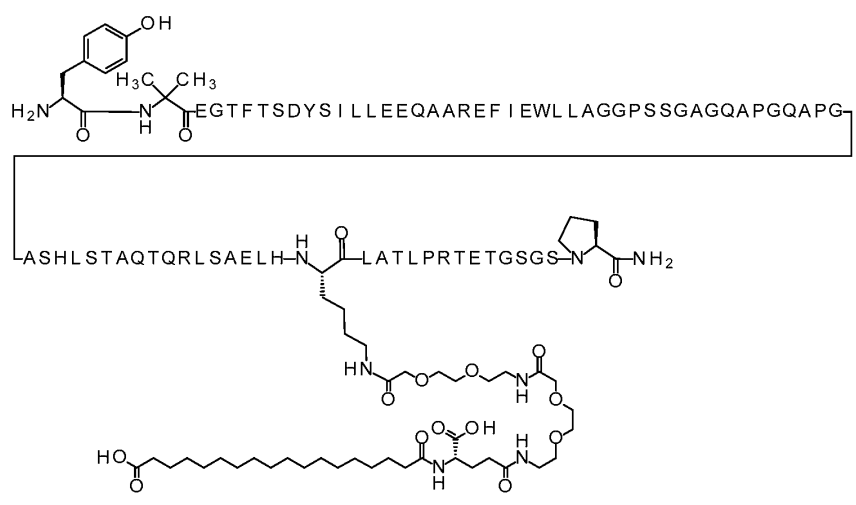
25 X_{13} represents Thr (T) or is absent,

X_{14} represents Leu (L) or is absent.

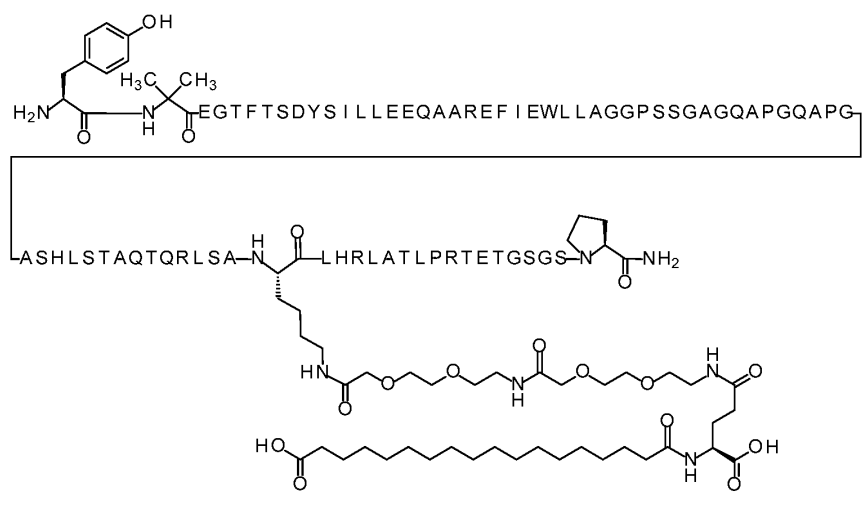
In some embodiments, said GLP-1-/GIP-/amylin-receptor tri-agonist comprises a
protraction moiety allowing for extended half-life.

30 Preferred GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention are:

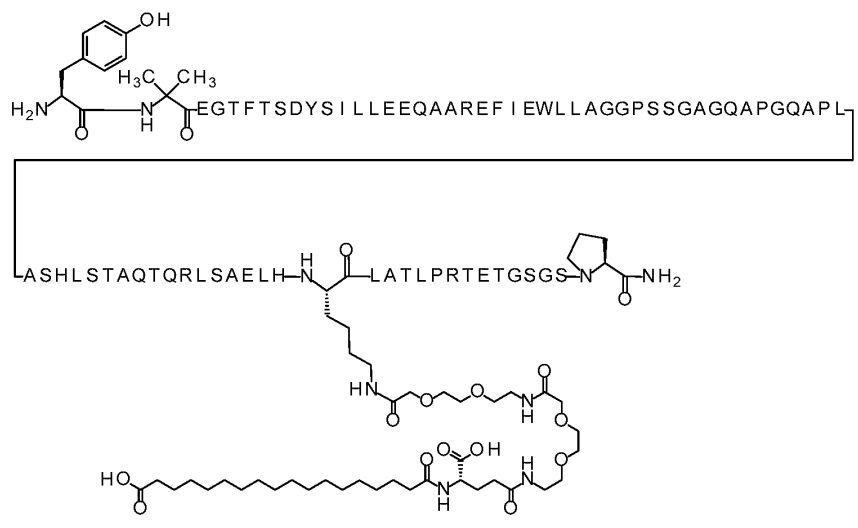
compound 52, that is:



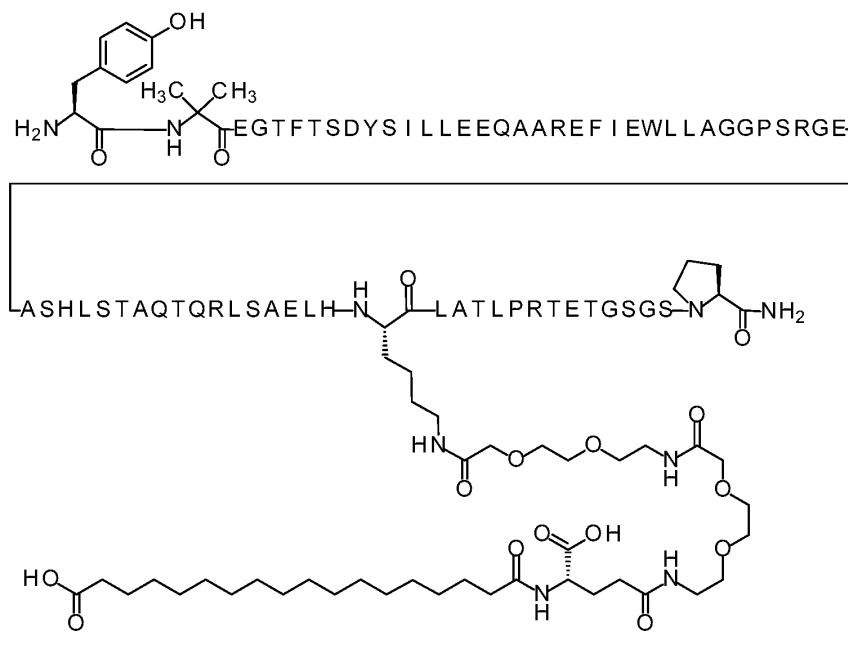
compound 55, that is:



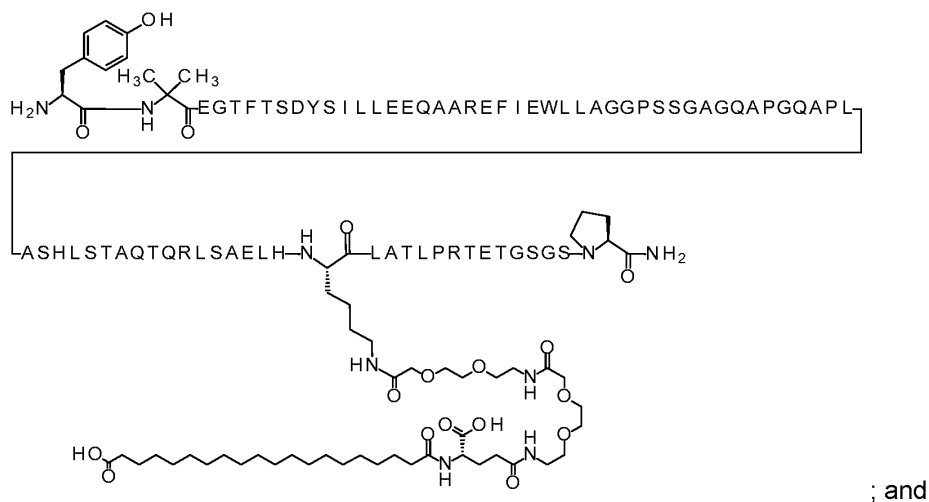
compound 58, that is:



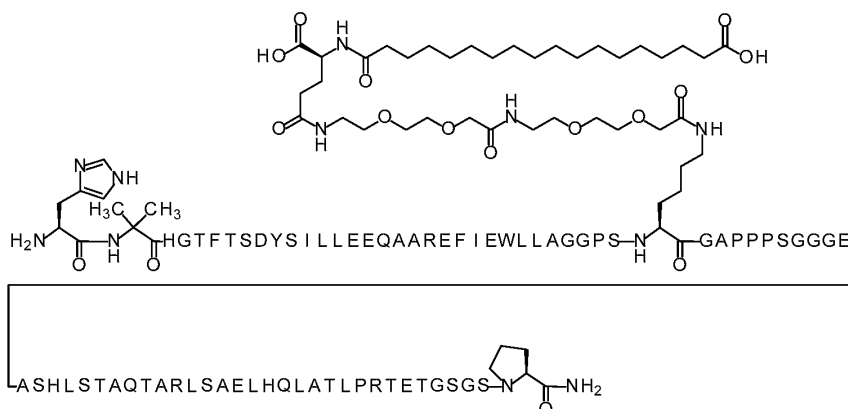
5 compound 68, that is:



compound 77, that is:



5 compound 101, that is:



In a third aspect the invention relates to a balanced GLP-1-/GIP-/amylin-receptor tri-agonist that is capable of selectively activating or "agonising" all three of the GLP-1 receptor, the GIP receptor, and the amylin receptor to a similar level.

10 Also or alternatively, in a fourth aspect, the invention relates to a GLP-1/GIP-
/amylin-receptor tri-agonist with improved pharmacokinetic properties.

Also or alternatively, in a fifth aspect, the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist which is suitable for once weekly administration.

Also or alternatively, in a sixth aspect, the invention relates to a GLP-1-/GIP-/amylin-
15 receptor tri-agonist which is suitable for oral administration.

Also or alternatively, in a seventh aspect, the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist with improved chemical stability.

Also, in further aspects the present invention relates to pharmaceutical compositions comprising such GLP-1-/GIP-/amylin-receptor triple agonist and one or more

- 5 pharmaceutically acceptable excipients, as well as a GLP-1-/GIP-/amylin-receptor triple agonist for use as a medicament, in particularly for use in the treatment of subjects with an initial body mass index (BMI) of 27 or more, such as 30 or more, optionally in the presence of at least one weight-related co-morbidity.

- 10 The invention may also solve further problems that will be apparent from the disclosure of the exemplary embodiments and aspects.

BRIEF DESCRIPTION OF DRAWINGS

- Fig. 1 shows changes in body weight (in %) over time in a study in DIO rats, as described in Example 8, comparing vehicle (-●-); Compound 52 with a dose of 3 nmol/kg (-●-); Compound 52 with a dose of 10 nmol/kg (-▼-); Compound 77 with a dose of 3 nmol/kg (-x-); and Compound 77 with a dose of 10 nmol/kg (-▲-). DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14.

- Fig. 2 shows daily food intake (in kcal) at baseline (day -4 to day 0) and during treatment (day 0-28) in a study in DIO rats, as described in Example 8, comparing vehicle (-●-); Compound 52 with a dose of 3 nmol/kg (-●-); Compound 52 with a dose of 10 nmol/kg (-▼-); Compound 77 with a dose of 3 nmol/kg (-x-); and Compound 77 with a dose of 10 nmol/kg (-▲-). DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14.

- Fig. 3 shows cumulative food intake over time in a study in DIO rats, as described in Example 8a, comparing vehicle (-●-); Compound 52 with a dose of 3 nmol/kg (-●-); Compound 52 with a dose of 10 nmol/kg (-▼-); Compound 77 with a dose of 3 nmol/kg (-x-); and Compound 77 with a dose of 10 nmol/kg (-▲-). DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14.

SEQUENCE LISTING

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

SEQ ID NO: 1 represents the amino acid sequence of Formula II of Z1.

SEQ ID NO: 2 represents the amino acid sequence of Formula III of Z2.

SEQ ID NO: 3 represents the amino acid sequence of Formula IIa of Z1.

SEQ ID NO: 4 represents the amino acid sequence of Formula IIIa of Z2.

SEQ ID NO: 5 represents the most encompassing amino acid sequence of Formula I Z1—L1—Z2.

SEQ ID NO: 6 represents the amino acid sequence of Formula V of Z1.

5 SEQ ID NO: 7 represents the amino acid sequence of Formula VI of Z1.

SEQ ID NO: 8 represents the amino acid sequence of Formula VII of Z2.

SEQ ID NO: 9 represents the amino acid sequence of Formula VIII of Z2.

SEQ ID NO: 11 represents the amino acid sequence of the peptide backbone within reference compound 1.

10 SEQ ID NO: 12 represents the amino acid sequence of the peptide backbone within reference compound 2.

SEQ ID NO: 13 represents the amino acid sequence of the peptide backbone within reference compound 3.

15 SEQ ID NO: 14 represents the amino acid sequence of the peptide backbone within reference compound 4.

SEQ ID NO: 15 represents the amino acid sequence of the peptide backbone within reference compound 5 (tirzepatide).

SEQ ID NO: 16 represents the amino acid sequence of the peptide backbone within reference compound 6 (cagrilintide).

20 SEQ ID NO: 17 represents the amino acid sequence of the peptide backbone within reference compound 7 (semaglutide).

SEQ ID NOs: 20-124 represent the amino acid sequences of the peptide backbones in compounds 10 to 115 and 210.

25 SEQ ID NOs: 125-159 represent the amino acid sequences of exemplified peptide linkers L1.

SEQ ID NO: 161 represents the amino acid sequence of Formula X of Z1.

SEQ ID NO: 162 represents the amino acid sequence of Formula Xa of Z1.

SEQ ID NO: 163 represents the amino acid sequence of Formula XI of Z1.

SEQ ID NO: 164 represents the amino acid sequence of Formula XII of Z2.

30 SEQ ID NO: 165 represents the amino acid sequence of Formula XIIa of Z2.

SEQ ID NO: 166 represents the amino acid sequence of Formula XIII of Z2.

SEQ ID NOs: 170-242 represent the amino acid sequences of the peptide backbones in compounds 120 to 197 and 211 to 221.

Table 1: Amino acid sequences of the peptide backbone within compounds of the invention

Compound no.	SEQ ID NO:	Amino acid sequences
peptide backbone within compound no. 10	20	YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGEASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 11	21	YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSKGGEASELST AALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 12	22	HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGEASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 13	23	YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGGASELSTAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 14	24	HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGGASELSTAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 15	25	HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGEASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 16	26	HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSG GGEASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 17	27	HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGGEASELS TAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 18	28	YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSKGGEASELST AALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 19	29	YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSKGGEASELST AALGRLSAELHRLATLPRTETGSGSP

peptide backbone within compound no. 20	30	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGASELSTA ALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 21	31	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGALAQT LAQTASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 22	32	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPSL AQTALAQTASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 23	33	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGALAQT LGTNEASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 24	34	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGALAQT LFVNQASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 25	35	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASEL STAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 26	36	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASEL STAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 27	37	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASEL STAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 28	38	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASEL STAALARLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 29	39	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASEL STAALGRLSAELHYLATLPRTETGSGSP
peptide backbone within compound no. 30	40	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPSG GGASELSTAALGRLSAELHRLATLPRTETGSGSP

peptide backbone within compound no. 31	41	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKAPPPSG GGEASELSTAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 32	42	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAEASEL STAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 33	43	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPEAS ELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 34	44	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAEASHLS TAQTQRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 35	45	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAEASHLS TAQTARLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 36	46	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAEASHLS TAQTARLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 37	47	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGEASHLSTAQTARLSAELHRLADLPRTETGSGSP
peptide backbone within compound no. 38	48	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGGQAPGQ APGEASHLSTAQTQRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 39	49	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGGQEPGQ EPGEASHLSTAQTQRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 40	50	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGGGGEG GGEGEASHLSTAQTQRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 41	51	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGEASELSTAALGRLSAELHRLADLPRTETGSGSP

peptide backbone within compound no. 42	52	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKAPPPSG GGEASELSTAALGRLSAELHRLATLPRRTETGSGSP
peptide backbone within compound no. 43	53	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGEASQLS TAALGRLSAELHRLADLPRRTETGSGSP
peptide backbone within compound no. 44	54	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGALAQTL GTNEASELSTAALGRLSAELHRLATLPRRTETGSGSP
peptide backbone within compound no. 45	55	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGALAQTL GTNEASHLSTAQTQRLSAELHELATLPRRTETGSGSP
peptide backbone within compound no. 46	56	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGAGQAPG QAPGASHLSTAALGRLSAELHRLATLPRRTETGSGSP
peptide backbone within compound no. 47	57	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGAGQAPG QAPGASHLSTAQTQRLSAELHELATLPRRTETGSGSP
peptide backbone within compound no. 48	58	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALAQT LAQTASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 49	59	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAPPPSG GGEASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 50	60	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 51	61	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALAQT LAQTASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compounds 52, 115, 210	62	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHKLATLPRRTETGSGSP

peptide backbone within compound no. 53	63	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAALGRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 54	64	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASKLSTAQTQRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 55	65	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 56	66	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLKTLPRRTETGSGSP
peptide backbone within compound no. 57	67	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 58	68	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 59	69	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALAQT LAQT LASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 60	70	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALAQT LAQT LASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 61	71	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGEASHLSTAQTARLSAELHRLADLPRTETGSGSP
peptide backbone within compound no. 62	72	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHRLADLPRTETGSGSP
peptide backbone within compound no. 63	73	HXHGFTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHTLATLPRTETGSGSP

peptide backbone within compound no. 64	74	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHQLATLPRTETGSGSP
peptide backbone within compound no. 65	75	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHGLATLPRTETGSGSP
peptide backbone within compound no. 66	76	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHHLATLPRTETGSGSP
peptide backbone within compound no. 67	77	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGEASELS TAALGRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 68	78	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGEASHLS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 69	79	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGEASHLS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 70	80	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGAPPPSG GGEASELSTAALGRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 71	81	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGAPPPSG GGEASHLSTQTQGRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 72	82	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGAPPPSG GGEASHLSTQTQGRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 73	83	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGEASQLS TAALGRLSAELHRLATLPRTETGSGSP
peptide backbone within compound no. 74	84	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSGGEASHLS TAQTQRLSAELHKLATLPRTETGSGSP

peptide backbone within compound no. 75	85	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGGEASHLS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 76	86	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 77	87	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 78	88	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 79	89	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 80	90	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHRLKTLPRTEGSGSP
peptide backbone within compound no. 81	91	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 82	92	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 83	93	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHRLKTLPRTEGSGSP
peptide backbone within compound no. 84	94	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 85	95	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAQTQRLSAELHKLATLPRTETGSGAP

peptide backbone within compound no. 86	96	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGASELSTAALGRLSAELHRLATLPRRTETGSGSP
peptide backbone within compound no. 87	97	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAALGRLSAKLHRLATLPRRTETGSGSP
peptide backbone within compound no. 88	98	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAALGRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 89	99	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAALGRLSAELHRLKTLPRRTETGSGSP
peptide backbone within compound no. 90	100	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGEASHLS TAALGRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 91	101	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLKTLPRRTETGSGSP
peptide backbone within compound no. 92	102	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLATLPKTETGSGSP
peptide backbone within compound no. 93	103	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 94	104	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGEASHLS TAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 95	105	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSGGGGEASH LSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 96	106	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSGGGGEASH LSTAQTQRLSAELHKLATLPRRTETGSGSP

peptide backbone within compound no. 97	107	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG ASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 98	108	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTARLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 99	109	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALQAPG QAPLASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 100	110	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGEASHLSTAQTARLSAELHRLADLPRRTETGSGSP
peptide backbone within compound no. 101	111	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSKGAPPPSG GGEASHLSTAQTARLSAELHQLATLPRRTETGSGSP
peptide backbone within compound no. 102	112	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHQLKTLPRRTETGSGSP
peptide backbone within compound no. 103	113	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTARLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 104	114	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTARLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 105	115	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALQAPG QAPLASHLSTAQTARLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 106	116	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALQAPG QAPGASHLSTAQTARLSAELHRLKTLPRRTETGSGSP
peptide backbone within compound no. 107	117	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALQAPG QAPGASHLSTAQTARLSAELHRLATLPKTETGSGSP

peptide backbone within compound no. 108	118	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGALQAPG QAPLASHLSTAQTARLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 109	119	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAPPPSG GGEASHLSTAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 110	120	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAPPPSG GGEASHLSTAQTQRLSAKLHRLATLPRTETGSGSP
peptide backbone within compound no. 111	121	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHRLKTLPRTEGSGSP
peptide backbone within compound no. 112	122	HXHGTFTSDYSILLEEQAAAREFIEWLLAGGPSSKGAPPPSG GGEASHLSTAQTARLSAELHRLADLPRTETGSGSP
peptide backbone within compound no. 113	123	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTARLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 114	124	YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAALARLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 120, 160, 161, 192 and 220	170	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 121	171	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGQAPGQ APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 122	172	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQLGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 123	173	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQLGRLSAELHQLATLPRTETGSGSP

peptide backbone within compound no. 124	174	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHELATLPRRTETGSGSP
peptide backbone within compounds 125, 142 and 221	175	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 126	176	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAALGRLSAELHELATLPRRTETGSGSP
peptide backbone within compound no. 127	177	YXEGTFTSDYSKLLLEEIAAREFIEWLIAGGPSSGAGQAPG QAPGASHLSTAALGRLSAELHELATLPRRTETGSGSP
peptide backbone within compound no. 128	178	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQTARLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 129	179	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQLGRLSAELHQLATLPRRTETGSGSP
peptide backbone within compound no. 130	180	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGASHL STAQLGRLSAELHQLATLPRRTETGSGSP
peptide backbone within compounds 131 and 193	181	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGAGASHLS TAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 132	182	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASQLSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 133	183	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASELSTAQTQRLSAELHKLATLPRRTETGSGSP
peptide backbone within compound no. 134	184	YXEGTFTSDYSILLEEEIAAREFIEWLLAGGPSSGEASHLST AQTQRLSAELHKLATLPRRTETGSGSP

peptide backbone within compound no. 135	185	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPAASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 136	186	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPEASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 137	187	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPPASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 138	188	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPSASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 139	189	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPVASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 140	190	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGGGGA SHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 141	191	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGGGGA GELSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 143	192	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGQAPGQ APLASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 144	193	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASELSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 145	194	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASELSTAQTARLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 146 and 211	195	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSEAGQAPGQ APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP

peptide backbone within compounds 147 and 212	196	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGEGQAPG QAPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 148 and 213	197	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGEAPGQ APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 149 and 214	198	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQEPG QAPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 150 and 215	199	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAEG QAPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 151 and 216	200	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPEQ APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 152 and 217	201	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGE APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 153 and 218	202	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QEPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compounds 154 and 219	203	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAEGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 155	204	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGQEPGQ EPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 156	205	YXEGTFTSDYSILLEEIAAREFIAWLIAGGPSSGAGQEPGQ EPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 157	206	YXEGTFTSDYSILLEEIAAREFIQWLIAGGPSSGAGQEPGQ EPGASHLSTAQTQRLSAELHKLATLPRTETGSGSP

peptide backbone within compound no. 158	207	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGASHLS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 159	208	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 162	209	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASHLSTAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 163	210	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASELSTAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 164	211	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGE APGASHLSTAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 165	212	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSSEAGQAPGQ APGASHLSTAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 166	213	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGE APGASELSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 167	214	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSSEAGQAPGQ APGASELSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 168	215	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSSEAGQAPGE APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 169	216	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASELSTAALGRLSAELHELATLPRTETGSGSP
peptide backbone within compound no. 170	217	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG QAPGASELSTAALGRLSAELHQLATLPRTETGSGSP

peptide backbone within compound no. 171	218	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG EAPGASELSTAALGRLSAELHQLATLPRTETGSGSP
peptide backbone within compound no. 172	219	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG EAPGASELSTAALGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 173	220	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG EAPGASELSTAQLGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 174	221	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG EAPGASELSTAQTGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 175	222	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGQAPG EAPGASELSTAQTQRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 176	223	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGASELS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 177	224	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSEAGASHLS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 178	225	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSEAGASELS TAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 179	226	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGASHLS TAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 180	227	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGASQLS TAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 181	228	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSEAGASQLS TAQTQRLSAELHKLATLPRTETGSGSP

peptide backbone within compound no. 182	229	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSEAGASQLS TAQTQRLSAELHKLATEPRTETGSGSP
peptide backbone within compound no. 183	230	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGASEL STAQLGRLSAELHQLATLPRTETGSGSP
peptide backbone within compound no. 184	231	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGASHL STAQLGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 185	232	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSEAGASHLS TAQLGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 186	233	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSGAGASEL STAQLGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 187	234	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSEAGASELS TAQLGRLSAELHQLATLPRTETGSGSP
peptide backbone within compound no. 188	235	YXEGTFTSDYSKLLLEEIAAREFIEWLLAGGPSSEAGASELS TAQLGRLSAELHQLATEPRTETGSGSP
peptide backbone within compound no. 189	236	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGEAPGE APGASHLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 190	237	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGEAPGQ APGASQLSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 191	238	YXEGTFTSDYSILLEEIAAREFIEWLIAGGPSSGAGEAPGQ APGASELSTAQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 194	239	YXEGTFTSDYSILLEEIAAQEFIEWLLQGGPSSGAGASELS TAQTQRLSAELHKLATLPRTETGSGSP

peptide backbone within compound no. 195	240	YXEGTFTSDYSILLEEIAAQEFIEWLLQGGPSSGAGEAPG QAPGASELSTAQQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 196	241	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGE APLASHLSTAQQTQRLSAELHKLATLPRTETGSGSP
peptide backbone within compound no. 197	242	YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPG QAPLASHLSTAQQTQRLSAELHKLATEPRTETGSGSP

wherein X represents always Aib.

DESCRIPTION

The present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

5 Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide comprising a maximum of 4 amino acid substitutions relative to II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

10 wherein the amino acid at position X2 represents Aib;

- L1 is a peptide linker; and
 - Z2 is a peptide comprising a C-terminal amide and a maximum of 10 amino acid
- tions relative to Formula III (SEQ ID NO: 2);

ASELSTAALGRLSAELHELATLPRTETGSGSP (III).

15 In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

$$Z1-L1-Z2 \quad (I),$$

comprising one lysine (Lys, K) residue; wherein:

- 20 • Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,
and wherein

25

Z1 comprises an amino acid sequence according to Formula X (SEQ ID NO: 161):



wherein

- 5 X₂₁ represents His (H) or Tyr (Y),
 X₂₂ represents Aib,
 X₂₃ represents Glu (E) or His (H),
 X₂₄ represents Ile (I) or Lys (K),
 X₂₅ represents Gln (Q) or Ile (I),
 X₂₆ represents Arg (R) or Gln (Q),
10 X₂₇ represents Ala (A), Glu (E) or Gln (Q),
 X₂₈ represents Leu (L) or I (Ile),
 X₂₉ represents Ala (A) or Gln (Q),
 X₃₀ represents Arg (R), Gly (G), Lys (K) or Ser (S),
 X₃₁ represents Gly (G), Glu (E) or Lys (K);
15 • L1 is a peptide linker; and
 • Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10

amino acid substitutions relative to Formula III (SEQ ID NO: 2):



and

- 20 Z2 comprises an amino acid sequence according to Formula XII (SEQ ID NO: 164):



wherein

- X₃₂ represents Gly (G) or Ser (S),
 X₃₃ represents Gln (Q), Glu (E), His (H) or Lys (K),
25 X₃₄ represents Ala (A) or Gln (Q),
 X₃₅ represents Gln (Q), Leu (L) or Thr (T),
 X₃₆ represents Ala (A), Gly (G) or Gln (Q),
 X₃₇ represents Glu (E) or Lys (K),
 X₃₈ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
30 X₃₉ represents Ala (A) or Lys (K),
 X₄₀ represents Asp (D) or Thr (T),
 X₄₁ represents Leu (L) or Glu (E),
 X₄₂ represents Arg (R) or Lys (K),
 X₄₃ represents Ala (A) or Ser (S).

35

In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



5 comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X_2 represents Aib,

10 and wherein

Z1 comprises an amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

X_1 represents His (H) or Tyr (Y),

15 X_2 represents Aib,

X_3 represents Glu (E) or His (H),

X_4 represents Arg (R), Gly (G), Lys (K) or Ser (S),

X_5 represents Gly (G) or Lys (K);

- L1 is a peptide linker; and

20 • Z2 is a peptide comprising a C-terminal amide and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



and

Z2 comprises an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

X_6 represents Gln (Q), Glu (E), His (H) or Lys (K),

X_7 represents Ala (A) or Gln (Q),

X_8 represents Gln (Q), Leu (L) or Thr (T),

30 X_9 represents Ala (A), Gly (G) or Gln (Q),

X_{10} represents Glu (E) or Lys (K),

X_{11} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X_{12} represents Ala (A) or Lys (K),

X_{13} represents Asp (D) or Thr (T),

35 X_{14} represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

5 Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

 YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

10 wherein the amino acid at position X₂ represents Aib,
and wherein

Z1 comprises an amino acid sequence according to Formula Xa (SEQ ID NO: 162):

 YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

wherein

15 X₅₁ represents Aib,

 X₅₂ represents Ile (I) or Lys (K),

 X₅₃ represents Arg (R) or Gln (Q),

 X₅₄ represents Ala (A), Glu (E) or Gln (Q),

 X₅₅ represents Leu (L) or I (Ile),

20 X₅₆ represents Ala (A) or Gln (Q),

 X₅₇ represents Gly (G) or Glu (E);

• L1 is a peptide linker; and

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

25 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

 AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

30 wherein

 X₅₈ represents Gly (G) or Ser (S),

 X₅₉ represents Gln (Q), Glu (E), or His (H),

 X₆₀ represents Ala (A) or Gln (Q),

 X₆₁ represents Leu (L) or Thr (T),

35 X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E).

The compounds disclosed herein are agonists at each of the receptors GLP-1, GIP, and amylin. Hence the compound of the present invention is a GLP-1 receptor agonist and a GIP receptor agonist and an agonist for the amylin receptor (i.e. an amylin receptor agonist). It is capable of activating or "agonising" all three of the GLP-1 receptor, the GIP receptor, and the amylin receptor: it is a "GLP-1-/GIP-/amylin-receptor tri-agonist". The GLP-1-/GIP-/amylin-receptor tri-agonist may provide a similar level of activation of all three GLP-1, GIP, and amylin receptors; then it is referred to as a "balanced GLP-1-/GIP-/amylin-receptor tri-agonist" or in short a "balanced tri-agonist".

A "receptor agonist" or "agonist" may be defined as a ligand, such as a compound, that binds to and activates a biological receptor to produce a biological response. A full agonist may be defined as one that elicits a response of the same magnitude as the natural ligand (see e.g., "Principles of Biochemistry", AL Lehninger, DL Nelson, MM Cox, Second Edition, Worth Publishers, 1993, page 763). Receptors can be activated by either endogenous agonists, such as endogenous hormones, or exogenous agonists, such as pharmaceutical drugs.

In the context of the current invention, a "co-agonist" is a compound capable of binding to and activating two different biological receptors, e.g. a compound comprising two different ligands, each of which binds to a given biological receptor, to produce a biological response that is characteristic of the natural ligands. In an analogous way, a "triple agonist" or "tri-agonist" is a compound capable of binding to and activating three different biological receptors, e.g. a compound comprising three different ligands, each of which binds to a given biological receptor, to produce a biological response that is characteristic of the natural ligands.

A "GLP-1 receptor agonist" may be defined as a compound which is capable of binding to the GLP-1 receptor and capable of activating it. A "full" GLP-1 receptor agonist may be defined as a GLP-1 receptor agonist which is capable of eliciting a magnitude of GLP-1 receptor response that is similar to native glucagon like peptide 1 (GLP-1). Semaglutide, disclosed in WO 2006/097537, Example 4, is an example of an exogenous GLP-1 receptor agonist.

A "GIP receptor agonist" may be defined as a compound which is capable of binding to the GIP receptor and capable of activating it. A "full" GIP receptor agonist may be defined

as a GIP receptor agonist which is capable of eliciting a magnitude of GIP receptor response that is similar to native glucose-dependent insulinotropic polypeptide (GIP).

A "GLP-1/GIP receptor co-agonist" may be defined as a compound which is capable of binding to both, the GLP-1 receptor, and the GIP receptor and capable of activating both
5 receptors. An example of a GLP-1/GIP co-agonist is tirzepatide, which is described in WO 2016/111971.

An "amylin receptor agonist" may be defined as a compound which is capable of binding to the amylin receptors (AMRAs) and the calcitonin receptor (CTR) and capable of activating it. Amylin receptors consist of heterodimers of two components: the calcitonin
10 receptor (CTR) and one of three receptor activity-modifying proteins (RAMP1-3), resulting in three possible complexes, AMR1-3. Unless otherwise specified herein, "amylin receptor" at least refers to amylin receptor 3 (AMR3). Nonetheless, some concomitant activity on the other receptors can be expected. A "full" amylin receptor agonist may be defined as an
15 amylin receptor agonist which is capable of eliciting a magnitude of amylin receptor response that is similar to native amylin. An amylin receptor agonist will often also be a calcitonin receptor agonist. Examples of amylin receptor agonists are human amylin, human calcitonin and cagrilintide (disclosed in WO 2012/168432). It is noted that all headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way.

20 The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention, and does not pose a limitation on the scope of the invention unless otherwise claimed.

In order that the present invention may be more readily understood, certain terms are first defined.

25 In what follows, Greek letters may be represented by their symbol or the corresponding written name, for example: α = alpha; β = beta; γ = gamma; ϵ = epsilon; 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.

Unless otherwise indicated in the specification, terms presented in singular form generally also include the plural situation. The term "a" or "an" is intended to mean "one or
30 more."

The term "comprise" and variations thereof such as "comprises" and "comprising," when preceding the recitation of a step or an element, are intended to mean that the addition of further steps or elements is optional and not excluded. As disclosed herein the open-ended terms like "comprises" and "comprising" might be replaced with closed terms such as
35 "consists of", "consisting of", and the like.

The term "about" is used herein to mean approximately, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by 10 percent, up or down (higher or lower).

Amino acids are molecules containing an amine 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 canonical amino acids (which are genetically encoded), and unnatural amino acids. Non-limiting examples of unnatural amino acids are Aib (α -aminoisobutyric acid or 2-aminoisobutyric acid), deamino histidine (alternative name 3-(imidazol-4-yl)propanoic acid, abbreviated Imp (imidazopropionyl)) and the d-isomers of the canonical amino acids. All amino acid residues within the peptide for which the optical isomer is not stated is herein to be understood to mean the L-isomer, unless otherwise specified.

Herein, "amino acid substitution" or "substitution" refers to one or more amino acid(s) being replaced with the same number of amino acid(s) in the backbone of the peptide. Substitutions may be, but are not limited to, conservative substitutions. For example, an amino acid may be substituted to an amino acid with similar biochemical properties, for example, a basic amino acid may be substituted to another basic amino acid (e.g. lysine to arginine), an acidic amino acid may be substituted to another acidic amino acid (e.g. glutamate to aspartate), a neutral amino acid may be substituted to another neutral amino acid (e.g. threonine to serine), a charged amino acid may be substituted to another charged amino acid (e.g. glutamate to lysine), a hydrophilic amino acid may be substituted to another hydrophilic amino acid (e.g. asparagine to glutamine), a hydrophobic amino acid may be substituted to another hydrophobic amino acid (e.g. alanine to valine), a polar amino acid may be substituted to another polar amino acid (e.g. serine to threonine), an aromatic amino acid may be substituted to another aromatic amino acid (e.g. phenylalanine to tryptophan) and an aliphatic amino acid may be substituted to another aliphatic amino acid (e.g. leucine to isoleucine).

The term "excipient" as used herein broadly refers to any component other than the Active Pharmaceutical Ingredient (API).

The term "identity" or "sequence identity" as known in the art, refers to a relationship between the sequences of two or more polypeptides, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptides, as determined by the number of matches between strings of two or more

amino acid residues. "Identity" measures the percentage of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related polypeptides can be readily calculated by known methods, e.g. using Needleman (Needleman *et al.* J.

- 5 Mol. Biol. 1970; 48:443-453) from EMBOSS-6.6.0 using the parameters 10 and 0.5 for gaps opening and extensions, respectively (gapopen=10, gapextend=0.5) or e.g. calculated by: (1) comparing two optimally aligned sequences over a window of comparison (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window, etc.), (2) determining the number of positions containing identical monomers (e.g., same amino acids
10 occurs in both sequences) to yield the number of matched positions, (3) dividing the number of matched positions by the total number of positions in the comparison window (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), and (4) multiplying the result by 100 to yield the percent "sequence identity". For example, if peptides A and B are both 20 amino acids in length and have identical amino acids at all but
15 1 position, then peptide A and peptide B have 95 % sequence identity.

The term "polypeptide" or "peptide" as used herein includes oligopeptides and refers to a single chain of amino acids connected by one or more amide (or peptide) bonds. The terms "polypeptide" and "peptide" shall be used interchangeably herein.

- 20 The term "half-life" or "plasma half-life" as used herein refers to the time required for half the quantity of a substance administered to a person to be metabolized or eliminated from the serum or plasma of the person by normal biological processes.

- The term "treatment" and variations thereof, as used herein, refers to the medical therapy of any human subject in need thereof. The term includes administering a therapeutically effective amount of a peptide as disclosed herein sufficient to reduce or
25 eliminate at least one symptom of the disorder in question. "Treatment", however, need not be a cure. The timing and purpose of said treatment may vary from one individual to another, according to the status quo of the subject's health. Thus, said treatment may be prophylactic, palliative, symptomatic and/or curative. In terms of the present invention, prophylactic, palliative, symptomatic and/or curative treatments may represent separate aspects of the
30 invention.

As used herein the terms "preventing", "prevent" or "prevention" or variations thereof refers to protecting a subject from developing at least one symptom of a disease or reducing the severity of a symptom of a disorder.

GLP-1-/GIP-/amylin-receptor tri-agonist

The compounds disclosed herein are herein referred to as “GLP-1-/GIP-/amylin-receptor tri-agonist “ or “GLP-1 receptor-GIP receptor-amylin receptor tri-agonists” or “GLP-1-/GIP-/amylin-receptor triple agonist “ or “GLP-1 receptor-GIP receptor-amylin receptor triple agonists”.

The GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide Z1, which is a GLP-1-/GIP receptor co-agonist, a peptide linker L1, and a peptide Z2, which is an amylin receptor agonist. The GLP-1-/GIP-/amylin-receptor tri-agonist is a compound that binds to each of the three GLP-1, GIP, and amylin receptors and is capable of activating each receptor GLP-1R, GIPR, and amylin receptor, thus eliciting a response at each receptor.

The peptide Z1 disclosed herein may have a maximum of 4 amino acid substitutions, relative to Formula II (SEQ ID NO: 1). The peptide Z2 disclosed herein may have a maximum of 10 amino acid substitution, relative to Formula III (SEQ ID NO: 2).

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 thereof, i.e. a compound as defined herein or a pharmaceutically acceptable salt, amide, or ester thereof.

The compound disclosed herein may be a potent GLP-1 receptor agonist.

The compound disclosed herein may be a potent GIP receptor agonist.

The compound disclosed herein may be a potent amylin receptor agonist.

The *in vitro* potency of the agonists may be measured as described in the assays of Example 4. The term “potency” is used to describe the effect of a given compound in assays where a sigmoidal relationship between log concentration and the effect of a compound has been established. Furthermore, the response should be variable from 0 to 100%. The potency of the compound may be described by means of its EC(effective concentration)₅₀ values. EC₅₀ represents the concentration of compound upon which 50% of its maximal effect is observed in the assay, e.g., as described in Example 4. The lower the EC₅₀ value, the more potent the compound.

The compound disclosed herein may provide a similar level of activation of all three GLP-1, GIP, and amylin receptors; that is, it may be “balanced” and is referred to as a “balanced GLP-1-/GIP-/amylin-receptor tri-agonist” or in short a “balanced tri-agonist”.

Relatively “balanced” receptor activation is advantageous because the relative ratio of the

compound's GLP-1, GIP, and amylin receptor agonist activities is locked to the molecule; it is not possible to titrate the three receptor agonists, relative to one another. Ultimately, where a molecule is "balanced", it may be dosed such that all three hormone systems are activated without side-effects outweighing benefits.

5 A tri-agonist whose potency ratio (A/B) of the potency (A) of the receptor with lowest potency (i.e. the highest numerical EC₅₀ value) divided by the potency (B) of the receptor with highest potency (i.e. the lowest numerical EC₅₀ value) is less than 50 is defined as a "balanced tri-agonist" or "balanced GLP-1-/GIP-/amylin-receptor tri-agonist" (based on the assays in the absence of human serum albumin (HSA), as shown in Example 4 Table 7a and
10 Table 8a). For example, compound 10 has an EC₅₀ of 13.83 pM on the GLP-1 receptor, an EC₅₀ of 2.11 pM on the GIP receptor, and an EC₅₀ of 6.8 pM on the amylin receptor. Hence it has lowest potency on the hGLP-1 and (A) corresponds to 13.83 pM, and it has its highest potency on the GIP receptor with 2.11 pM, which corresponds to (B). Hence the potency ratio (A/B) is 13.83 pM (A) divided by 2.11 pM (B) is equal to 7 (rounded accordingly), this means
15 that compound 10 is a "balanced tri-agonist".

A compound that is potent on one receptor and much less potent on the other(s) would be "unbalanced". Such an "unbalanced tri-agonist" is defined as compound having a potency ratio (A/B) of 50 or more. For example, reference compound 1 has a potency ratio (A/B) of 287 (i.e., 1516.05 pM (= A) divided by 5.29 pM (= B), rounded accordingly). For
20 example, this reference compound being potent (i.e. EC₅₀ value of <30 pM) on the GIP and amylin receptors and less potent (i.e. EC₅₀ value of 1516 pM) on the GLP-1 receptor, would be unable to achieve optimal efficacy from all three hormone systems because side-effects arising from activation of the GIP and amylin receptors would prevent administration of a dose sufficiently high to achieve activation of the GLP-1 hormone system as well. The
25 opposite situation might occur if the compound were potent on one receptor, e.g. the GLP-1 receptor (for example, having an EC₅₀ value of <50 pM) and considerably less potent on the two other receptors, e.g. GIP and amylin receptors (for example, having an EC₅₀ value of >500 pM each).

Peptide

30 The present invention relates in one aspect to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide comprising a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X2 represents Aib;

- 5 • L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide and a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



10 The GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide, Z1-L1-Z2 comprising a peptide Z1, a peptide linker L1, and a peptide Z2.

The peptide Z1 is a GLP-1-/GIP receptor co-agonist which is capable of binding to both, the GLP-1 receptor, and the GIP receptor and capable of activating both receptors. The C-terminus of peptide Z1 is attached to the peptide linker L1 via a peptide bond.

15 L1 is a peptide linker. Its N-terminus is attached to the C- terminus of Z1 and its C-terminus is attached to the N-terminus of Z2 via a peptide bond.

The peptide Z2 is an amylin receptor agonist which is capable of binding to at least amylin receptor and capable of activating it. The N-terminus of Z2 is attached to the C-terminus of L1 via a peptide bond. The C-terminus of Z2 is modified with an amide group, which is considered essential for bioactivity. In a preferred embodiment, the amine group of the C-terminal amide is NH₂.

The molecular format may be a single chain peptide backbone comprising one lysine (Lys, K) residue. The one lysine (Lys, K) residue may be present in the peptide Z1 portion of the peptide backbone, or the one lysine (Lys, K) residue may be present in the peptide Z2 portion of the peptide backbone. The one lysine residue may be covalently bound to a protraction moiety, which may be referred to herein as "L_P-P", wherein "L_P" is an optional linker and "P" is a protractor. The peptide backbone of the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention typically comprises about 66 to about 80 amino acid residues linked together by peptide bonds.

30 The peptide Z1 disclosed herein may have a maximum of 4 amino acid substitutions, relative to Formula II (SEQ ID NO: 1), wherein said substitution(s) can take place in any of the positions 1 to 34, preferably said substitution(s) take place in position 1, 3, 12, 17, 20, 24, 27, 28, 33 and/or 34. The present invention encompasses variants of the GLP-1-/GIP-/amylin-receptor tri-agonist, as disclosed herein, wherein peptide Z1 may

35 comprise 1, 2, 3 or 4 amino acid substitution(s) relative to Formula II (SEQ ID NO: 1).

Preferred substitutions include a conservative substitution, those which, instead of the amino acid residue, which appears in the sequence, comprises an amino acid with similar biochemical properties or a structural analogue of the amino acid residue.

The peptide Z2 disclosed herein may have a maximum of 10 amino acid substitution, relative to Formula III (SEQ ID NO: 2), wherein said substitution(s) can take place in any of the positions 1 to 32, preferably said substitution(s) take place in position 2, 3, 7, 8, 9, 10, 15, 18, 20, 21, 22, 24 and/or 31. The present invention encompasses variants of the GLP-1-/GIP-/amylin-receptor tri-agonist, as disclosed herein, wherein peptide Z2 may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitution(s) relative to Formula III (SEQ ID NO: 2). Preferred substitutions include a conservative substitution, as above explained.

In some embodiments of the present invention the peptide Z1 comprises an amino acid sequence which has at least 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identity (i.e., a sequence identity) to Formula II (SEQ ID NO: 1), and the peptide Z2 comprises an amino acid sequence which has at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identity (i.e., a sequence identity) relative to Formula III (SEQ ID NO: 2).

In some embodiments of the invention the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein does not comprise a cysteine (Cys, C) residue and/or does not comprise a disulfide bridge. The term "disulfide bridge" in reference to human amylin and analogues thereof, refers to a functional group with the structure R-S-S-R' and may also be referred to as an "SS-bond".

The GLP-1-/GIP-/amylin-receptor tri-agonist may exhibit a variety of properties rendering it useful as a medicament, as described herein.

The present invention relates in another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib,
and wherein

Z1 comprises or consists of an amino acid sequence according to Formula X (SEQ ID NO: 161):

$X_{21}X_{22}X_{23}GTFTSDYSX_{24}LLEEX_{25}AAX_{26}EFIX_{27}WLX_{28}X_{29}GGPSX_{30}X_{31}$ (X),

wherein

X_{21} represents His (H) or Tyr (Y),

X_{22} represents Aib,

5 X_{23} represents Glu (E) or His (H),

X_{24} represents Ile (I) or Lys (K),

X_{25} represents Gln (Q) or Ile (I),

X_{26} represents Arg (R) or Gln (Q),

X_{27} represents Ala (A), Glu (E) or Gln (Q),

10 X_{28} represents Leu (L) or I (Ile),

X_{29} represents Ala (A) or Gln (Q),

X_{30} represents Arg (R), Gly (G), Lys (K) or Ser (S),

X_{31} represents Gly (G), Glu (E) or Lys (K);

• L1 is a peptide linker; and

15 • Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

20 Z2 comprises or consists of an amino acid sequence according to Formula XII (SEQ ID NO:164):

$AX_{32}X_{33}LSTAX_{34}X_{35}X_{36}RLSAX_{37}LHX_{38}LX_{39}X_{40}X_{41}PX_{42}TETGSGX_{43}P$ (XII),

wherein

X_{32} represents Gly (G) or Ser (S),

X_{33} represents Gln (Q), Glu (E), His (H) or Lys (K),

25 X_{34} represents Ala (A) or Gln (Q),

X_{35} represents Gln (Q), Leu (L) or Thr (T),

X_{36} represents Ala (A), Gly (G) or Gln (Q),

X_{37} represents Glu (E) or Lys (K),

30 X_{38} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X_{39} represents Ala (A) or Lys (K),

X_{40} represents Asp (D) or Thr (T),

X_{41} represents Leu (L) or Glu (E),

X_{42} represents Arg (R) or Lys (K),

35 X_{43} represents Ala (A) or Ser (S).

The present invention relates in another aspect to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



5 comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X_2 represents Aib,

10 and wherein

Z1 comprises or consists of an amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

15 X_1 represents His (H) or Tyr (Y),

X_2 represents Aib,

X_3 represents Glu (E) or His (H),

X_4 represents Arg (R), Gly (G), Lys (K) or Ser (S),

X_5 represents Gly (G) or Lys (K);

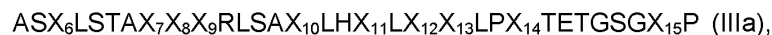
20 • L1 is a peptide linker; and

- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



and

25 Z2 comprises or consists of an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

30 X_6 represents Gln (Q), Glu (E), His (H) or Lys (K),

X_7 represents Ala (A) or Gln (Q),

X_8 represents Gln (Q), Leu (L) or Thr (T),

X_9 represents Ala (A), Gly (G) or Gln (Q),

X_{10} represents Glu (E) or Lys (K),

X_{11} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or

35 Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ala (A) or Ser (S).

5

In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



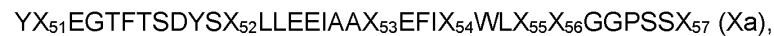
comprising one lysine (Lys, K) residue; wherein:

- 10 • Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib,
and wherein

- 15 Z1 comprises or consists of an amino acid sequence according to Formula Xa (SEQ ID NO: 162):



wherein

- 20 X₅₁ represents Aib,
X₅₂ represents Ile (I) or Lys (K),
X₅₃ represents Arg (R) or Gln (Q),
X₅₄ represents Ala (A), Glu (E) or Gln (Q),
X₅₅ represents Leu (L) or I (Ile),
X₅₆ represents Ala (A) or Gln (Q),
25 X₅₇ represents Gly (G) or Glu (E);

- L1 is a peptide linker; and

- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



30 and

Z2 comprises or consists of an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):



wherein

- 35 X₅₈ represents Gly (G) or Ser (S),

5
X₅₉ represents Gln (Q), Glu (E), or His (H),
X₆₀ represents Ala (A) or Gln (Q),
X₆₁ represents Leu (L) or Thr (T),
X₆₂ represents Ala (A), Gly (G) or Gln (Q),
X₆₃ represents Gln (Q), Glu (E), or Lys (K),
X₆₄ represents Leu (L) or Glu (E).

10 In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

15 X₁X₂X₃ represents YAibE (Tyr-Aib-Glu) or HAibH (His-Aib-His)
X₄ represents Arg (R), Gly (G) or Ser (S),
X₅ represents Gly (G).

20 In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

25 X₁ represents His (H),
X₂ represents Aib,
X₃ represents His (H),
X₄ represents Lys (K),
X₅ represents Gly (G).

30 In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula V (SEQ ID NO: 6):



wherein

35 X₂ represents Aib,
X₄ is Arg (R) or Ser (S).

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula VI (SEQ ID NO: 7):

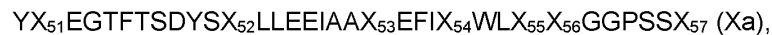


5 wherein X_2 represents Aib.

In some embodiments of the present invention the peptide Z1 may comprise an amino acid sequence which has at least 90, 91, 92 93, 94, 95, 96, 97, 98 or 99 % identity relative to Formula VI (SEQ ID NO: 7).

10

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):



15 wherein

X_{51} represents Aib,

X_{52} represents Ile (I) or Lys (K),

X_{53} represents Arg (R),

X_{54} represents Glu (E),

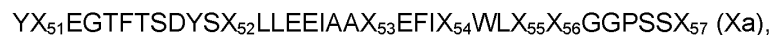
20 X_{55} represents Leu (L) or I (Ile),

X_{56} represents Ala (A),

X_{57} represents Gly (G) or Glu (E).

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):

25



wherein

X_{51} represents Aib,

30 X_{52} represents Ile (I) or Lys (K),

X_{53} represents Arg (R),

X_{54} represents Glu (E),

X_{55} represents Leu (L),

X_{56} represents Ala (A),

35 X_{57} represents Gly (G).

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula XI (SEQ ID NO: 163):

5 YX₅₁EGTFTSDYSX₅₂LLEEIAAREFIEWLLAGGPSSG (XI),

wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K).

10 In one embodiment peptide Z2 may comprise or consist of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Lys (K),

15 X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),

20 X₁₂ represents Ala (A),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R),

X₁₅ represents Ala (A) or Ser (S);

or

25 X₆ represents Gln (Q), Glu (E), or His (H),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Lys (K),

30 X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),

X₁₂ represents Ala (A),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R),

X₁₅ represents Ala (A) or Ser (S);

35 or

- 5 X₆ represents Gln (Q), Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Lys (K),
X₁₂ represents Ala (A),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R),
10 X₁₅ represents Ala (A) or Ser (S);
or
X₆ represents Gln (Q), Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
15 X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
X₁₂ represents Lys (K),
X₁₃ represents Asp (D) or Thr (T),
20 X₁₄ represents Arg (R),
X₁₅ represents Ala (A) or Ser (S);
or
X₆ represents Gln (Q), Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
25 X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
X₁₂ represents Ala (A),
30 X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Lys (K),
X₁₅ represents Ala (A) or Ser (S).

35 In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

- 5 X₆ represents His (H),
X₇ represents Gln (Q),
X₈ represents Thr (T),
X₉ represents Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R) or Lys (K),
X₁₂ represents Ala (A) or Lys (K),
10 X₁₃ represents Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ser (S).

- 15 In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

- 20 X₆ represents His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R) or Gln (Q),
X₁₂ represents Lys (K),
25 X₁₃ represents Thr (T),
X₁₄ represents Arg (R),
X₁₅ represents Ser (S).

- 30 In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

- 35 X₆ represents His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R),
X₁₂ represents Ala (A),
5 X₁₃ represents Thr (T),
X₁₄ represents Lys (K),
X₁₅ represents Ser (S).

In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
10 according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),
X₇ represents Ala (A) or Gln (Q),
15 X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E) or Lys (K),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
X₁₂ represents Ala (A) or Lys (K),
20 X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ala (A) or Ser (S).

In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
25 according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
30 X₈ represents Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q) or Glu (E),
X₁₂ represents Ala (A),
35 X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R),

X₁₅ represents Ser (S).

In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
5 according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Glu (E) or His (H),

X₇ represents Ala (A) or Gln (Q),

10 X₈ represents Leu (L) or Thr (T),

X₉ represents Ala (A) or Gly (G),

X₁₀ represents Glu (E),

X₁₁ represents Arg (R), Gln (Q) or Glu (E),

X₁₂ represents Ala (A),

15 X₁₃ represents Thr (T),

X₁₄ represents Arg (R),

X₁₅ represents Ser (S).

In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
20 according to Formula VII (SEQ ID NO: 8):

ASHLSTAQTQRLSAKLHRLATLPRTETGSGSP (VII).

In one embodiment peptide Z2 may comprise or consist of the amino acid sequence
according to Formula VIII (SEQ ID NO: 9):

ASHLSTAQTQRLSAELHKLATLPRTETGSGSP (VIII).

25

In some embodiments of the present invention the peptide Z2 may comprise an
amino acid sequence which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identity
relative to Formula VII (SEQ ID NO: 8) or Formula VIII (SEQ ID NO: 9).

30 In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present
invention may comprise a peptide Z2 comprising or consisting of the amino acid sequence
according to Formula XIIa (SEQ ID NO: 165):

AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

wherein

35 X₅₈ represents Ser (S),

X₅₉ represents His (H),
X₆₀ represents Gln (Q),
X₆₁ represents Thr (T),
X₆₂ represents Gln (Q),
5 X₆₃ represents Lys (K),
X₆₄ represents Leu (L).

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z2 comprising or consisting of the amino acid sequence
10 according to Formula XIII (SEQ ID NO: 166):

ASX₅₉LSTAQTQRLSAELHKLATLPRTETGSGSP (XIII),

wherein

X₅₉ represents Glu (E) or His (H).

15 In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of an amino acid sequence which has at least 90, 91, 92 93, 94, 95, 96, 97, 98 or 99 % identity relative to Formula VI (SEQ ID NO: 7), and a peptide Z2 comprising or consisting of an amino acid sequence which
20 has at least 90, 91, 92 93, 94, 95, 96, 97, 98 or 99 % identity relative to Formula VII (SEQ ID NO: 8) or Formula VIII (SEQ ID NO: 9).

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

25 wherein

X₁ represents His (H) or Tyr (Y),

X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G) or Ser (S),

30 X₅ represents Gly (G); and

a peptide Z2 comprising or consisting of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

35 X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

5 X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

10

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



15 wherein

X₁ represents His (H) or Tyr (Y),

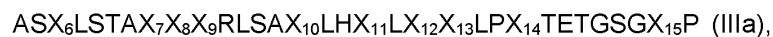
X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G) or Ser (S),

20 X₅ represents Gly (G); and

a peptide Z2 comprising or consisting of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

25 X₆ represents Glu (E) or His (H),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

30 X₁₁ represents Arg (R), Gln (Q) or Lys (K),

X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

35

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



5 wherein

X_1 represents His (H) or Tyr (Y),

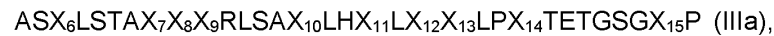
X_2 represents Aib,

X_3 represents Glu (E) or His (H),

X_4 represents Arg (R), Gly (G) or Ser (S),

10 X_5 represents Gly (G); and

a peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

15 X_6 represents Glu (E) or His (H),

X_7 represents Ala (A) or Gln (Q),

X_8 represents Gln (Q), Leu (L) or Thr (T),

X_9 represents Ala (A), Gly (G) or Gln (Q),

X_{10} represents Glu (E),

20 X_{11} represents Lys (K),

X_{12} represents Ala (A),

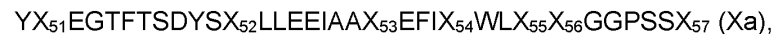
X_{13} represents Thr (T),

X_{14} represents Arg (R),

X_{15} represents Ala (A) or Ser (S).

25

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide Z1 comprising or consisting of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):



30 wherein

X_{51} represents Aib,

X_{52} represents Ile (I) or Lys (K),

X_{53} represents Arg (R),

X_{54} represents Glu (E),

35 X_{55} represents Leu (L) or I (Ile),

X₅₆ represents Ala (A),

X₅₇ represents Gly (G) or Glu (E); and

a peptide Z2 comprising or consisting of the amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

5 AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTEGSGSP (XIIa),

wherein

X₅₈ represents Ser (S),

X₅₉ represents His (H),

X₆₀ represents Gln (Q),

10 X₆₁ represents Thr (T),

X₆₂ represents Gln (Q),

X₆₃ represents Lys (K),

X₆₄ represents Leu (L).

15 In one embodiment the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs 20 to 124 and 170 to 242, and wherein X represents Aib.

 In a particular embodiment the invention relates to a GLP-1-/GIP-/amylin-receptor
20 tri-agonist, wherein the amino acid sequence of the peptide comprises or consists of
 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQR
LSAELHKLATLPRTEGSGSP (SEQ ID NO: 62), wherein X represents Aib; or

 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQR
LSAKLHRLATLPRTEGSGSP (SEQ ID NO: 65), wherein X represents Aib; or

25 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRL
SAELHKLATLPRTEGSGSP (SEQ ID NO: 68), wherein X represents Aib; or

 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSRGEASHLSTAQTQRLSAELHKLATL
PRTEGSGSP (SEQ ID NO: 78), wherein X represents Aib; or

30 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRL
SAELHKLATLPRTEGSGSP (SEQ ID NO: 87), wherein X represents Aib; or

 HXHGFTFTSDYSILLEEQAAREFIEWLLAGGPSSKGAPPPSGGGEASHLSTAQTARLS
AELHQLATLPRTEGSGSP (SEQ ID NO: 111), wherein X represents Aib; or

 YXEGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
SAELHKLATLPRTEGSGSP (SEQ ID NO: 170); wherein X represents Aib.

35

In one aspect of the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist which is capable of activating the human GIP, GLP-1, and amylin receptors *in vitro*.

When tested as described in "GLP-1 receptor assay" (preferably method A; in the absence of HSA), the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have an
5 EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM.

When tested as described in "GIP receptor assay" (preferably method A; in the absence of HSA), the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have an
10 EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM.

When tested as described in "Amylin receptor assay" (preferably method A; in the absence of HSA), the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have an
15 EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein agonise, or activate, the amylin receptor. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may be tested for amylin
20 activity as described in Example 4.

The more potent the compound, the lower its EC₅₀ value. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay (see Example 4) of about 100 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 90 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional
25 assay of about 80 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 75 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 70 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a
30 human GLP-1 receptor functional assay of about 60 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 50 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 40 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1 receptor functional assay of about 30 pM or
35 less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC₅₀ in a human GLP-1

receptor functional assay of about 25 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GLP-1 receptor functional assay of about 20 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GLP-1 receptor functional assay of about 15 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GLP-1 receptor functional assay of about 10 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GLP-1 receptor functional assay of about 5 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have a similar potency as that of semaglutide or tirzepatide.

The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay (see Example 4) of about 125 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 100 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 90 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 80 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 75 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 70 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 60 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 50 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 40 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 30 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 25 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 20 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 15 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 10 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 9 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 8 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 7 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional assay of about 6 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human GIP receptor functional

assay of about 5 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have a similar potency as that of tirzepatide.

The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay (see Example 4) of about 125 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 100 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 90 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 80 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 75 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 70 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 60 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 50 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 40 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 30 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 25 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 20 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 15 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 10 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 9 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 8 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 7 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 6 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist may have an EC_{50} in a human amylin receptor functional assay of about 5 pM or less. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may have a similar potency as that of cagrilintide.

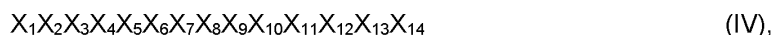
In another aspect the invention relates to a balanced GLP-1-/GIP-/amylin-receptor tri-agonist which activates the human GIP, GLP-1, and amylin receptors *in vitro*, when

measured without HSA in assays as described in Example 4, and which has a potency ratio of less than 50.

Peptide linker

The GLP-1-/GIP-/amylin-receptor tri-agonist peptide backbone disclosed herein
 5 comprise a peptide linker L1, which may comprise 1 to 14 amino acid residues, in particularly canonical amino acid residues. The peptide linker may comprise 1 to 10 amino acid residues, in particularly canonical amino acid residues, such as 2 to 10, 3 to 10, 4 to 10, 5 to 10, 6 to 10, 7 to 10, 8 to 10, 9 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, or 1 to 3 amino acid residues. Specifically, the peptide linker may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino
 10 acid residues, in particularly canonical amino acid residues.

The peptide linker L1 may be represented by Formula IV:



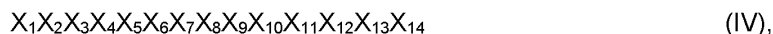
wherein any of X_{1-14} is independently selected from any naturally occurring, or canonical, amino acid residue(s), and wherein any of X_{2-14} may be absent.

15 The GLP-1-/GIP-/amylin-receptor tri-agonist peptide backbone disclosed herein comprise a peptide linker L1, wherein the peptide linker L1 comprises or consists of the amino acid sequence according to Formula IV. Thus, the GLP-1-/GIP-/amylin-receptor tri-agonist may comprise a peptide or consist of a peptide according to the amino acid sequence of SEQ ID No: 5.

20 Any of X_{1-14} may be selected from any nonaromatic amino acid residue. Any of X_{1-14} may be a charged amino acid. Any of X_{1-14} may be a polar amino acid. Any of X_{1-14} may be a hydrophobic amino acid.

Any of X_{1-14} may be independently selected from the group consisting of alanine (Ala, A), glutamic acid (Glu, E), glutamine (Gln, Q), glycine (Gly, G), leucine (Leu, L),
 25 phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), valine (Val, V) and asparagine (Asn, N). Preferably any of X_{1-14} may be selected from the group consisting of alanine (Ala, A), glutamic acid (Glu, E), glutamine (Gln, Q), glycine (Gly, G), leucine (Leu, L) and proline (Pro, P).

In one embodiment the peptide linker L1 may be represented by Formula IV, or
 30 comprises or consists of the amino acid sequence according to Formula IV:



wherein

X_1 represents Ala (A), Glu (E), Gly (G),

X_2 represents Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P) or is absent,

- X₃ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or is absent,
 X₄ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or is absent,
 X₅ represents Glu (E), Gly (G), Pro (P), Ser (S), Thr (T) or is absent,
 X₆ represents Glu (E), Gly (G), Leu (L), Gln (Q) or is absent,
 5 X₇ represents Ala (A), Gln (Q), Glu (E), Gly (G), Phe (F) or is absent,
 X₈ represents Ala (A), Gln (Q), Glu (E), Gly (G), Thr (T), Pro (P), Val (V) or is absent,
 X₉ represents Glu (E), Asn (N), Pro (P), Thr (T) or is absent,
 X₁₀ represents Ala (A), Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P), Ser (S), Val (V) or
 is absent,
 10 X₁₁ represents Ala (A) or is absent,
 X₁₂ represents Gln (Q) or is absent,
 X₁₃ represents Thr (T) or is absent,
 X₁₄ represents Leu (L) or is absent.
- 15 The peptide linker L1 may be any one of the peptide linkers represented by SEQ ID
 NOs 125-159. The peptide linker L1 may be any one of the peptide linkers listed in Table 2.

Table 2: Peptide linkers L1

SEQ ID NO	Peptide linker	SEQ ID NO	Peptide linker
-	A	132	ALAQTLFVNQ
-	E	133	ALAQTLGTNE
-	G	134	ALQAPGQAPG
-	AE	135	ALQAPGQAPL
-	GE	136	AGQAPGQAPG
125	APPE	137	AGQAPGQAPL
126	GGGE	138	GGGEGGGE
127	AGQAPG	139	GQAPGQAPGE
128	APPPSGGG	140	GQEPGQEPGE
129	APPPSGGGE	141	APPPSLAQTLAQTL
130	APPPSGGGG	150	EGQAPGQAPG
131	ALAQTLAQTL	151	AGQEPGQAPG
-	AG	152	AGQAEGQAPG
142	AGGGG	153	AGQAPEQAPG
143	AGEAPGQAPG	154	AGQAPGEAPG

144	AGEAPGEAPG	155	AGQAPGQEPG
145	AGQAPGQAPA	156	AGQAPGQAEQ
146	AGQAPGQAPE	157	AGQEPGQEPG
147	AGQAPGQAPP	158	AGQAPGQAP
148	AGQAPGQAPS	159	AGQAPGEAPL
149	AGQAPGQAPV		

The peptide linker L1 may be selected from the group consisting of A, E, G, AE, GE, APPE (SEQ ID NO: 125), GGGE (SEQ ID NO: 126), AGQAPG (SEQ ID NO: 127), APPPSGGG (SEQ ID NO: 128), APPPSGGGE (SEQ ID NO: 129), APPPSGGGG (SEQ ID NO: 130), ALAQTLAQTL (SEQ ID NO: 131), ALAQTLFVNQ (SEQ ID NO: 132), ALAQTLGTNE (SEQ ID NO: 133), ALQAPGQAPG (SEQ ID NO: 134), ALQAPGQAPL (SEQ ID NO: 135), AGQAPGQAPG (SEQ ID NO: 136), AGQAPGQAPL (SEQ ID NO: 137), GGGEGGGEGE (SEQ ID NO: 138), GQAPGQAPGE (SEQ ID NO: 139), GQEPGQEPGE (SEQ ID NO: 140) APPPSLAQTLAQTL (SEQ ID NO: 141), AG, AGGGG (SEQ ID NO: 142), AGEAPGQAPG (SEQ ID NO: 143), AGEAPGEAPG (SEQ ID NO: 144), AGQAPGQAPA (SEQ ID NO: 145), AGQAPGQAPE (SEQ ID NO: 146), AGQAPGQAPP (SEQ ID NO: 147), AGQAPGQAPS (SEQ ID NO: 148), AGQAPGQAPV (SEQ ID NO: 149), EGQAPGQAPG (SEQ ID NO: 150), AGQEPGQAPG (SEQ ID NO: 151), AGQAEGQAPG (SEQ ID NO: 152), AGQAPEQAPG (SEQ ID NO: 153), AGQAPGEAPG (SEQ ID NO: 154), AGQAPGQEPG (SEQ ID NO: 155), AGQAPGQAEQ (SEQ ID NO: 156), AGQEPGQEPG (SEQ ID NO: 157), AGQAPGQAP (SEQ ID NO: 158) and AGQAPGEAPL (SEQ ID NO: 159).

In a preferred embodiment the peptide linker L1 may be E, GE, APPPSGGGE (SEQ ID NO: 129), AGQAPGQAPG (SEQ ID NO: 136), or AGQAPGQAPL (SEQ ID NO: 137) or AGQAPGEAPG (SEQ ID NO: 154).

20

In one embodiment the backbone of the peptide Z1—L1—Z2 comprises 66 to 80 amino acid residues.

In one embodiment the backbone of the peptide Z1—L1—Z2 comprises 67, 68, 75 or 76 amino acid residues.

25

In one embodiment the backbone of the peptide Z1—L1—Z2 comprises 76 amino acid residues.

In one embodiment the backbone of the peptide Z1—L1—Z2 comprises or consists of an amino acid sequence according to SEQ ID NO: 5.

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide according to Formula I: Z1—L1—Z2, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs 5, 20 to 124 and 170 to 242.

5 Protraction moiety

In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist which may further comprise a protraction moiety. In such cases the peptide is referred to as a "peptide derivative". The addition of the term "derivative" thus means that a protraction moiety is present and a compound or compounds comprising a protraction moiety are referred to as "derivative" or "derivatives".

The term "protraction moiety" as used herein refers to a moiety having half-life extending properties and comprising a "protractor P" and an optional "linker L_P", and may be represented by the general formula "L_P-P", in which L_P is said optional linker and P is said protractor.

The term "protractor" as used herein refers to a molecule which is capable of increasing the plasma half-life of the peptide to which it is attached. The term "protraction" thus refers to half-life extension and a protractor or protraction moiety serves the purpose of extending the plasma half-life of the peptides as disclosed herein.

Furthermore, the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention have a long plasma half-life relative to dosing interval, thus reducing the variability in steady state exposure, and thus making a once weekly administration possible. The compound disclosed herein may be orally bioavailable, hence suitable for oral administration of subjects in need thereof. Both the peptide backbone and the protraction moiety have been engineered and refined in order to achieve a compound having all of the above properties.

Each protraction moiety L_P-P covalently attaches to the epsilon amino group of a lysine residue in the peptide backbone of the inventive GLP-1-/GIP-/amylin-receptor tri-agonist. The protraction moiety L_P-P may attach to the epsilon position (i.e., amino group) of the one lysine (Lys, K) residue. The protraction moiety L_P-P may attach to the epsilon position (i.e., amino group) of a lysine (Lys, K) residue in the peptide Z1 portion of the peptide backbone (the "Z1" in Z1-L1-Z2), such as a lysine (Lys, K) residue at position X₄ or X₅ of Formula IIa (SEQ ID NO: 3) or at positions 33 or 34 of Formula IIa (SEQ ID NO: 3), or at position 12 of Formula Xa (SEQ ID NO: 162) or at position X₅₂ of Formula Xa (SEQ ID NO: 162).

The protraction moiety L_P -P may attach to the epsilon position (i.e., amino group) of a lysine (Lys, K) residue in the peptide linker L1 portion of the peptide backbone (the "L1" in Z1-L1-Z2).

5 The protraction moiety L_P -P may attach to the epsilon position (i.e. amino group) of a lysine (Lys, K) residue in the peptide Z2 portion of the peptide backbone (the "Z2" in Z1-L1-Z2), such as a lysine (Lys, K) residue at any one of positions X_6 , X_{10} , X_{11} , X_{12} , or X_{14} of Formula IIIa (SEQ ID NO: 4) or at positions 3, 15, 18, 20 or 24 of Formula IIIa (SEQ ID NO: 4), or at position 18 of Formula XIIa (SEQ ID NO: 165) or at position X_{63} of Formula XIIa (SEQ ID NO: 165). The attachment point is generally referred to as R1.

10 When the optional linker L_P is present, the protraction moiety L_P -P covalently attaches to the peptide backbone via the linker L_P . When the linker L_P is absent, P covalently attaches to the peptide backbone.

The GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention, as disclosed herein, comprises or consists of a peptide comprising one lysine (Lys, K) residue, to which a
15 single protraction moiety is covalently attached/conjugated (at the epsilon amino group). A protraction moiety may consist of one protractor P. A protraction moiety may comprise one linker L_P and one protractor P. A protraction moiety may comprise one linker L_P and two or more protractors (in this case referred to as P1, P2 or P3 and so forth). The two protractors (P1 and P2) may be identical, or the two protractors (P1 and P2) may be non-identical.

20 Where the peptide derivative comprises two or three protractors (P1, P2, P3), the protractors are preferably similar, more preferably substantially identical, or, most preferably, identical.

In the context of chemical moieties such as the protraction moieties disclosed herein, similarity and/or identity may be determined using any suitable computer program and/or algorithm known in the art.

25 The protraction moiety may be capable of non-covalently binding to albumin, thereby promoting the circulation of the peptide derivative of the present invention in the blood stream and prolonging its plasma half-life. Thus, the skilled person may also refer to the protraction moiety as being an "albumin binding moiety".

30 Protractor P

The protractor P may comprise an acyl group. The acyl group may be branched or unbranched. The acyl group may be saturated or unsaturated. The protractor P may comprise a fatty acid acyl group. The fatty acid acyl group may be branched or unbranched. The fatty acid acyl group may be saturated or unsaturated.

35 The protractor P may comprise a distal carboxylic acid group.

The protractor P may comprise a fatty acid group.

The protractor P may comprise a fatty acid group and an amide group.

The protractor P may comprise a distal carboxylic acid group and an amide group.

The protractor P may comprise an alkyl group.

5 The protractor P may comprise an aryl group.

The protractor P may comprise a tetrazole group.

The protractor P may comprise a sulfonic acid group.

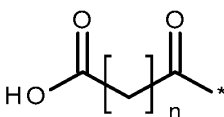
The protractor P may comprise a phenoxy group.

The protractor P may comprise a benzoic acid group.

10 The protractor P may comprise a phosphonic acid group.

The protractor may comprise a group defined by:

Chem. 1a: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 6-30, which may also be referred to as a $\text{C}_{(n+2)}$ diacid (e.g. C_{18} diacid) or as

Chem. 1b:  , wherein n is an integer in the range of 6-30. The

15 asterisk (*) shows the point of attachment of the radical.

The protractor P may comprise 8-32 carbon atoms. The protractor may comprise 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 carbon atoms.

20 The protractor P may comprise 6-30 consecutive $-\text{CH}_2-$ groups. The protractor P may comprise a carbon chain comprising at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 consecutive $-\text{CH}_2-$ groups.

The protractor P may comprise 12-26 carbon atoms. The protractor P may comprise 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 26 carbon atoms.

25 The protractor P may comprise 10-26 consecutive $-\text{CH}_2-$ groups. The protractor P may comprise a carbon chain comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 26 consecutive $-\text{CH}_2-$ groups.

The protractor P may comprise 16-22 carbon atoms. The peptide derivative of the present invention may comprise a single protraction moiety with a protractor P comprising a carbon chain a side chain comprising 16, 17, 18, 19, 20, 21 or 22 carbon atoms.

30 The protractor P may comprise 14-20 consecutive $-\text{CH}_2-$ groups. The protractor P may comprise a carbon chain comprising 14, 15, 16, 17, 18, 19 or 20 consecutive $-\text{CH}_2-$ groups.

The protractor may P comprise 16-22 consecutive carbon atoms and 14-20 consecutive -CH₂- groups.

The protractor P may comprise 16 consecutive carbon atoms and 14 consecutive -CH₂- groups. The protractor P may be a C₁₆ diacid, which may be defined by the formula
5 HOOC-(CH₂)₁₄-CO-*

The protractor P may comprise 18 consecutive carbon atoms and 16 consecutive -CH₂- groups. The protractor P may be a C₁₈ diacid, which may be defined by the formula
HOOC-(CH₂)₁₆-CO-*

The protractor P may comprise 20 consecutive carbon atoms and 18 consecutive -CH₂- groups. The protractor P may be a C₂₀ diacid, which may be defined by the formula
10 HOOC-(CH₂)₁₈-CO-*

The protractor P may comprise 22 consecutive carbon atoms and 20 consecutive -CH₂- groups. The protractor P may be a C₂₂ diacid, which may be defined by the formula
HOOC-(CH₂)₂₀-CO-*

15 The term "fatty acid" refers to aliphatic mono- or dicarboxylic acids having from 4 to 28 carbon atoms, it may be branched or un-branched, it is preferably un-branched, and it may be saturated or unsaturated, it is preferably saturated.

As described above, the peptide derivative disclosed herein comprise one lysine (Lys, K) residue and hence one protraction moiety (L_P-P), wherein the protraction moiety is
20 attached to the peptide backbone described herein via the epsilon position (i.e., amino group) of the lysine (Lys, K) residue (via an amide bond formed between a carboxylic acid group in the protraction moiety and the epsilon amino group of the lysine residue). The protraction moiety may be attached to the epsilon position of the one lysine (Lys, K) residue in the peptide backbone.

25 In one embodiment the protraction moiety may attach to the epsilon position of the lysine (Lys, K) residue in the peptide Z1 of the peptide backbone (the "Z1" in Z1-L1-Z2). In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) at position X₄ or X₅ of Formula IIa (SEQ ID NO: 3) of peptide Z1. In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) at position X₅₂ of Formula Xa
30 (SEQ ID NO: 162) of peptide Z1. In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) at any one of positions 12, 33 or 34 of peptide Z1, preferably at position 12 or position 33 of peptide Z1.

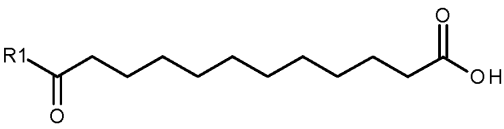
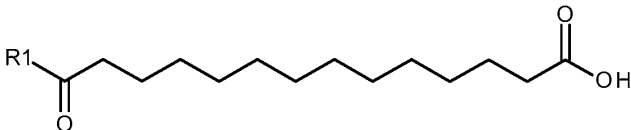
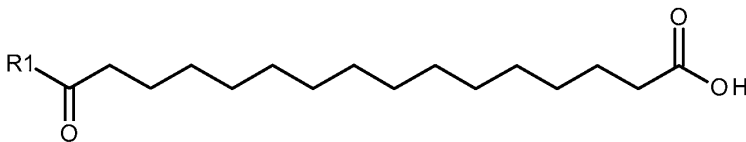
In one embodiment the protraction moiety may attach to the epsilon position of the lysine (Lys, K) residue in the linker L1 portion of the peptide backbone (the "L1" in Z1-L1-Z2).

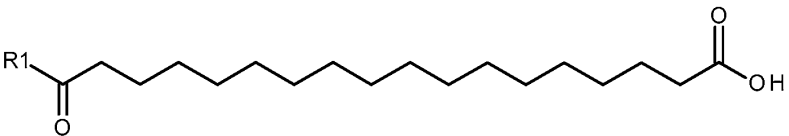
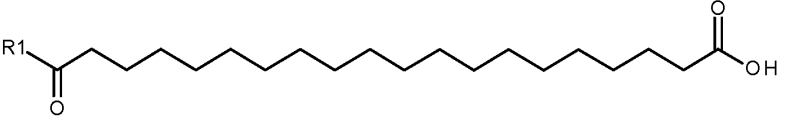
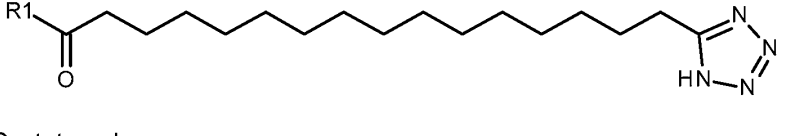
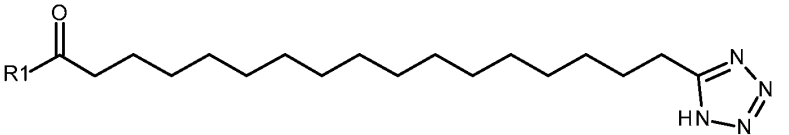
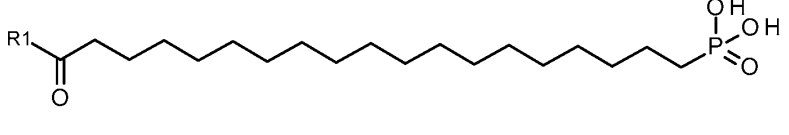
In one embodiment the protraction moiety may attach to the epsilon position of the lysine (Lys, K) residue in the peptide Z2 of the peptide backbone (the “Z2” in Z1-L1-Z2). In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) at any one of positions X₆, X₁₀, X₁₁, X₁₂, or X₁₄ of Formula IIIa (SEQ ID NO: 4) of peptide Z2. In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) at position X₆₃ of Formula XIIa (SEQ ID NO: 165) of peptide Z2. In particular, the protraction moiety may attach to the epsilon position of the lysine (Lys, K) residue at any one of positions 3, 15, 18, 20 or 24 of peptide Z2. In a preferred embodiment the protraction moiety may attach to the epsilon position of the lysine (Lys, K) residue at position 15 of peptide Z2 or at position 18 of peptide Z2.

In some embodiments the GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide derivative as disclosed herein may comprise a protractor P, which is selected from any one of those depicted in

Table 3. R1 represents the site of attachment to (a) the backbone of the peptide derivative - more specifically the epsilon amino group of the lysine or (b) the present optional linker L_P. Based on the disclosure herein, the skilled person will be able to determine also other chemical moieties for use as a protractor in a specific peptide derivative as disclosed herein, optionally after some limited routine experiments.

Table 3: Examples of protractors (P)

Protractor ID	Structure
Chem. 2	 C ₁₂ diacid
Chem. 3	 C ₁₄ diacid
Chem. 4	

	C ₁₆ diacid
Chem. 5	 C ₁₈ diacid
Chem. 6	 C ₂₀ diacid
Chem. 7	 C ₁₇ tetrazole
Chem. 8	 C ₁₈ tetrazole
Chem. 32	 C ₁₉ phosphonic acid

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide derivative comprising a protractor P, being a C₁₂-C₂₀ diacid.

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide derivative comprising a protractor P selected from the group consisting of C₁₆ diacid, C₁₈ diacid, and C₂₀ diacid.

In a preferred embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist comprises a peptide derivative comprising a protractor P being a C₁₈ diacid or a C₂₀ diacid.

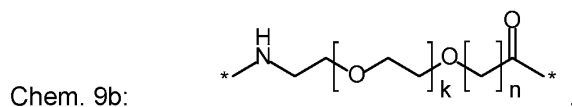
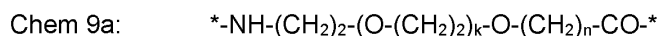
Linker L_P

In one embodiment the protractor is attached/conjugated directly onto the backbone of the peptide derivative, i.e., without use of a linker L_P (i.e., by way of a covalent bond, e.g. an amide bond).

- 5 In other embodiments the protractor is covalently conjugated to the peptide derivative using a linker L_P , hence, as described above, the protraction moiety (L_P -P) comprises an optional linker L_P . The linker L_P may comprise several "linker elements". The linker elements may be selected so that they improve the overall properties of the molecule, e.g., so that they improve the oral bioavailability, the conversion of half-life or the protracting effect, thus improving the overall exposure profile upon oral administration of the compound.

The linker L_P may comprise Ado, Aeep or Aeep, Ahx, Ala, ϵ -Lys, Glu, γ Glu, Gly, Ser, sulfonamide, Thr and/or Trx.

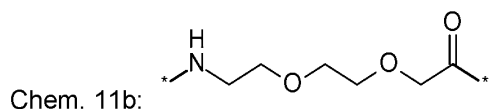
- The linker L_P may comprise at least a moiety which may be represented by the following chemical formula (wherein the asterisks (*) show the points of attachment of the radicals):



- 20 wherein k is an integer in the range of 1-5, and n is an integer in the range of 1-5. When k=1 and n=1, the linker element may be designated Ado, or 8-amino-3,6-dioxaoctanoyl, which may be represented by the following chemical formula:



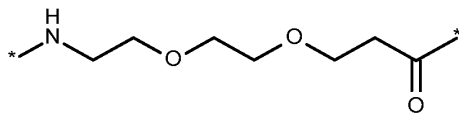
- 25 or



When k=1 and n=2, the linker element may be designated Aeep, which may be represented by the following chemical formula:

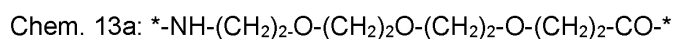
- 30 Chem. 12a: $^*\text{-NH-(CH}_2)_2\text{-O-(CH}_2)_2\text{-O-(CH}_2)_2\text{-CO-}^*$

or



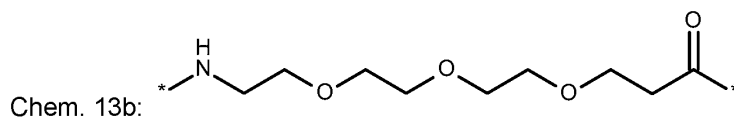
Chem. 12b:

- 5 When $k=2$ and $n=2$, the linker element may be designated Aeeep, which may be represented by the following chemical formula:



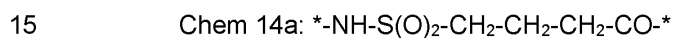
or

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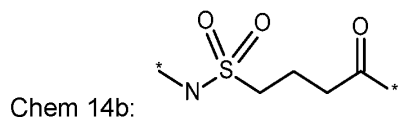


Chem. 13b:

The linker L_P may comprise a sulfonamide-C4 moiety. A sulfonamide-C4 group is a sulfonamide group attached to a 4-butanoyl group, having the following chemical formula:

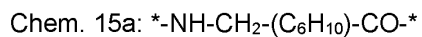


or



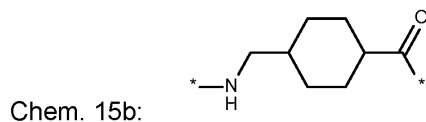
Chem 14b:

- 20 The linker L_P may comprise Trx. Trx is also referred to as Tranexamic acid, trans-4-(aminomethyl)cyclohexanecarboxylic acid, having the following chemical formula:



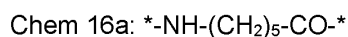
or

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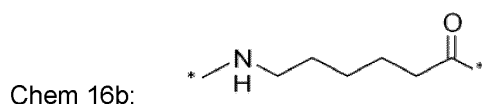
Chem. 15b:

The linker L_P may comprise Ahx. Ahx is also referred to as Aminocaproic acid, 6-aminohexanoic acid having the following chemical formula:



5

or



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The linker L_P may comprise epsilon-lysine (ϵ -Lys).

The linker L_P may comprise lysine (Lys).

The linker L_P may comprise alanine (Ala).

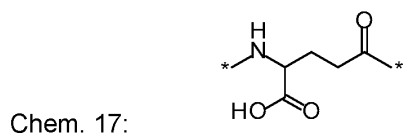
The linker L_P may comprise glycine (Gly).

The linker L_P may comprise serine (Ser).

15

The linker L_P may comprise glutamic acid (Glu).

The linker L_P may comprise a Glu di-radical, such as



20

wherein the Glu di-radical may be included p times, where p is an integer in the range of 1-3. Any one of above disclosed amino acids, which are used as linker L_P or as part of linker L_P , may be used as L-isomer or as D-isomer.

Chem. 17 may also be referred to as gamma-Glu, or briefly γ Glu, due to the fact that it is the gamma carboxy group of the amino acid glutamic acid which is here used for connection to the epsilon amino group of lysine. As described above, the other linker element may, for example, be another Glu residue, or an Ado molecule. The amino group of Glu in turn forms an amide bond with the carboxy group of the protruding moiety, or with the carboxy group of, e.g., an Ado molecule, if present, or with the gamma-carboxy group of, e.g., another Glu, if present.

25

The peptide derivative disclosed herein may comprise a linker L_P which is selected from any one of those depicted in Table 4 below. R1 represents the residue in the peptide backbone to which the protraction moiety is attached to, and P represents the protractor.

In some embodiments the peptide derivative comprises a protraction moiety,
5 wherein the protractor Chem. 4 or Chem. 5 or Chem. 6 is attached to the peptide backbone using the linker designated L_P1 , L_P2 , L_P3 , L_P4 , L_P5 or L_P6 in Table 4 below.

In some embodiments the peptide derivative comprises a protraction moiety,
wherein the protractor Chem. 5 is attached to the peptide backbone using the linker
designated L_P1 , L_P2 , L_P3 , L_P4 , L_P5 or L_P6 in Table 4 below, thus wherein said protraction
10 moiety comprises Chem. 18, Chem. 19, Chem. 20, Chem. 21, Chem. 33 or Chem. 34 as
linker L_P and Chem. 5 as protractor P.

In some embodiments the peptide derivative comprises a protraction moiety,
wherein the protractor Chem. 6 is attached to the peptide backbone using the linker
designated L_P1 , L_P2 , L_P3 , L_P4 , L_P5 or L_P6 in Table 4 below, thus wherein said protraction
15 moiety comprises Chem. 18, Chem. 19, Chem. 20, Chem. 21, Chem. 33 or Chem. 34 as
linker L_P and Chem. 6 as protractor P.

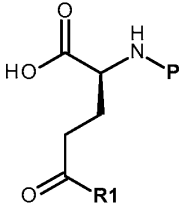
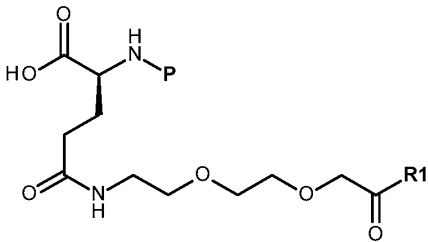
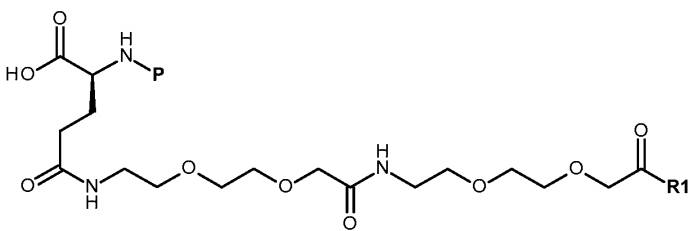
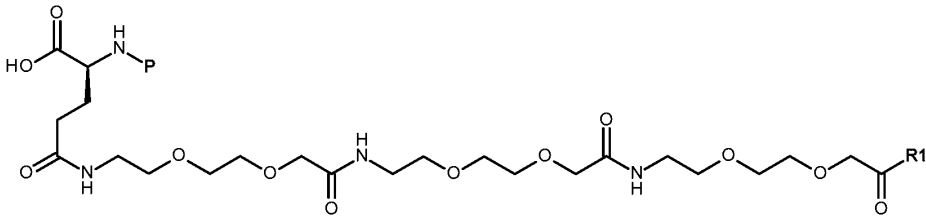
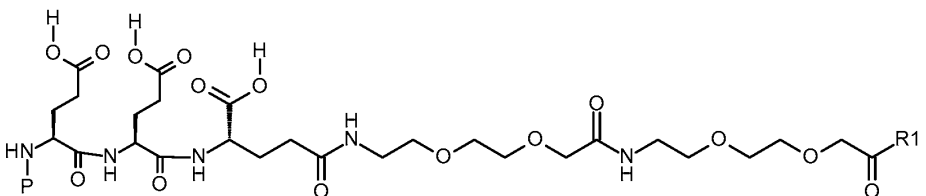
In a preferred embodiment the peptide derivative comprises a protraction moiety,
wherein said protraction moiety comprises Chem. 20 or Chem. 21 as linker L_P and Chem. 5
or Chem. 6 as protractor P.

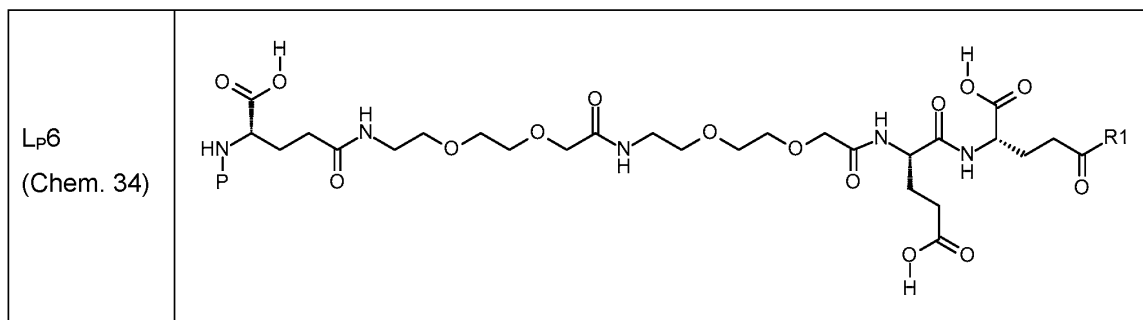
20 In a preferred embodiment the peptide derivative comprises a protraction moiety,
wherein the protractor Chem. 5 is attached to the peptide backbone using the linker
designated L_P3 in Table 4 below, thus wherein said protraction moiety comprises Chem. 20
as linker L_P and Chem. 5 as protractor P.

In a preferred embodiment the peptide derivative comprises a protraction moiety,
25 wherein the protractor Chem. 6 is attached to the peptide backbone using the linker
designated L_P3 in Table 4 below, thus wherein said protraction moiety comprises Chem. 20
as linker L_P and Chem. 6 as protractor P.

Based on the disclosure herein, the skilled person will be able to determine the
optimal L_P linker for use in a specific peptide derivative as disclosed herein, optionally after
30 some limited routine experiments.

Table 4: Examples of optional linkers (L_P) of the protraction moiety

Linker L_P ID	Structure
L_{P1} (Chem. 18)	
L_{P2} (Chem. 19)	
L_{P3} (Chem. 20)	
L_{P4} (Chem. 21)	
L_{P5} (Chem. 33)	

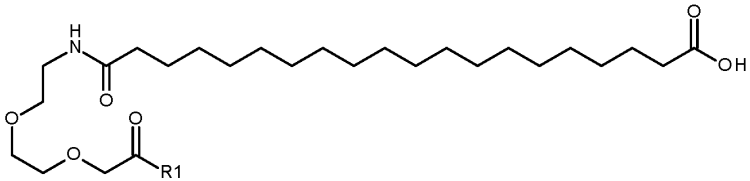
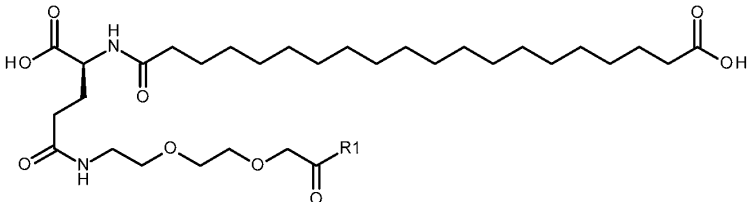
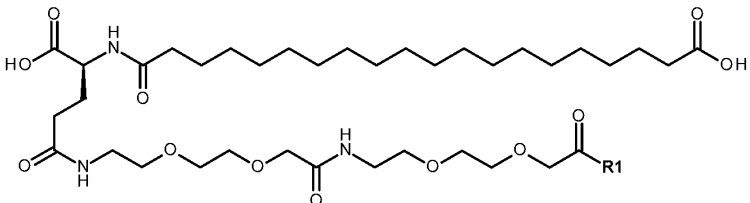
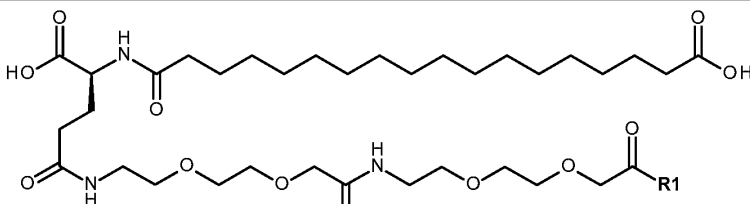
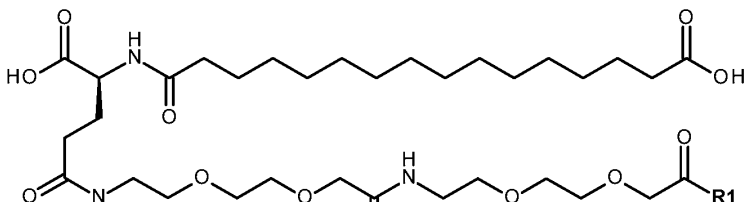
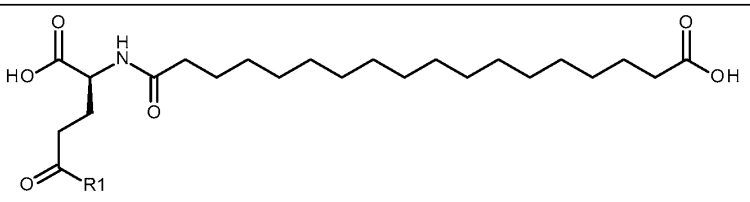


In some embodiments the peptide derivative comprises a protraction moiety which is selected from the group presented in Table 5. R1 represents the residue in the peptide backbone to which the protraction moiety is attached to.

5

Table 5: Examples of protraction moieties

Protraction moiety	Structure
C ₂₀ diacid gamma-Glu (Chem. 22)	
C ₂₀ diacid gamma-Glu gamma-Glu (Chem. 23)	
C ₂₀ diacid gamma-Glu gamma-Glu gamma-Glu (Chem. 24)	

C₂₀ diacid Ado (Chem. 25)	
C₂₀ diacid gamma-Glu Ado (Chem. 26)	
C₂₀ diacid gamma-Glu 2xAdo (Chem. 27)	
C₁₈ diacid gamma-Glu 2xAdo (Chem. 28)	
C₁₆ diacid gamma-Glu 2xAdo (Chem. 29)	
C₁₈ diacid gamma-Glu (Chem. 30)	

C ₁₈ diacid gamma-Glu 3xAdo (Chem. 31)	
C ₁₉ phosphonic acid gamma-Glu 2xAdo (Chem. 35)	
(Chem. 36) C ₂₀ diacid 2xGlu gamma-Glu 2xAdo	
(Chem. 37) C ₂₀ diacid gamma-Glu 2xAdo Glu gamma-Glu	

In one embodiment the free lysine may serve as a conjugation site for attaching one C₁₆ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[4S)-4-carboxy-4-(15-carboxypentadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]), and thus the peptide derivative of the present invention comprises a

5 protraction moiety, wherein said protraction moiety is a C₁₆ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

In one embodiment a free lysine may serve as a conjugation site for attaching one C₁₈ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]), and thus the peptide derivative of the present invention comprises a

10

protraction moiety, wherein said protraction moiety is a C₁₈ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

In one embodiment a free lysine may serve as a conjugation site for attaching one C₂₀ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[[(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]]], and thus the peptide derivative of the present invention comprises a protraction moiety, wherein said protraction moiety is a C₂₀ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

In one embodiment a free lysine may serve as a conjugation site for attaching one C₁₈ diacid gamma-Glu fatty acid moiety (IUPAC name [(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]), and thus the peptide derivative of the present invention comprises a protraction moiety, wherein said protraction moiety is a C₁₈ diacid (S) gamma-Glu fatty acid moiety.

In one embodiment a free lysine may serve as a conjugation site for attaching one C₂₀ diacid gamma-Glu fatty acid moiety (IUPAC name [(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]), and thus the peptide derivative of the present invention comprises a protraction moiety, wherein said protraction moiety is a C₂₀ diacid (S) gamma-Glu fatty acid moiety.

In a preferred embodiment a free lysine may serve as a conjugation site for attaching one C₁₈ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[[(4S)-4-carboxy-4-(17-carboxypentadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]]], and thus the peptide derivative of the present invention comprises a protraction moiety, wherein said protraction moiety is a C₁₈ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

In a preferred embodiment a free lysine may serve as a conjugation site for attaching one C₂₀ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[[(4S)-4-carboxy-4-(19-carboxynonadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]]], and thus the peptide derivative of the present invention comprises a protraction moiety, wherein said protraction moiety is a C₂₀ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

In a most preferred embodiment a free lysine may serve as a conjugation site for attaching one C₁₈ diacid gamma-Glu 2xAdo fatty acid moiety (IUPAC name [2-[2-[2-[[2-[2-[2-[[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]]], and thus the peptide derivative of the present invention

comprises a protraction moiety, wherein said protraction moiety is a C₁₈ diacid (S) gamma-Glu 2xAdo fatty acid moiety.

Peptide derivative

As described above, the GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention may comprise a peptide linker and further comprise a protraction moiety. Therefore, the present invention relates in another aspect to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib,
and

- Z1 comprises or consists of an amino acid sequence according to Formula X (SEQ ID NO: 161):



wherein

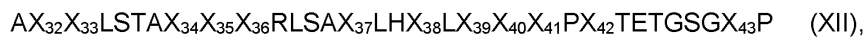
- X₂₁ represents His (H) or Tyr (Y),
- X₂₂ represents Aib,
- X₂₃ represents Glu (E) or His (H),
- X₂₄ represents Ile (I) or Lys (K),
- X₂₅ represents Gln (Q) or Ile (I),
- X₂₆ represents Arg (R) or Gln (Q),
- X₂₇ represents Ala (A), Glu (E) or Gln (Q),
- X₂₈ represents Leu (L) or I (Ile),
- X₂₉ represents Ala (A) or Gln (Q),
- X₃₀ represents Arg (R), Gly (G), Lys (K) or Ser (S),
- X₃₁ represents Gly (G), Glu (E) or Lys (K);

- L1 is a peptide linker;
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):



and

Z2 comprises or consists of an amino acid sequence according to Formula XII (SEQ ID NO: 164):



wherein

- 5 X_{32} represents Gly (G) or Ser (S),
 X_{33} represents Gln (Q), Glu (E), His (H) or Lys (K),
 X_{34} represents Ala (A) or Gln (Q),
 X_{35} represents Gln (Q), Leu (L) or Thr (T),
 X_{36} represents Ala (A), Gly (G) or Gln (Q),
10 X_{37} represents Glu (E) or Lys (K),
 X_{38} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
 X_{39} represents Ala (A) or Lys (K),
 X_{40} represents Asp (D) or Thr (T),
15 X_{41} represents Leu (L) or Glu (E),
 X_{42} represents Arg (R) or Lys (K),
 X_{43} represents Ala (A) or Ser (S); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

- 20 In another aspect the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X_2 represents Aib,

and

- 30 Z1 comprises or consists of an amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

- X_1 represents His (H) or Tyr (Y),
 X_2 represents Aib,
35 X_3 represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₅ represents Gly (G) or Lys (K);

• L1 is a peptide linker;

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10

5 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

10 ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

15 X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),

20 X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

25 In another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula

30 II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

and

Z1 comprises or consists of an amino acid sequence according to Formula Xa
35 (SEQ ID NO: 162):

YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K),

5 X₅₃ represents Arg (R) or Gln (Q),

X₅₄ represents Ala (A), Glu (E) or Gln (Q),

X₅₅ represents Leu (L) or I (Ile),

X₅₆ represents Ala (A) or Gln (Q),

X₅₇ represents Gly (G) or Glu (E);

10 • L1 is a peptide linker;

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

15 Z2 comprises or consists of an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

wherein

X₅₈ represents Gly (G) or Ser (S),

20 X₅₉ represents Gln (Q), Glu (E), or His (H),

X₆₀ represents Ala (A) or Gln (Q),

X₆₁ represents Leu (L) or Thr (T),

X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

25 X₆₄ represents Leu (L) or Glu (E); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

In yet another aspect the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

30 Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

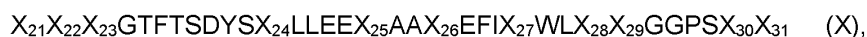
• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

35 wherein the amino acid at position X₂ represents Aib,

and

Z1 comprises or consists of an amino acid sequence according to Formula X (SEQ ID NO: 161):



5 wherein

X_{21} represents His (H) or Tyr (Y),

X_{22} represents Aib,

X_{23} represents Glu (E) or His (H),

X_{24} represents Ile (I) or Lys (K),

10 X_{25} represents Gln (Q) or Ile (I),

X_{26} represents Arg (R) or Gln (Q),

X_{27} represents Ala (A), Glu (E) or Gln (Q),

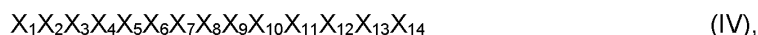
X_{28} represents Leu (L) or I (Ile),

X_{29} represents Ala (A) or Gln (Q),

15 X_{30} represents Arg (R), Gly (G), Lys (K) or Ser (S),

X_{31} represents Gly (G), Glu (E) or Lys (K);

• L1 is a peptide linker comprising or consisting of the amino acid sequence according to Formula IV:



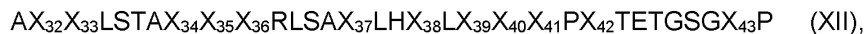
20 which is selected from the group consisting of E, GE, APPPSGGGE (SEQ ID NO: 129), AGQAPGQAPG (SEQ ID NO: 136), AGQAPGQAPL (SEQ ID NO: 137) and AGQAPGEAPG (SEQ ID NO: 154);

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

25 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula XII (SEQ ID NO: 164):



30 wherein

X_{32} represents Gly (G) or Ser (S),

X_{33} represents Gln (Q), Glu (E), His (H) or Lys (K),

X_{34} represents Ala (A) or Gln (Q),

X_{35} represents Gln (Q), Leu (L) or Thr (T),

35 X_{36} represents Ala (A), Gly (G) or Gln (Q),

X_{37} represents Glu (E) or Lys (K),
 X_{38} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
 X_{39} represents Ala (A) or Lys (K),
 X_{40} represents Asp (D) or Thr (T),
 X_{41} represents Leu (L) or Glu (E),
 X_{42} represents Arg (R) or Lys (K),
 X_{43} represents Ala (A) or Ser (S); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

In yet another aspect the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:



comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X_2 represents Aib,

and

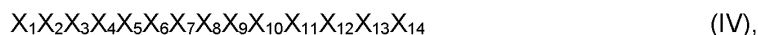
Z1 comprises or consists of an amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

X_1 represents His (H) or Tyr (Y),
 X_2 represents Aib,
 X_3 represents Glu (E) or His (H),
 X_4 represents Arg (R), Gly (G), Lys (K) or Ser (S),
 X_5 represents Gly (G) or Lys (K);

• L1 is a peptide linker comprising or consisting of the amino acid sequence according to Formula IV:



which is selected from the group consisting of E, GE, APPPSGGGE (SEQ ID NO: 129), AGQAPGQAPG (SEQ ID NO: 136), AGQAPGQAPL (SEQ ID NO: 137) and AGQAPGEAPG (SEQ ID NO: 154);

• Z2 is a peptide comprising a C-terminal amide and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

5 Z2 comprises or consists of an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),
10 X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E) or Lys (K),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or
15 Tyr (Y),
X₁₂ represents Ala (A) or Lys (K),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ala (A) or Ser (S); and
20 wherein the peptide is a peptide derivative comprising a protraction moiety.

In yet another aspect the invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

Z1—L1—Z2 (I),

25 comprising one lysine (Lys, K) residue; wherein:

• Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

30 and

Z1 comprises or consists of an amino acid sequence according to Formula Xa (SEQ ID NO: 162):

YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

wherein

35 X₅₁ represents Aib,

X_{52} represents Ile (I) or Lys (K),
 X_{53} represents Arg (R) or Gln (Q),
 X_{54} represents Ala (A), Glu (E) or Gln (Q),
 X_{55} represents Leu (L) or I (Ile),
 X_{56} represents Ala (A) or Gln (Q),
 X_{57} represents Gly (G) or Glu (E);

5

• L1 is a peptide linker comprising or consisting of the amino acid sequence according to Formula IV:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (IV),

10 which is selected from the group consisting of E, GE, APPPSGGGE (SEQ ID NO: 129), AGQAPGQAPG (SEQ ID NO: 136), AGQAPGQAPL (SEQ ID NO: 137) and AGQAPGEAPG (SEQ ID NO: 154);

• Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

15 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

$AX_{58}X_{59}LSTAX_{60}X_{61}X_{62}RLSAELHX_{63}LATX_{64}PRTETGSGSP$ (XIIa),

20 wherein

X_{58} represents Gly (G) or Ser (S),
 X_{59} represents Gln (Q), Glu (E), or His (H),
 X_{60} represents Ala (A) or Gln (Q),
 X_{61} represents Leu (L) or Thr (T),
 X_{62} represents Ala (A), Gly (G) or Gln (Q),
 X_{63} represents Gln (Q), Glu (E), or Lys (K),
 X_{64} represents Leu (L) or Glu (E); and

25

wherein the peptide is a peptide derivative comprising a protraction moiety.

30 The GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention may be a peptide derivative which comprise any one of the above disclosed peptides Z1, any one of the above disclosed peptides Z2, any one of the above disclosed peptide linkers L1 and any one of the above disclosed protraction moieties, and based on the disclosure herein, the skilled person will be able to determine the optimal combination to come to a specific peptide

derivative which is a potent GLP-1-/GIP-/amylin-receptor tri-agonist having specific properties as described below.

When tested as described in "GLP-1 receptor assay" (preferably method A; in the absence of HSA), the peptide derivative disclosed herein may have an EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM.

When tested as described in "GIP receptor assay" (preferably method A; in the absence of HSA), the peptide derivative disclosed herein may have an EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM.

When tested as described in "Amylin receptor assay" (preferably method A; in the absence of HSA), the peptide derivative disclosed herein may have an EC₅₀ value of less than 125 pM, preferably less than 100 pM, such as less than 75 pM, even more preferably less than 50 pM, such as less than 40 pM, and most preferably less than 30 pM, such as less than 20 pM, such as less than 10 pM, such as less than 5 pM.

The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, which activates the human GIP, GLP-1 and amylin receptors *in vitro*, when measured without HSA in assays as described in Example 4, and which has a wherein potency ratio (A/B) of less than 50.

The balanced GLP-1-/GIP-/amylin-receptor tri-agonist of the present invention activates the human GIP, GLP-1, and amylin receptors *in vitro*, when measured without HSA in assays as described in Example 4, and has a potency ratio (A/B), i.e., potency (A) of the receptor with lowest potency divided by potency (B) of the receptor with highest potency, of less than 50. The tri-agonists disclosed herein may have a potency ratio (A/B) of less than 50, preferably less than 20, such as less than 19, less than 18, less than 17, less than 16, even more preferred less than 15, such as less than 14, less than 13, less than 12, and most preferred less than 11, such as less than 10, less than 9, less than 8 and less than 7. Half-life is an important parameter as a long half-life indicates that less frequent administration of a compound may be possible. Based on the disclosure herein, the skilled person will be able to determine the protraction moiety for use in a specific peptide derivative as disclosed herein, optionally after some limited routine experiments. Hence in a fourth aspect, the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist having improved pharmacokinetic properties. The GLP-1-/GIP-/amylin-receptor tri-agonists or the peptide

derivatives of the present invention have a long half-life relative to dosing interval, thus reducing the variability in steady state exposure.

The *in vivo* pharmacology, including half-life, of the GLP-1-/GIP-/amylin-receptor tri-agonist described herein may be assessed as described in Example 6. In some
5 embodiments, the half-life is half-life ($t_{1/2}$) *in vivo* in minipigs after i.v. administration, e.g., as described in Example 6. The half-life of the GLP-1-/GIP-/amylin-receptor tri-agonist in animal subjects may be as long as about 100 hours, or longer. The half-life of the GLP-1-/GIP-
/amylin-receptor tri-agonist in animal subjects may be at least 40 hours, preferably at least 100 hours. The half-life of the GLP-1-/GIP-/amylin-receptor tri-agonist may be more than 40,
10 45, 55, 60, 60,65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, or 140 hours. The half-life of the GLP-1-/GIP-/amylin-receptor tri-agonist may be 40-145 hours, such as 90-140 hours, such as 85-125 hours.

In a fifth aspect, the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist which is suitable for once weekly administration. The GLP-1-/GIP-/amylin-receptor
15 tri-agonists or the peptide derivatives of the present invention have a long half-life relative to dosing interval, thus reducing the variability in steady state exposure, and thus making a once weekly administration possible.

In a sixth aspect, the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist which is suitable for oral administration. The GLP-1-/GIP-/amylin-receptor tri-agonist
20 described herein may be orally bioavailable; that is, present in the bloodstream following per oral administration. Therefore, the compound is suitable for oral administration of subjects in need thereof.

In a seventh aspect, the present invention relates to a GLP-1-/GIP-/amylin-receptor tri-agonist having improved chemical stability. The term "chemical stability" refers to chemical
25 (in particular covalent) changes in the polypeptide structure leading to formation of chemical degradation products, such as high molecular weight proteins (HMWPs), deamidation, isomerization and hydrolysis products potentially having a reduced biological potency, and/or increased immunogenic effect as compared to the intact polypeptide. The chemical stability may be determined by measuring the purity loss, e.g., by measuring the amount of chemical
30 degradation products at various time-points after exposure to different environmental conditions, e.g., by SEC-HPLC, and/or LCMS, e.g., as described in Example 7 herein. The GLP-1-/GIP-/amylin-receptor tri-agonist of the invention has a purity loss per week of less than 10.0 percent, preferably less than 6.0 percent, such as 5.0 or 4.0 percent, more preferably less than 3.0 percent, and most preferred less than 1.5 percent, upon incubation
35 at 37 °C and as determined in Example 7 described herein.

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may reduce food intake in a subject, e.g., normal weight rats or DIO rats. Administration of the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein may result in an acute reduction in the intake of food. The *in vivo* effect of the GLP-1-/GIP-/amylin-receptor tri-agonist on food intake in rats may be assessed as described in Example 5 or Example 8. A reduction of food intake of 100 % (hypothetical value), relative or compared to vehicle, means that the rat does not eat.

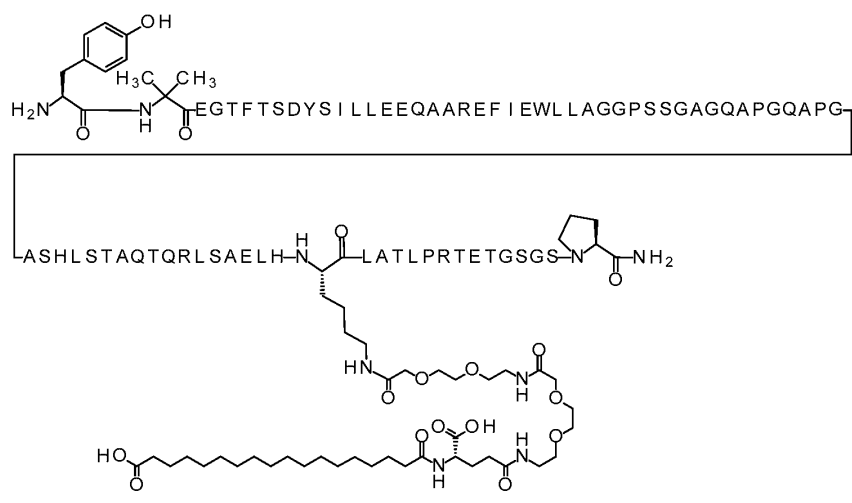
In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 10 nmol/kg, may reduce food intake at day 1 (0-24 hours) by at least 10 % compared to vehicle, preferably by at least 50 % compared to vehicle, such as at least 70 % compared to vehicle. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 10 nmol/kg, may reduce food intake at day 1 (0-24 hours) by 1 % to 100 %, when compared to vehicle, such as 15 % to 95 %, preferably by 40 % to 85 % when compared to vehicle, even more preferably by 50 % to 80 %, when compared to vehicle.

In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 10 nmol/kg, may reduce food intake at day 2 (24-48 hours) by at least 15 % compared to vehicle, preferably by at least 50 % compared to vehicle, such as at least 70 % compared to vehicle. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 10 nmol/kg, may reduce food intake at day 2 (24-48 hours) by 1 % to 100 %, when compared to vehicle, such as 15 % to 95 %, preferably by 40 % to 95 % when compared to vehicle, even more preferably by 70 % to 95 %, when compared to vehicle.

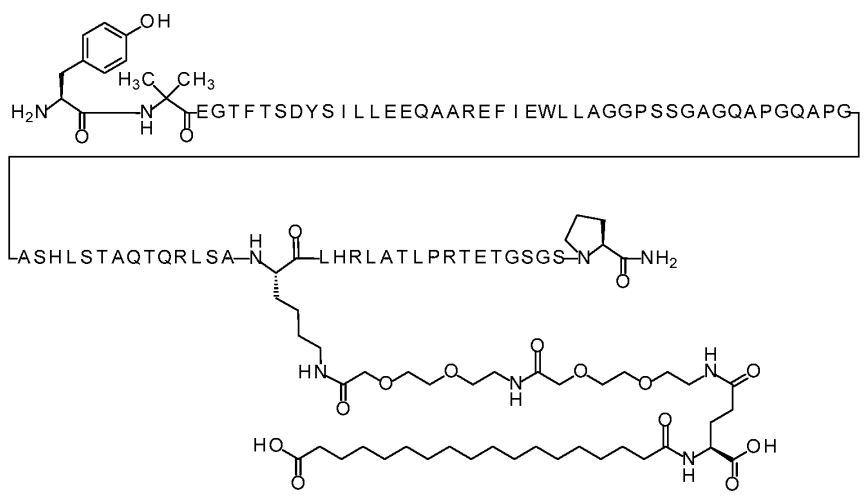
In one embodiment the GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 30 nmol/kg, may reduce food intake at day 1 (0-24 hours) by at least 15 % compared to vehicle, preferably by at least 35 % compared to vehicle. The GLP-1-/GIP-/amylin-receptor tri-agonist disclosed herein, after a single subcutaneous administration of 30 nmol/kg, may reduce food intake at day 2 (24-48 hours) by at least 15 % compared to vehicle, preferably by at least 35 % compared to vehicle.

Both the peptide backbone and the protraction moiety have been engineered and refined to achieve a peptide derivative having all of the above properties.

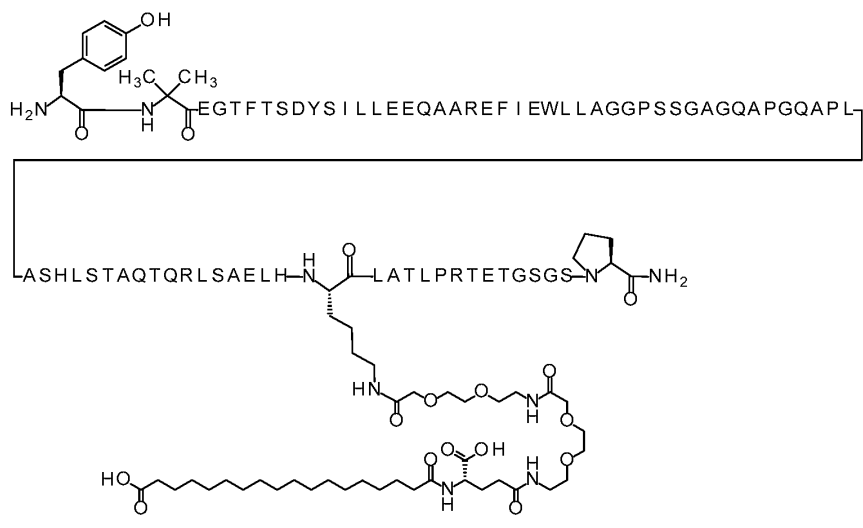
Preferred GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention are compound 52, that is:



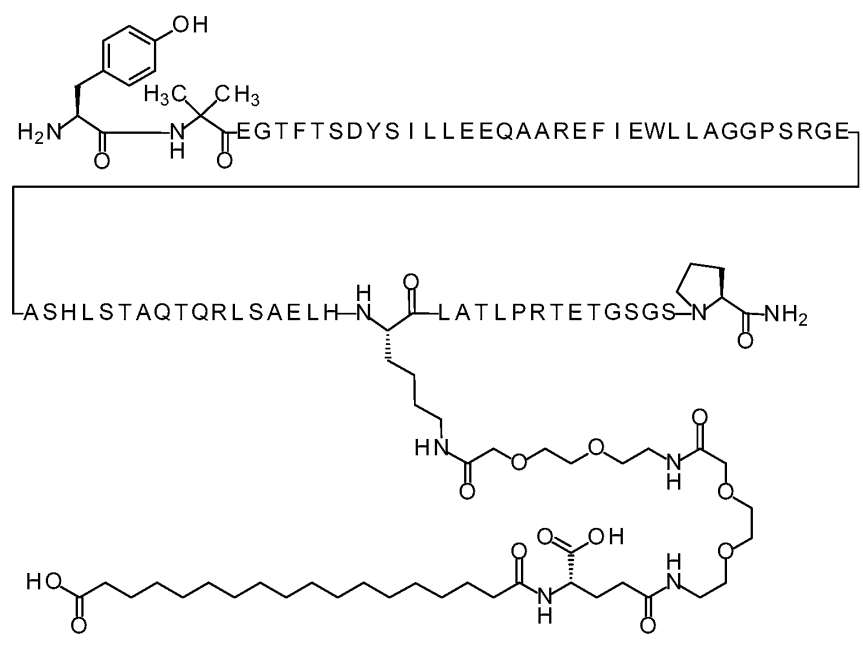
compound 55, that is:



compound 58, that is:



5 compound 68, that is:



Pharmaceutically acceptable salt

The compounds of the invention may be in the form of a pharmaceutically acceptable salt, or amide.

Salts are 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 nor (i.e., a neutral salt). Basic salts produce hydroxide ions and acid salts produce hydronium ions in water.

The salts of the compounds of the invention may be formed with added cations or anions between anionic or cationic groups, respectively. These groups may be situated in the peptide moiety, and/or in the protraction moiety of the compounds of the invention.

Non-limiting examples of anionic groups of the compounds of the invention include free carboxylic groups in the protraction moiety, if any, as well as in the peptide backbone. The peptide backbone may include free carboxylic groups at internal amino acid residues such as Asp (D) and Glu (E).

Non-limiting examples of cationic groups in the peptide backbone include the free amino group at the N-terminus, if present, as well as any free amino group of internal basic amino acid residues such as His (H), Arg (R), and Lys (K).

The amide of the compound of the invention may, e.g., be formed during peptide synthesis (based on the used resin) or by the reaction of a free carboxylic acid group with an amine or a substituted amine, or by reaction of a free or substituted amino group with a carboxylic acid. The amide formation may be at any free carboxylic group in the protraction moiety, the free amino group at the N-terminus of the peptide, and/or any free or substituted amino group in the peptide backbone.

In one aspect, the derivative of the invention is in the form of a pharmaceutically acceptable salt, preferably in the form of a trifluoroacetate salt.

Methods of production

The tri-agonists disclosed herein may 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 Dorwald, "Organic Synthesis on Solid Phase", Wiley-VCH Verlag GmbH, 2000; and "Fmoc Solid Phase Peptide Synthesis", Edited by W.C. Chan and P.O. White, Oxford University Press, 2000. In some embodiments, methods for preparing the triple agonists are described herein. In some embodiments, the methods for preparing the triple agonists as described herein comprises a step of solid phase peptide synthesis.

Also, or alternatively, the compounds, the peptide sequence or parts of the peptide sequences may be produced by recombinant methods, e.g., by culturing a host cell containing a DNA sequence encoding the tri-agonist 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 tri-agonists that include non-natural amino acids and/or covalently attached substituents (protraction moieties) may be produced as described under 'General method for peptide synthesis' in the experimental part. Or see e.g., Hodgson et al: "The synthesis of peptides and proteins containing non-natural amino acids", Chemical Society Reviews, vol. 33, no. 7 (2004), p. 422-430.

The tri-agonists as described herein which include a protraction moiety may, e.g., be produced as described under 'General method for peptide synthesis' in the experimental part. In some embodiments, the protraction moiety is built as part of the solid phase peptide synthesis or produced separately and attached via the one lysine residue after the solid phase peptide synthesis.

Specific examples of methods of preparing a number of the tri-agonists as described herein are provided below.

A further aspect of the invention relates to a method for preparing the receptor tri-agonists described herein.

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

Pharmaceutical compositions

In a further aspect the present invention relates to a pharmaceutical composition comprising said GLP-1-/GIP-/amylin-receptor triple agonist. Disclosed herein is a pharmaceutical composition comprising the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein, and one or more pharmaceutically acceptable excipients. Pharmaceutical composition comprising the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein, and one or more pharmaceutically acceptable excipients, may be prepared using methods known to the person skilled in the art.

The term "pharmaceutically acceptable excipient" refers to any ingredient in the pharmaceutical composition which is not the active pharmaceutical ingredient, or the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein. The term "pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and includes excipients that are acceptable for human pharmaceutical use. Such excipients can for example be solid, liquid or semisolid.

The excipient may be functional or inert and may serve various purposes, e.g. as a buffer, an isotonicity agent, a carrier, a vehicle, a filler, a binder, a lubricant, a glidant, a disintegrant, a flow control agent, a crystallization inhibitor, a solubilizer, a stabilizer, a colouring agent, a flavouring agent, a surfactant, emulsifier, or combinations thereof and/or to improve administration, and/or absorption of the active pharmaceutical ingredient(s). The amount of each excipient used may vary within ranges conventional in the art.

Techniques and excipients which may be used are described in e.g., *Handbook of Pharmaceutical Excipients* (e.g., 8th edition, Sheskey et al., Eds., American Pharmaceuticals Association and Pharmaceutical Press, publications department of the Royal Pharmaceutical Society of Great Britain (2017) and later editions) and *Remington: The Science and Practice of Pharmacy* (e.g., 23rd edition, Remington and Allen, Eds., Pharmaceutical Press (2021) and later editions).

The pharmaceutical composition comprising the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein may be for oral administration.

The pharmaceutical composition comprising the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein may be a solid pharmaceutical composition (e.g., a tablet or capsule) containing the active pharmaceutical ingredient, for example as a freeze-dried or spray-dried composition, and may be used as is, dissolved prior to use, or combined with excipients in the formulation.

The pharmaceutical composition may be a solid pharmaceutical composition comprising the compound disclosed herein, a salt of *N*-[8-(2-hydroxybenzoyl)amino]caprylate, preferably sodium *N*-(8-(2-hydroxybenzoyl)amino)caprylate, and one or more further excipients, as is described in the art. For example, the solid pharmaceutical composition may be as described in WO 2012/080471, WO 2013/139694, WO 2013/189988, WO 2019/149880, WO 2019/215063, WO 2021/219710 or WO 2023/012263 A1.

Alternatively, the pharmaceutical composition comprising the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein may be a liquid composition, such as an aqueous composition. Such liquid compositions may be suitable for oral administration or for

parenteral administration, for example intravenous, intramuscular, or subcutaneous administration.

Liquid compositions that are 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 procedure, the compound described herein is dissolved in a suitable buffer at a suitable pH. The composition may be sterilized, for example, by sterile filtration. Techniques and excipients which may be used to prepare liquid formulations are described in e.g., *Handbook of Pharmaceutical Excipients* (e.g., 8th edition, Sheskey et al., Eds., American Pharmaceuticals Association and Pharmaceutical Press, publications department of the Royal Pharmaceutical Society of Great Britain (2017) and later editions) and *Remington: The Science and Practice of Pharmacy* (e.g., 23rd edition, Remington and Allen, Eds., Pharmaceutical Press (2021) and later editions). Preferably, in an embodiment wherein pharmaceutical composition is in a liquid formulation, the liquid formulation provides an improved stability.

The pharmaceutical compositions are typically administered to a subject already suffering from a disease, such as the indications described below, in an amount sufficient to cure, alleviate or partially arrest the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount". As will be understood by the person skilled in the art amounts effective for this purpose will depend on the severity of the disease as well as the weight and general state of the subject.

In some embodiments the dose of the compounds to be delivered by subcutaneous administration may be from about 0.1 mg to 500 mg of the compound per day, preferably from about 0.5 mg to 150 mg per day, per every second day, per every third day, per every fourth day, per every fifth day, per every sixth day or once weekly depending on the severity of the condition.

A suitable dose may also be adjusted for a particular compound based on the properties of that compound, including its *in vivo* half-life or mean residence time and its biological activity. For example, compounds to be delivered could in one embodiment be administered once daily, or in another embodiment once weekly. Accordingly, the pharmaceutical compositions may be used for dosing approximately once daily, such as once every 12-36 hours, such as once every 18-30 hours, such as approximately once every 24 hours, or may be used for dosing approximately once weekly, such as once every 6-8 days.

In one embodiment the present invention relates to an injection device comprising said pharmaceutical composition.

Indications

In a further aspect the present invention relates to the GLP-1-/GIP-/amylin-receptor
5 triple agonist as disclosed herein for use as a medicament.

The GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein may be used for the following medical treatments or indications:

- (i) prevention and/or treatment of all forms of diabetes, such as hyperglycaemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, non-insulin dependent
10 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, and/or delaying the progression of non-insulin requiring type 2 diabetes to
15 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, food cravings, bulimia nervosa and/or obesity induced by administration of an antipsychotic or a steroid; reduction of gastric motility; and/or delaying
20 gastric emptying;
- (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 cardiovascular diseases, such as delaying or reducing development of a major adverse cardiovascular event (MACE) selected from the
25 group consisting of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, revascularisation, hospitalisation for unstable angina pectoris, and hospitalisation for heart failure.
- (vi) prevention and/or treatment of non-alcoholic fatty liver disease (NAFLD, otherwise known as metabolic-dysfunction associated fatty liver disease, MAFLD) and/or
30 non-alcoholic steatohepatitis (NASH, otherwise known as metabolic dysfunction-associated steatohepatitis, MASH);
- (vii) prevention and/or treatment of cognitive impairment, such as that caused by Alzheimer's disease;
- (viii) the prevention and/or treatment of chronic kidney disease;

(ix) the prevention and/or treatment of obstructive sleep apnoea.

In some embodiments the indication is (i). In some embodiments the indication is (ii). In a still further particular aspect the indication is (iii). In some embodiments the indication is (iv). In some embodiments the indication is (v). In some embodiments the indication is (vi). In some embodiments the indication is (vii). In some embodiments the indication is (viii). In some embodiments the indication is (ix). In some embodiments the indication is type 2 diabetes. In some embodiments the indication is overweight or obesity.

The term "treatment", as used herein, refers to the medical therapy of any human or other vertebrate subject in need thereof. Said subject is expected to have undergone physical examination by a medical practitioner, or a veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said specific treatment is beneficial to the health of said human or other vertebrate. The timing and purpose of said treatment may vary from one individual to another, according to the status quo of the subject's health. Thus, said treatment may be prophylactic (preventive), palliative, symptomatic and/or curative.

In some embodiments the indication is (i) and (iii). In some embodiments the indication is (ii) and (iii).

The World Health Organisation (WHO) defines overweight and obesity as being the abnormal or excessive accumulation of body fat that present a risk to an individual's overall health. Generally, all subjects suffering from obesity are also considered to be suffering from overweight. The subject suffering from obesity may be a human being, such as an adult human or a paediatric human, wherein "paediatric human" includes the infant, the child and the adolescents. Alternatively to overweight, also the term pre-obesity is used in this field. The WHO considers body mass index (BMI) to be the most convenient population-level measure of overweight and obesity. Body mass index (BMI) is a measure of body fat based on height and weight. The formula for calculation is $BMI = \text{weight in kilograms (kg)} / \text{height in meters squared (m}^2\text{)}$.

For adults, the WHO defines overweight, and obesity as follows: overweight means having a BMI greater than or equal to 25; obesity means having a BMI greater than or equal to 30.

For children, the WHO considers age when defining overweight and obesity. For children under the age of five, overweight means having a weight-for-height greater than two standard deviations above the WHO Child Growth Standards median; and obesity means having a weight-for-height greater than three standard deviations above the WHO Child

Growth Standards median. Overweight and obesity are defined as follows for children aged five to nineteen: overweight means having a BMI-for-age that is greater than one standard deviation above the WHO Growth Reference median; and obesity means having a BMI-for-age that is greater than two standard deviations above the WHO Growth Reference median.

5 Nonetheless, the diagnostic criteria for underweight, the normal range, pre-obesity/overweight and obesity can differ between countries/populations, as illustrated in Table 16 below for adults.

10 *Table 16: Definitions of underweight, the normal range, pre-obesity/overweight and obesity in adults*

Nutritional status	BMI (kg/m ²)				
	International/ European/ US classification	Chinese population	Japanese classification	Taiwanese classification	Korean classification
Underweight	<18.5	<18.5	<18.5	<18.5	
Normal weight	>=18.5 and <25	>=18.5 and <23	>=18.5 and <25	>=18.5 and <24	
Overweight (pre-obesity)	>=25 and <30	>=23 and <28		>=24 and <27	>=23 and <25
Obesity	>=30	>=28	>=25	>=27	>=25
Obesity class I	>=30 and <35		>=25 and <30	>=27 and <30	>=25 and <30
Obesity class II	>=35.0 and <40		>=30 and <35	>=30 and <35	>=30 and <35
Obesity class III	>=40		>=35 and <40	>=35	>=35
Obesity class IV			>=40		

Guidelines for the Asian population were published by Misra A et al. J Assoc Physicians India. 2009; 57:163-70. Guidelines for the Chinese population were issued in the 2006 edition of the *Guidelines for Prevention and Control of Overweight and Obesity in Chinese Adults*, compiled by the Chinese Working Group on Obesity. Guidelines for the Japanese population were issued, in 2016, by the Japanese Society for the Study of Obesity

(JASSO) in *Guidelines for the management of obesity disease*. Guidelines for the Taiwanese population were issued by the Taiwanese government's Health Promotion Administration (HPA), Ministry of Health and Welfare in 2023, in the 2nd edition of its "*Evidence-Based Guideline on Adult Obesity Prevention and Management*".

5 In some embodiments the subject suffering from obesity is human, such as an adult human or a paediatric human (including infants, children, and adolescents). A human subject suffering from obesity thus may have a BMI of 25 or more, or 27 or more, or 28 or more, or 30 or more; this subject may also be referred to as being obese. The obesity may be class I, class II, class III or class IV obesity (as defined in Table 16). In some embodiments the
10 human subject suffering from obesity may have a BMI of ≥ 35 or a BMI in the range of ≥ 30 to < 40 . In some embodiments the obesity is severe obesity or morbid obesity, wherein the human subject may have a BMI of ≥ 40 .

 In some embodiments the invention relates to a method for treatment or prevention of overweight, optionally in the presence of at least one weight-related co-morbidity. In one
15 embodiment the GLP-1-/GIP-/amylin-receptor triple agonist as disclosed herein is for use in the treatment of a subject with an initial body mass index (BMI) of 25 or more, 27 or more, or 28 or more, or 30 or more; optionally in the presence of at least one weight-related co-morbidity.

 In some embodiments the invention relates to use of the formulation for treatment or
20 prevention of overweight, optionally in the presence of at least one weight-related co-morbidity. In some embodiments the subject suffering from overweight is human, such as an adult human or a paediatric human (including infants, children, and adolescents). In some embodiments an adult human subject suffering from overweight may have a BMI of 23 or more, or 24 or more, or 25 or more, or 27 or more. In some embodiments a human subject
25 suffering from overweight has a BMI in the range of 24 to < 27 , in the range of 24 to < 28 , in the range of 25 to < 30 or in the range of 27 to < 30 . In some embodiments the weight-related co-morbidity is selected from the group consisting of hypertension, dysglycaemia (prediabetes or type 2 diabetes), dyslipidaemia, high cholesterol, cardiovascular disease and obstructive sleep apnoea.

30 In some embodiments, the tri-agonist as disclosed herein relates to a method for weight management. In some embodiments, the tri-agonist as disclosed herein relates to a method for reduction of appetite. In some embodiments, the tri-agonist as disclosed herein relates to a method for reduction of food intake. In some embodiments, the tri-agonist as disclosed herein relates to a method of preventing or treating overweight in a subject.

The term "reduction of body weight" may include treatment or prevention of obesity and/or overweight.

Administration of the compound disclosed herein may be as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management in an adult subject suffering from obesity, i.e. with an initial body mass index (BMI) of 25 or more, or 27 or more, or 28 or more, or 30 or more; or in an adult subject suffering from overweight, i.e. with an initial body mass index (BMI) of 23 or more, or 24 or more, or 25 or more, or 27 or more; optionally in the presence of at least one weight-related co-morbidity (e.g., hypertension, dysglycaemia (prediabetes or type 2 diabetes), dyslipidaemia, high cholesterol, cardiovascular disease or obstructive sleep apnoea).

Methods of production

The compounds disclosed herein 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* and mammalian BHK or CHO. Specific examples of methods of preparing the disclosed compounds are included in the examples.

A further aspect of the invention relates to a method for preparing the peptides described herein. In an embodiment, the method for preparing a compound as described herein comprises a step of solid phase peptide synthesis. The protraction moiety may be introduced sequentially as part of the solid phase peptide synthesis or produced separately and attached via the alanine or lysine residue after peptide synthesis.

Particular embodiments

1. A GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

5 Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide comprising a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

10 wherein the amino acid at position X₂ represents Aib;

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide and a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III).

2. The GLP-1/GIP-/amylin-receptor tri-agonist according to embodiment 11, wherein peptide Z1 comprises an amino acid sequence which has at least 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identity to Formula II (SEQ ID NO: 1), and peptide Z2 comprises an amino acid sequence which has at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identity relative to Formula III (SEQ ID NO: 2).

3. A GLP-1/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

30 wherein the amino acid at position X₂ represents Aib,
and

Z1 comprises or consists of an amino acid sequence according to Formula X (SEQ ID NO: 161):

$$X_{21}X_{22}X_{23}GTFTSDYSX_{24}LLEEX_{25}AAX_{26}EFIX_{27}WLX_{28}X_{29}GGPSX_{30}X_{31} \quad (X),$$

35 wherein

X₂₁ represents His (H) or Tyr (Y),

X₂₂ represents Aib,

X₂₃ represents Glu (E) or His (H),

X₂₄ represents Ile (I) or Lys (K),

5 X₂₅ represents Gln (Q) or Ile (I),

X₂₆ represents Arg (R) or Gln (Q),

X₂₇ represents Ala (A), Glu (E) or Gln (Q),

X₂₈ represents Leu (L) or I (Ile),

X₂₉ represents Ala (A) or Gln (Q),

10 X₃₀ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₃₁ represents Gly (G), Glu (E) or Lys (K);

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

15 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula XII (SEQ ID NO:164):

20 AX₃₂X₃₃LSTAX₃₄X₃₅X₃₆RLSAX₃₇LHX₃₈LX₃₉X₄₀X₄₁PX₄₂TETGSGX₄₃P (XII),

wherein

X₃₂ represents Gly (G) or Ser (S),

X₃₃ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₃₄ represents Ala (A) or Gln (Q),

X₃₅ represents Gln (Q), Leu (L) or Thr (T),

25 X₃₆ represents Ala (A), Gly (G) or Gln (Q),

X₃₇ represents Glu (E) or Lys (K),

X₃₈ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₃₉ represents Ala (A) or Lys (K),

30 X₄₀ represents Asp (D) or Thr (T),

X₄₁ represents Leu (L) or Glu (E),

X₄₂ represents Arg (R) or Lys (K),

X₄₃ represents Ala (A) or Ser (S).

4. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 1 to embodiment 3, wherein the one lysine (Lys, K) residue is present in the peptide Z1 or in the peptide Z2.
5. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 1 to 4, wherein the GLP-1-/GIP-/amylin-receptor triple agonist does not comprise a cysteine (Cys, C) residue.
6. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiments 1 to 5, wherein the GLP-1-/GIP-/amylin-receptor triple agonist does not comprise a disulfide bridge.
7. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiments 1 to 6, wherein:
- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):
$$\text{YX}_2\text{EGTFTSDYSILLEEQAAAREFIEWLLAGGPSKG} \quad (\text{II}),$$

wherein the amino acid at position X_2 represents Aib,
and
Z1 comprises or consists of an amino acid sequence according to Formula IIa (SEQ ID NO: 3):
$$\text{X}_1\text{X}_2\text{X}_3\text{GTFTSDYSILLEEQAAAREFIEWLLAGGPSX}_4\text{X}_5 \quad (\text{IIa}),$$

wherein
 X_1 represents His (H) or Tyr (Y),
 X_2 represents Aib,
 X_3 represents Glu (E) or His (H),
 X_4 represents Arg (R), Gly (G), Lys (K) or Ser (S),
 X_5 represents Gly (G) or Lys (K);
 - L1 is a peptide linker; and
 - Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):
$$\text{ASELSTAALGRLSAELHELATLPR TETGSGSP} \quad (\text{III}),$$

and
Z2 comprises or consists of an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

5 X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

10 X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

15 8. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiments 1 to 6, wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

20 wherein the amino acid at position X₂ represents Aib, and

Z1 comprises or consists of an amino acid sequence according to Formula Xa (SEQ ID NO: 162):

YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

25 wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K),

X₅₃ represents Arg (R) or Gln (Q),

X₅₄ represents Ala (A), Glu (E) or Gln (Q),

30 X₅₅ represents Leu (L) or I (Ile),

X₅₆ represents Ala (A) or Gln (Q),

X₅₇ represents Gly (G) or Glu (E);

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

35

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

5 AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

wherein

X₅₈ represents Gly (G) or Ser (S),

X₅₉ represents Gln (Q), Glu (E), or His (H),

X₆₀ represents Ala (A) or Gln (Q),

10 X₆₁ represents Leu (L) or Thr (T),

X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E).

15 9. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 8, wherein the peptide linker L1 comprises 1 to 14, 1 to 10, 4 to 10 or 9 to 10 amino acid residues.

20 10. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 9, wherein the peptide linker L1 comprises 1 to 14 amino acid residues selected from the group consisting of Ala (A), Glu (E), Gln (Q), Gly (G), Leu (L), Phe (F), Pro (P), Ser (S), Thr (T), Val (V), Asn (N).

25 11. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 10, wherein the peptide linker L1 comprises or consists of the amino acid sequence according to Formula IV:

X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄ (IV),

wherein

X₁ represents Ala (A), Glu (E), Gly (G),

30 X₂ represents Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P) or is absent,

X₃ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or absent,

X₄ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or absent,

X₅ represents Glu (E), Gly (G), Pro (P), Ser (S), Thr (T) or is absent,

X₆ represents Glu (E), Gly (G), Leu (L), Gln (Q) or is absent,

35 X₇ represents Ala (A), Gln (Q), Glu (E), Gly (G), Phe (F) or is absent,

X₈ represents Ala (A), Gln (Q), Glu (E), Gly (G), Thr (T), Pro (P), Val (V) or is absent,

X₉ represents Glu (E), Asn (N), Pro (P), Thr (T) or is absent,

X₁₀ represents Ala (A), Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P), Ser (S), Val (V) or is absent,

5 X₁₁ represents Ala (A) or is absent,

X₁₂ represents Gln (Q) or is absent,

X₁₃ represents Thr (T) or is absent,

X₁₄ represents Leu (L) or is absent.

10 12. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 11,

wherein the peptide linker L1 comprises or consists of the amino acid sequence according to Formula IV:

X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄ (IV),

15 which is selected from the group consisting of A, E, G, AE, AG, GE, APPE (SEQ ID NO: 125), GGGE (SEQ ID NO: 126), AGQAPG (SEQ ID NO: 127), APPPSGGG (SEQ ID NO: 128), APPPSGGGE (SEQ ID NO: 129), APPPSGGGG (SEQ ID NO:

130), ALAQTALAQT (SEQ ID NO: 131), ALAQTALFVNQ (SEQ ID NO: 132),

ALAQTALGTNE (SEQ ID NO: 133), ALQAPGQAPG (SEQ ID NO: 134),

20 ALQAPGQAPL (SEQ ID NO: 135), AGQAPGQAPG (SEQ ID NO: 136),

AGQAPGQAPL (SEQ ID NO: 137), GGEGGGEGE (SEQ ID NO: 138),

GQAPGQAPGE (SEQ ID NO: 139), GQEPGQEPGE (SEQ ID NO: 140),

APPPSLAQTALAQT (SEQ ID NO: 141), AGGGG (SEQ ID NO: 142), AGEAPGQAPG

(SEQ ID NO: 143), AGEAPGEAPG (SEQ ID NO: 144), AGQAPGQAPA (SEQ ID NO:

25 145), AGQAPGQAPE (SEQ ID NO: 146), AGQAPGQAPP (SEQ ID NO: 147),

AGQAPGQAPS (SEQ ID NO: 148), AGQAPGQAPV (SEQ ID NO: 149),

EGQAPGQAPG (SEQ ID NO: 150), AGQEPGQAPG (SEQ ID NO: 151),

AGQAEGQAPG (SEQ ID NO: 152), AGQAPEQAPG (SEQ ID NO: 153),

AGQAPGEAPG (SEQ ID NO: 154), AGQAPGQEPG (SEQ ID NO: 155),

30 AGQAPGQAEQ (SEQ ID NO: 156), AGQEPGQEPG (SEQ ID NO: 157),

AGQAPGQAP (SEQ ID NO: 158) and AGQAPGEAPL (SEQ ID NO: 159).

13. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 12,

wherein the amino acid sequence according to Formula IV:

35 X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄ (IV)

is selected from the group consisting of E, GE, APPPSGGGE (SEQ ID NO: 129), AGQAPGQAPG (SEQ ID NO: 136) AGQAPGQAPL (SEQ ID NO: 137), and AGQAPGEAPG (SEQ ID NO: 154).

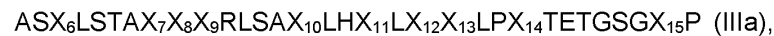
- 5 14. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 13, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



wherein

- 10 X_1 represents His (H) or Tyr (Y),
 X_2 represents Aib,
 X_3 represents Glu (E) or His (H),
 X_4 represents Arg (R), Gly (G) or Ser (S),
 X_5 represents Gly (G); and

- 15 wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

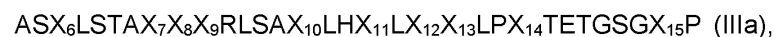


wherein

- 20 X_6 represents Gln (Q), Glu (E), His (H) or Lys (K),
 X_7 represents Ala (A) or Gln (Q),
 X_8 represents Gln (Q), Leu (L) or Thr (T),
 X_9 represents Ala (A), Gly (G) or Gln (Q),
 X_{10} represents Glu (E) or Lys (K),
 X_{11} represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
25 X_{12} represents Ala (A) or Lys (K),
 X_{13} represents Asp (D) or Thr (T),
 X_{14} represents Arg (R) or Lys (K),
 X_{15} represents Ala (A) or Ser (S).

- 30 15. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 14,

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



- 35 wherein

- X₆ represents Lys (K),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
5 X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
X₁₂ represents Ala (A),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R),
10 X₁₅ represents Ala (A) or Ser (S);
or
X₆ represents Gln (Q), Glu (E), or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
15 X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Lys (K),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
X₁₂ represents Ala (A),
X₁₃ represents Asp (D) or Thr (T),
20 X₁₄ represents Arg (R),
X₁₅ represents Ala (A) or Ser (S);
or
X₆ represents Gln (Q), Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
25 X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Lys (K),
X₁₂ represents Ala (A),
30 X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R),
X₁₅ represents Ala (A) or Ser (S);
or
X₆ represents Gln (Q), Glu (E) or His (H),
35 X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
5 X₁₂ represents Lys (K),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R),
X₁₅ represents Ala (A) or Ser (S);

or

10 X₆ represents Gln (Q), Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
15 X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Thr (T) or Tyr (Y),
X₁₂ represents Ala (A),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Lys (K),
X₁₅ represents Ala (A) or Ser (S).

20

16. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 15, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):



25

wherein

X₁X₂X₃ represents YAibE (Tyr-Aib-Glu) or HAibH (His-Aib-His)
X₄ represents Arg (R), Gly (G) or Ser (S),
X₅ represents Gly (G).

30

17. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 16, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula V (SEQ ID NO: 6):



wherein

35

X₂ represents Aib,

X₄ is Arg (R) or Ser (S).

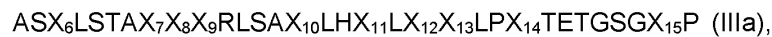
18. The GLP-1-/GIP-/amylin-receptor tri-agonist agonist according to any one of
embodiments 1 to 7 and 9 to 15,
5 wherein the peptide Z1 comprises or consists of the amino acid sequence according
to Formula IIa (SEQ ID NO: 3):



wherein

- X₁ represents His (H) or Tyr (Y),
10 X₂ represents Aib,
X₃ represents Glu (E) or His (H),
X₄ represents Arg (R), Gly (G) or Ser (S),
X₅ represents Gly (G); and

- wherein the peptide Z2 comprises or consists of the amino acid sequence according
15 to Formula IIIa (SEQ ID NO: 4):



wherein

- X₆ represents Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
20 X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E) or Lys (K),
X₁₁ represents Arg (R), Gln (Q) or Lys (K),
X₁₂ represents Ala (A) or Lys (K),
25 X₁₃ represents Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ala (A) or Ser (S).

19. The GLP-1-/GIP-/amylin-receptor tri-agonist agonist according to any one of
30 embodiments 1 to 7, 9 to 15 or embodiment 18,
wherein the peptide Z1 comprises or consists of the amino acid sequence according
to Formula IIa (SEQ ID NO: 3):



wherein

- 35 X₁ represents His (H) or Tyr (Y),

X₂ represents Aib,
X₃ represents Glu (E) or His (H),
X₄ represents Arg (R), Gly (G) or Ser (S),
X₅ represents Gly (G); and

5 wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Glu (E) or His (H),
10 X₇ represents Ala (A) or Gln (Q),
X₈ represents Gln (Q), Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Lys (K),
15 X₁₂ represents Ala (A),
X₁₃ represents Thr (T),
X₁₄ represents Arg (R),
X₁₅ represents Ala (A) or Ser (S).

20 20. The GLP-1-/GIP-/amylin-receptor tri-agonist agonist according to any one of embodiments 9 to 15, 18 and 19,
wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

25 wherein

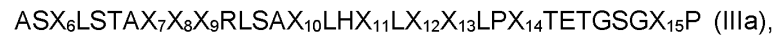
X₁ represents His (H) or Tyr (Y),
X₂ represents Aib,
X₃ represents Glu (E) or His (H),
X₄ represents Arg (R), Gly (G) or Ser (S),
30 X₅ represents Gly (G); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula VIII (SEQ ID NO: 9):

ASHLSTAQTQRLSAELHKLATLPRTETGSGSP (VIII).

21. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 20, wherein the amino acid sequence of the peptide Z1—L1—Z2 comprises or consists of
- 5 YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
SAELHKLATLPRTETGSGSP (SEQ ID NO: 62), or
YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRLS
AELHKLATLPRTETGSGSP (SEQ ID NO: 68), or
YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSRGEASHLSTAQTQRLSAELHKLATLP
RTETGSGSP (SEQ ID NO: 78), or
- 10 YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRLS
AELHKLATLPRTETGSGSP (SEQ ID NO: 87);
wherein in each amino acid sequence X represents Aib.
22. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiments 16 or 17,
15 wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula VII (SEQ ID NO: 8):
- ASHLSTAQTQRLSAKLHRLATLPRTETGSGSP (VII).
23. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 22,
20 wherein the amino acid sequence of the peptide Z1—L1—Z2 comprises or consists of
- YXEGTFTSDYSILLEEQAAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
SAKLHRLATLPRTETGSGSP (SEQ ID NO: 65).
24. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1
25 to 7, 9 to 15 and 18,
wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):
- $X_1X_2X_3$ GTFTSDYSILLEEQAAAREFIEWLLAGGPSX₄X₅ (IIa),
- 30 wherein
- X₁ represents His (H) or Tyr (Y),
X₂ represents Aib,
X₃ represents Glu (E) or His (H),
X₄ represents Arg (R), Gly (G) or Ser (S),
35 X₅ represents Gly (G); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

- 5 X₆ represents His (H),
 X₇ represents Ala (A) or Gln (Q),
 X₈ represents Leu (L) or Thr (T),
 X₉ represents Ala (A), Gly (G) or Gln (Q),
 X₁₀ represents Glu (E),
10 X₁₁ represents Arg (R) or Gln (Q),
 X₁₂ represents Lys (K),
 X₁₃ represents Thr (T),
 X₁₄ represents Arg (R),
 X₁₅ represents Ser (S).

15

25. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7, 9 to 15 and 18, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

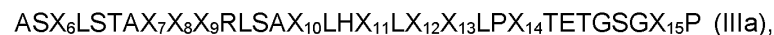


20

wherein

- X₁ represents His (H) or Tyr (Y),
 X₂ represents Aib,
 X₃ represents Glu (E) or His (H),
 X₄ represents Arg (R), Gly (G) or Ser (S),
25 X₅ represents Gly (G); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



wherein

- 30 X₆ represents His (H),
 X₇ represents Ala (A) or Gln (Q),
 X₈ represents Leu (L) or Thr (T),
 X₉ represents Ala (A), Gly (G) or Gln (Q),
 X₁₀ represents Glu (E),
35 X₁₁ represents Arg (R),

X₁₂ represents Ala (A),
X₁₃ represents Thr (T),
X₁₄ represents Lys (K),
X₁₅ represents Ser (S).

5

26. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7, 9 to 15 and 18, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

10

wherein

X₁ represents His (H) or Tyr (Y),
X₂ represents Aib,
X₃ represents Glu (E) or His (H),
X₄ represents Arg (R), Gly (G) or Ser (S),
X₅ represents Gly (G); and

15

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

20

X₆ represents His (H),
X₇ represents Gln (Q),
X₈ represents Thr (T),
X₉ represents Gln (Q),
X₁₀ represents Glu (E),
X₁₁ represents Arg (R) or Lys (K),
X₁₂ represents Ala (A) or Lys (K),
X₁₃ represents Thr (T),
X₁₄ represents Arg (R) or Lys (K),
X₁₅ represents Ser (S).

25

30

27. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 13,

wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

35

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

wherein

X₁ represents His (H),

X₂ represents Aib,

X₃ represents His (H),

5 X₄ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₅ represents Gly (G); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

10 wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

15 X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

20 X₁₅ represents Ala (A) or Ser (S).

28. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 27,

wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula IIa (SEQ ID NO: 3):

25 X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

wherein

X₁ represents His,

X₂ represents Aib,

X₃ represents His (H),

30 X₄ represents Lys (K),

X₅ represents Gly (G); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

35 wherein

X₆ represents Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Leu (L) or Thr (T),
X₉ represents Ala (A), Gly (G) or Gln (Q),
5 X₁₀ represents Glu (E),
X₁₁ represents Arg (R), Gln (Q) or Glu (E),
X₁₂ represents Ala (A),
X₁₃ represents Asp (D) or Thr (T),
X₁₄ represents Arg (R),
10 X₁₅ represents Ser (S).

29. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 27 or embodiment 28,

wherein in Formula IIIa (SEQ ID NO: 4)

15 X₆ represents Glu (E) or His (H),
X₇ represents Ala (A) or Gln (Q),
X₈ represents Leu (L) or Thr (T),
X₉ represents Ala (A) or Gly (G),
X₁₀ represents Glu (E),
20 X₁₁ represents Arg (R), Gln (Q) or Glu (E),
X₁₂ represents Ala (A),
X₁₃ represents Thr (T),
X₁₄ represents Arg (R),
X₁₅ represents Ser (S).

25

30. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 27 to 29,

wherein the amino acid sequence of the peptide Z1—L1—Z2 comprises or consists of

30 HXHGTFTSDYSILLEEQAAREFIEWLLAGGPSKGAPPPSGGGEASHLSTAQTARLSA
ELHQLATLPRTETGSGSP (SEQ ID NO: 111), wherein X represents Aib.

31. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 13,

wherein the peptide Z1—L1—Z2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs 20 to 124, wherein X represents Aib.

- 5 32. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 19, 20, 24, 25, 26, 27, or 28, wherein X₁ represents His (H).
33. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 18, 19, 20, 24, 25, or 26, wherein X₁ represents Tyr (Y).
- 10 34. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 19, 20, 24, 25, or 26, wherein X₃ represents Glu (E).
35. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 18, 19, 20, 24, 25, 26, 27, or 28, wherein X₃ represents His (H).
- 15 36. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 16, 17, 18, 19, 20, 24, 25, 26, or 27, wherein X₄ represents Arg (R).
- 20 37. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 16, 18, 19, 20, 24, 25, 26, or 27, wherein X₄ represents Gly (G).
38. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 27, or 28, wherein X₄ represents Lys (K).
- 25 39. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 16, 17, 18, 20, 20, 24, 25, 26 or 27 wherein X₄ represents Ser (S).
40. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 16, 18, 19, 20, 24, 25, 26, 27 or 28, wherein X₅ represents Gly (G).
- 30 41. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X₅ represents Lys (K).

42. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_6 represents Gln (Q).
- 5 43. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 27, 28 or 29, wherein X_6 represents Glu (E).
44. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 16, 19, 24, 25, 26, 27, 28 or 29, wherein X_6 represents His (H).
- 10 45. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_6 represents Lys (K).
46. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 26, 27, 28 or 29, wherein X_7 represents Ala (A).
- 15 47. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 17, 19, 24, 25, 26, 27, 28 or 29, wherein X_7 represents Gln (Q).
48. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 20 14, 15, 18, 19, or 27, wherein X_8 represents Gln (Q).
49. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 27, 28 or 29, wherein X_8 represents Leu (L).
- 25 50. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 26, 27, 28 or 29, wherein X_8 represents Thr (T).
51. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 27, 28 or 29, wherein X_9 represents Ala (A).
- 30 52. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 26, 27, 28 or 29, wherein X_9 represents Gly (G).
53. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 35 14, 15, 18, 19, 24, 25, 26, 27 or 28, wherein X_9 represents Gln (Q).

54. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 25, 26, 27, 28 or 29, wherein X_{10} represents Glu (E).
- 5 55. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18 or 27, wherein X_{10} represents Lys (K).
56. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 24, 25, 26, 27, 28, or 29, wherein X_{11} represents Arg (R).
- 10 57. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 24, 27, 28, or 29, wherein X_{11} represents Gln (Q).
58. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 27, 28 or 29, wherein X_{11} represents Glu (E).
- 15 59. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_{11} represents Gly (G).
60. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_{11} represents His (H).
- 20 61. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 26, or 27, wherein X_{11} represents Lys (K).
- 25 62. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_{11} represents Thr (T).
63. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, or 27, wherein X_{11} represents Tyr (Y).
- 30 64. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 25, 26, 27, 28 or 29, wherein X_{12} represents Ala (A).

65. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 24, 26, or 27, wherein X_{12} represents Lys (K).
- 5 66. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 27, or 28, wherein X_{13} represents Asp (D).
67. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 16, 18, 24, 25, 26, 27, 28 or 29, wherein X_{13} represents Thr (T).
- 10 68. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 24, 26, 27, 28 or 29, wherein X_{14} represents Arg (R).
69. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 25, 26 or 27, wherein X_{14} represents Lys (K).
- 15 70. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19 or 27, wherein X_{15} represents Ala (A).
71. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 7, 14, 15, 18, 19, 23, 24, 25, 26, 27, 28 or 29 wherein X_{15} represents Ser (S).
- 20 72. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 8, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):
- 25 $YX_{51}EGTFTSDYSX_{52}LLEEIAAX_{53}EFIX_{54}WLX_{55}X_{56}GGPSSX_{57}$ (Xa),
wherein
 X_{51} represents Aib,
 X_{52} represents Ile (I) or Lys (K),
 X_{53} represents Arg (R),
30 X_{54} represents Glu (E),
 X_{55} represents Leu (L) or I (Ile),
 X_{56} represents Ala (A),
 X_{57} represents Gly (G) or Glu (E).

73. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 8 or embodiment 72, wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula XIIa (SEQ ID NO: 165):



5

wherein

 X_{58} represents Ser (S), X_{59} represents His (H), X_{60} represents Gln (Q), X_{61} represents Thr (T),

10

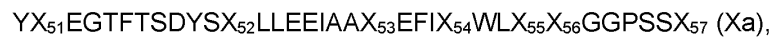
 X_{62} represents Gln (Q), X_{63} represents Lys (K), X_{64} represents Leu (L).

74. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the

15

embodiments 8, 72 or 73,

wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):



wherein

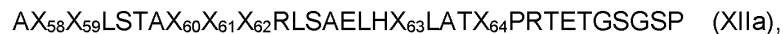
20

 X_{51} represents Aib, X_{52} represents Ile (I) or Lys (K), X_{53} represents Arg (R), X_{54} represents Glu (E), X_{55} represents Leu (L) or I (Ile),

25

 X_{56} represents Ala (A), X_{57} represents Gly (G) or Glu (E); and

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula XIIa (SEQ ID NO: 165):



30

wherein

 X_{58} represents Ser (S), X_{59} represents His (H), X_{60} represents Gln (Q), X_{61} represents Thr (T),

35

 X_{62} represents Gln (Q),

X₆₃ represents Lys (K),

X₆₄ represents Leu (L).

75. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the
5 embodiments 8 or 72 to 74,
 wherein the peptide Z1 comprises or consists of the amino acid sequence according
 to Formula Xa (SEQ ID NO: 162):

YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

 wherein

- 10 X₅₁ represents Aib,
 X₅₂ represents Ile (I) or Lys (K),
 X₅₃ represents Arg (R),
 X₅₄ represents Glu (E),
 X₅₅ represents Leu (L),
15 X₅₆ represents Ala (A),
 X₅₇ represents Gly (G).

76. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the
 embodiments 8 or 72 to 75,
20 wherein the peptide Z1 comprises or consists of the amino acid sequence according
 to Formula XI (SEQ ID NO: 163):

YX₅₁EGTFTSDYSX₅₂LLEEIAAREFIEWLLAGGPSSG (XI),

 wherein

- X₅₁ represents Aib,
25 X₅₂ represents Ile (I) or Lys (K).

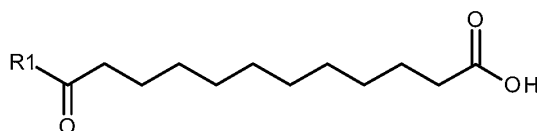
77. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the
 embodiments 8 or 72 to 77,
 wherein the peptide Z2 comprises or consists of the amino acid sequence according
30 to Formula XIII (SEQ ID NO: 166):

ASX₅₉LSTAQTQRLSAELHKLATLPRTETGSGSP (XIII),

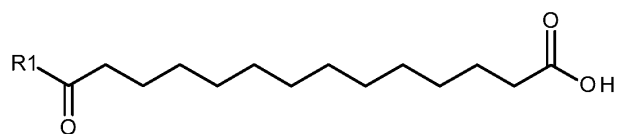
 wherein

 X₅₉ represents Glu (E) or His (H).

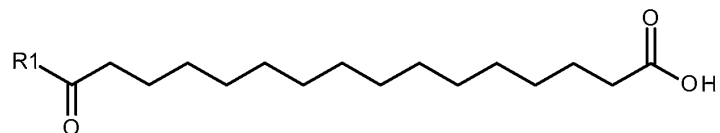
78. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 7 and 9 to 13,
wherein the peptide Z1—L1—Z2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs 170 to 242, wherein X represents Aib.
79. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments wherein the backbone of the peptide Z1—L1—Z2 comprises 66 to 80 amino acid residues.
80. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments wherein the backbone of the peptide Z1—L1—Z2 comprises 67, 68, 75 or 76 amino acid residues, preferably 76 amino acid residues.
81. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments wherein the peptide is a peptide derivative comprising a protraction moiety.
82. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety comprises a protractor P being a C₁₂-C₂₀ diacid.
83. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety comprise a protractor P selected from the group consisting of:



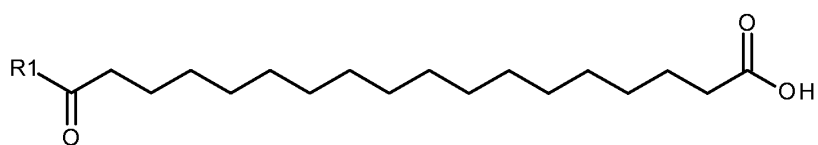
(Chem. 2);



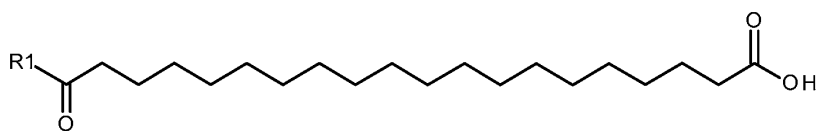
(Chem. 3);



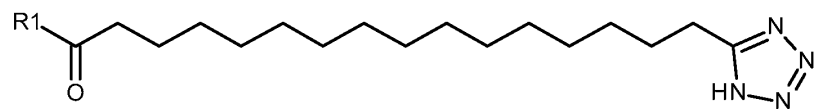
(Chem. 4);



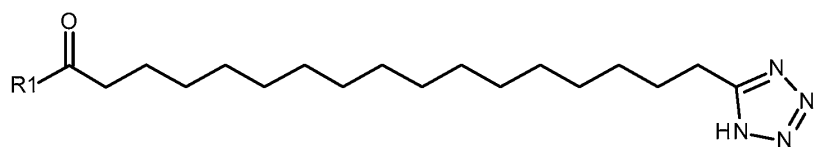
(Chem. 5);



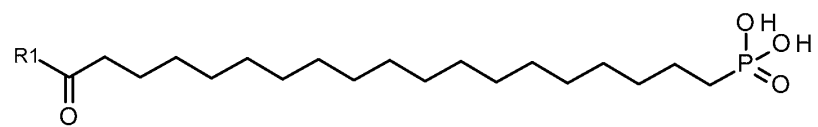
(Chem. 6);



(Chem. 7);



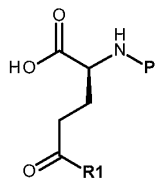
(Chem. 8); and



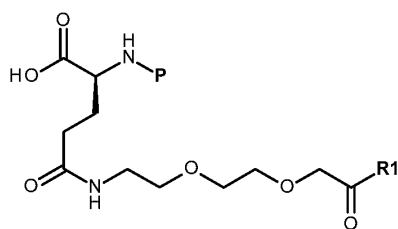
(Chem. 32).

84. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety comprises a protractor P selected from the group consisting of C₁₆ diacid, C₁₈ diacid, C₂₀ diacid, and C₁₉ phosphonic acid; preferably the protractor P is a C₁₈ diacid or a C₂₀ diacid.
85. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein the protraction moiety is attached to the epsilon position of the one lysine (Lys, K) residue.
86. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein the protraction moiety is attached to the epsilon position of the lysine (Lys, K) residue in the peptide Z1 or to the epsilon position of the lysine (Lys, K) residue in the peptide Z2.
87. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein the protraction moiety is attached to the epsilon position of the lysine (Lys, K) residue at position 12 or position 33 or position 34 of peptide Z1, preferably at position 12 or position 33 of peptide Z1.
88. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein the protraction moiety is attached to the epsilon position of the lysine (Lys, K) residue at positions 3, 15, 18, 20, or 24 of peptide Z2.
89. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein the protraction moiety is attached to the epsilon position of the lysine (Lys, K) residue at position 15 of peptide Z2 or at position 18 of peptide Z2.
90. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, wherein the peptide is a peptide derivative comprising a protraction

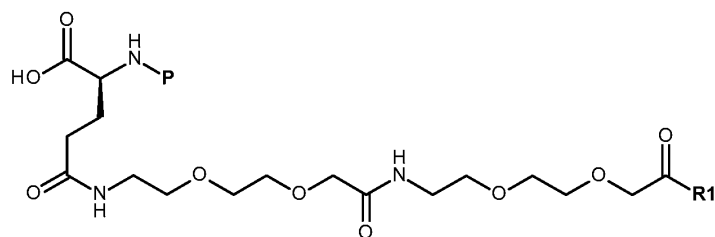
moiety, wherein said protraction moiety further comprises a linker L_P selected from the group consisting of:



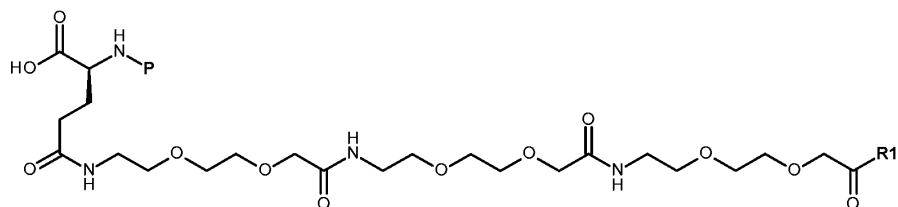
(Chem. 18);



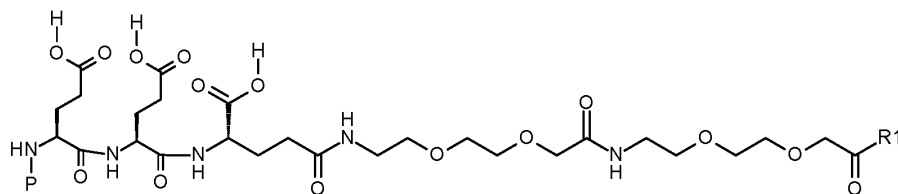
(Chem. 19);



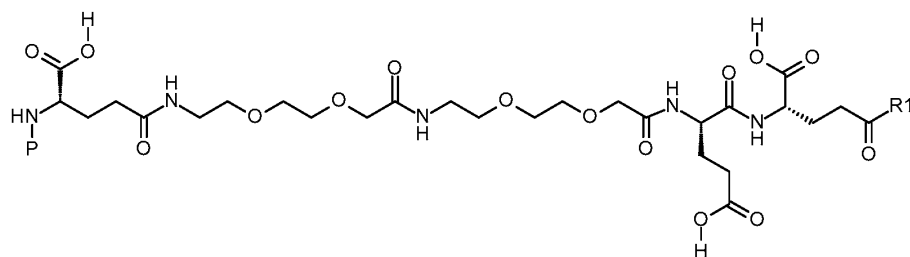
(Chem. 20);



(Chem. 21);



(Chem. 33); and



(Chem. 34).

91. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
embodiments, wherein the peptide is a peptide derivative comprising a protraction
moiety, wherein said protraction moiety comprises Chem. 20 or Chem. 21 as linker L_P
and Chem. 5 or Chem. 6 as protractor P.
92. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
embodiments, wherein the peptide is a peptide derivative comprising a protraction
moiety,
wherein said protraction moiety consists of (i) a linker L_P selected from the group
presented in Table 4 and (ii) a protractor P selected from the group presented in
Table 3, preferably said protraction moiety is selected from the group presented in
Table 5.
93. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
embodiments, wherein the peptide is a peptide derivative comprising a protraction
moiety, wherein said protraction moiety is a C₁₈ diacid (S) gamma-Glu 2xAdo fatty
acid moiety (Chem. 28) or a C₂₀ diacid (S) gamma-Glu 2xAdo fatty acid moiety
(Chem. 27).
94. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
embodiments,
wherein the peptide Z1 comprises or consists of the amino acid sequence according
to Formula IIa (SEQ ID NO: 3):
- $$X_1X_2X_3GTFTSDYSILLEEQAAREFIEWLLAGGPSX_4X_5 \quad (IIa),$$
- wherein
X₁ represents His (H) or Tyr (Y),
X₂ represents Aib,

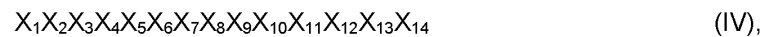
X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G) or Ser (S),

X₅ represents Gly (G);

wherein the peptide linker L1 comprises or consists of the amino acid sequence

5 according to Formula IV:

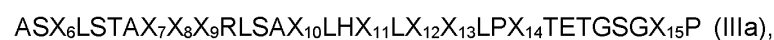


which is selected from the group consisting of E, GE, APPPSGGGE (SEQ ID NO:

129), AGQAPGQAPG (SEQ ID NO: 136) and AGQAPGQAPL (SEQ ID NO: 137);

wherein the peptide Z2 comprises or consists of the amino acid sequence according

10 to Formula IIIa (SEQ ID NO: 4):



wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

15 X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),

20 X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

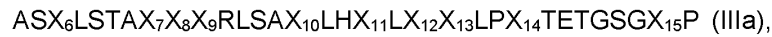
X₁₅ represents Ala (A) or Ser (S); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

25 95. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 94, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety comprises a protractor P being a C₁₂-C₂₀ diacid.

30 96. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 95, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety further comprises a linker L_P selected from the group presented in Table 4.

97. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 94 to 96, wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula IIIa (SEQ ID NO: 4):



5 wherein

X₆ represents Glu (E) or His (H),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

10 X₁₀ represents Glu (E),

X₁₁ represents Lys (K),

X₁₂ represents Ala (A),

X₁₃ represents Thr (T),

X₁₄ represents Arg (R),

15 X₁₅ represents Ala (A) or Ser (S).

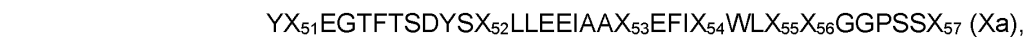
98. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 94 to 97, wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula VIII (SEQ ID NO: 9):



99. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 94 to 98, wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula VII (SEQ ID NO: 8):



100. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 1 to 93, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula Xa (SEQ ID NO: 162):



wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K),

35 X₅₃ represents Arg (R) or Gln (Q),

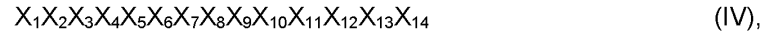
X₅₄ represents Ala (A), Glu (E) or Gln (Q),

X₅₅ represents Leu (L) or I (Ile),

X₅₆ represents Ala (A) or Gln (Q),

X₅₇ represents Gly (G) or Glu (E);

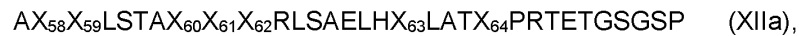
5 wherein the peptide linker L1 comprises or consists of the amino acid sequence according to Formula IV:



which is selected from the group consisting of E, AG, AGQAPGQAPG (SEQ ID NO: 136), AGQAPGQAPL (SEQ ID NO: 137), AGGGG (SEQ ID NO: 142),

10 AGEAPGQAPG (SEQ ID NO: 143), and AGQAPGEAPG (SEQ ID NO: 154);

wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula XIIa (SEQ ID NO: 165):



wherein

15 X₅₈ represents Gly (G) or Ser (S),

X₅₉ represents Gln (Q), Glu (E), or His (H),

X₆₀ represents Ala (A) or Gln (Q),

X₆₁ represents Leu (L) or Thr (T),

X₆₂ represents Ala (A), Gly (G) or Gln (Q),

20 X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E); and

wherein the peptide is a peptide derivative comprising a protraction moiety.

25 101. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 100, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety comprises a protractor P being a C₁₂-C₂₀ diacid.

30 102. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 101, wherein the peptide is a peptide derivative comprising a protraction moiety, wherein said protraction moiety further comprises a linker L_P selected from the group presented in Table 4.

35 103. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 100 to 102, wherein the peptide Z1 comprises or consists of the amino acid sequence according to Formula XI (SEQ ID NO: 163):

YX₅₁EGTFTSDYSX₅₂LLEEIAAREFIEWLLAGGPSSG (XI),

wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K).

5

104. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 100 to 103, wherein the peptide Z2 comprises or consists of the amino acid sequence according to Formula XIII (SEQ ID NO: 166):

ASX₅₉LSTAQTQRLSAELHKLATLPRTETGSGSP (XIII),

10

wherein

X₅₉ represents Glu (E) or His (H).

105. A GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

15

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

20

wherein the amino acid at position X₂ represents Aib,

and wherein

Z1 comprises or consists of an amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAREFIEWLLAGGPSX₄X₅ (IIa),

25

wherein

X₁ represents His (H) or Tyr (Y),

X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G), Lys (K) or Ser (S),

30

X₅ represents Gly (G) or Lys (K);

- L1 is a peptide linker comprising or consisting of 1 to 14, 1 to 10, 4 to 10 or 9 to 10 amino acid residues; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

35

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and
Z2 comprises or consists of an amino acid sequence according to Formula IIIa
(SEQ ID NO: 4):

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or
Tyr (Y),

X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S);

wherein the peptide is a peptide derivative comprising a protraction moiety, wherein
said protraction moiety comprises a protractor P being a C₁₆-C₂₀ diacid and further
comprises a linker L_P selected from the group presented in Table 4.

106. A GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to
Formula I:

Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to
Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

and wherein

Z1 comprises or consists of an amino acid sequence according to Formula Xa
(SEQ ID NO: 162):

YX₅₁EGTFTSDYSX₅₂LLEEIAAX₅₃EFIX₅₄WLX₅₅X₅₆GGPSSX₅₇ (Xa),

wherein

X₅₁ represents Aib,

X₅₂ represents Ile (I) or Lys (K),

X₅₃ represents Arg (R) or Gln (Q),

X₅₄ represents Ala (A), Glu (E) or Gln (Q),

X₅₅ represents Leu (L) or I (Ile),

X₅₆ represents Ala (A) or Gln (Q),

5 X₅₇ represents Gly (G) or Glu (E);

- L1 is a peptide linker comprising or consisting of 1 to 14, 1 to 10, 4 to 10 or 9 to 10 amino acid residues; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

10 ASELSTAALGRLS AELHELATLPRTETGSGSP (III),

and

Z2 comprises or consists of an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLS AELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

15 wherein

X₅₈ represents Gly (G) or Ser (S),

X₅₉ represents Gln (Q), Glu (E), or His (H),

X₆₀ represents Ala (A) or Gln (Q),

X₆₁ represents Leu (L) or Thr (T),

20 X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E);

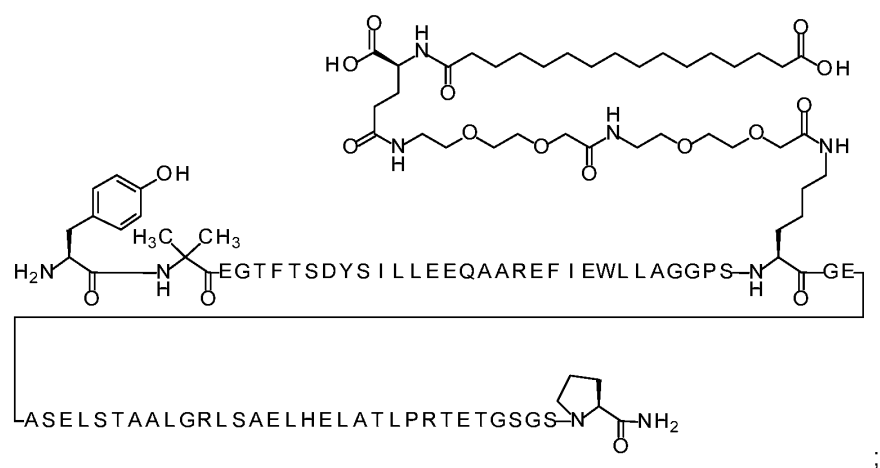
wherein the peptide is a peptide derivative comprising a protraction moiety,

wherein said protraction moiety comprises a protractor P being a C₁₆-C₂₀ diacid

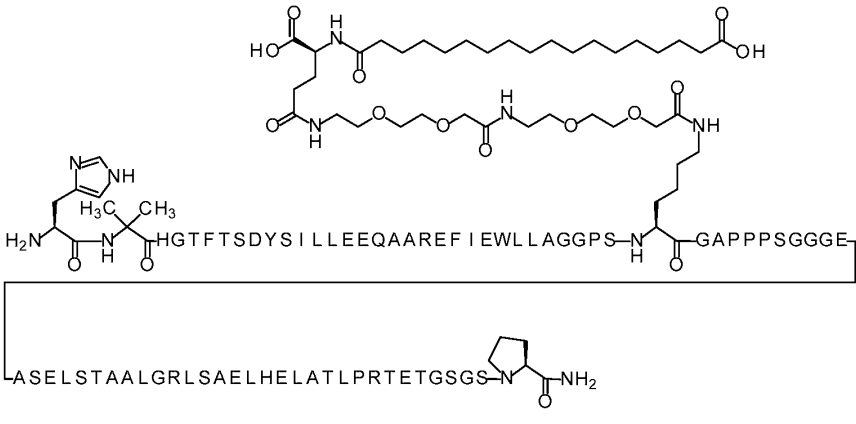
25 and further comprises a linker L_P selected from the group presented in Table 4.

107. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments selected from:

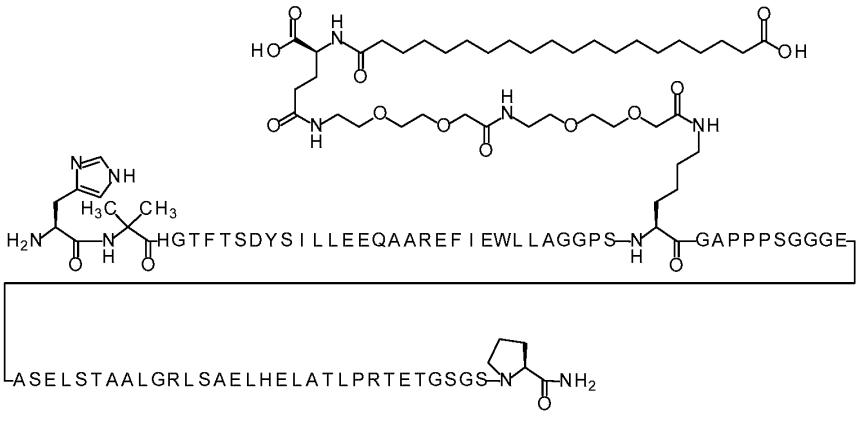
compound 11



compound 15

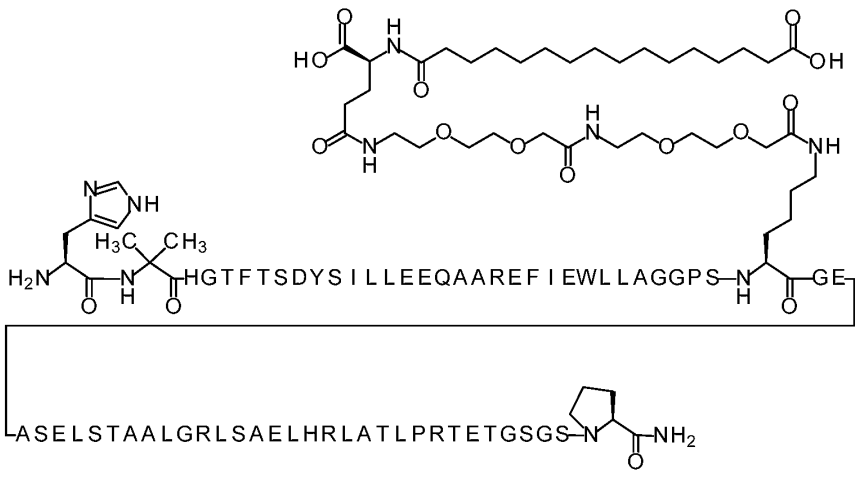


compound 16

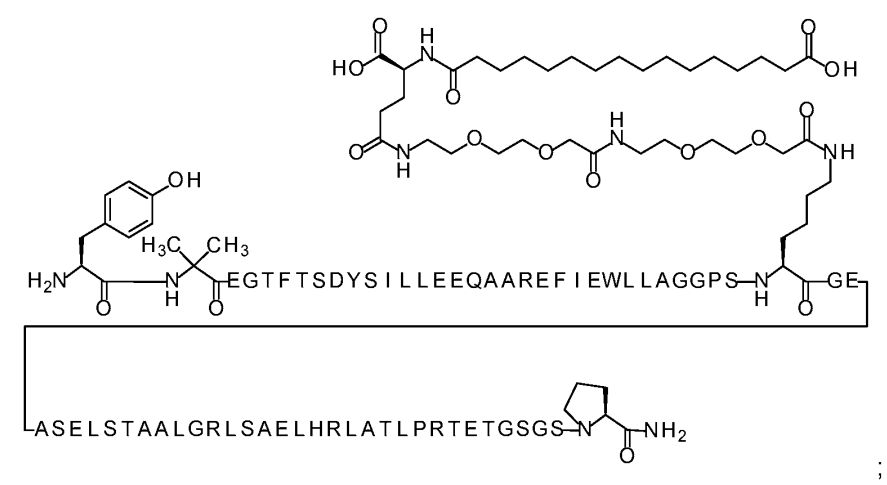


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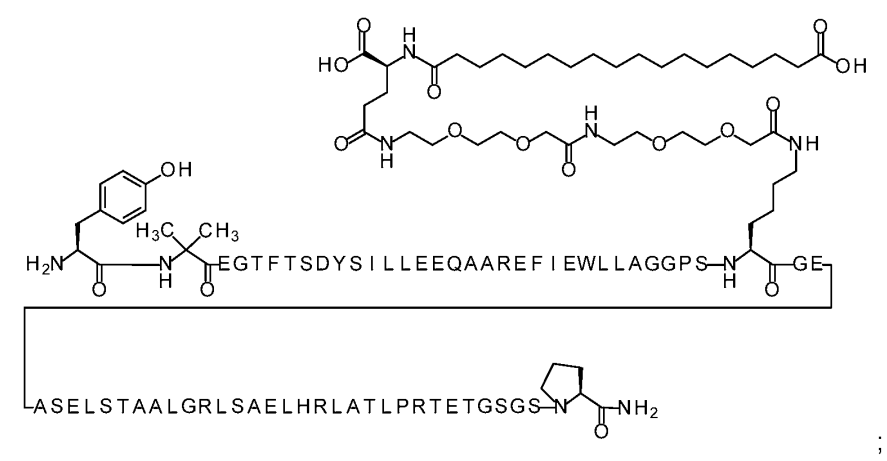
compound 17



compound 18

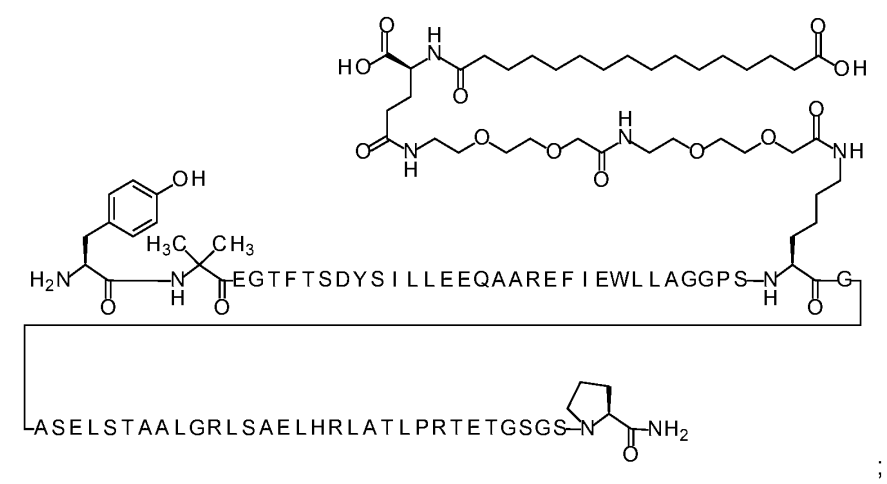


compound 19



5

compound 20

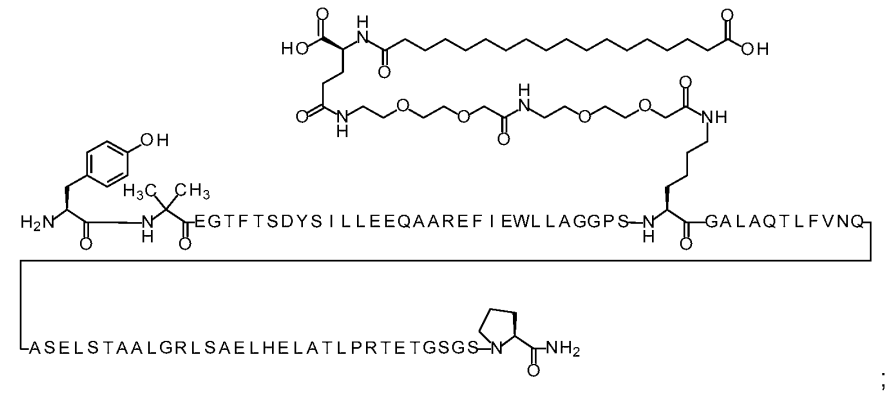


compound 22

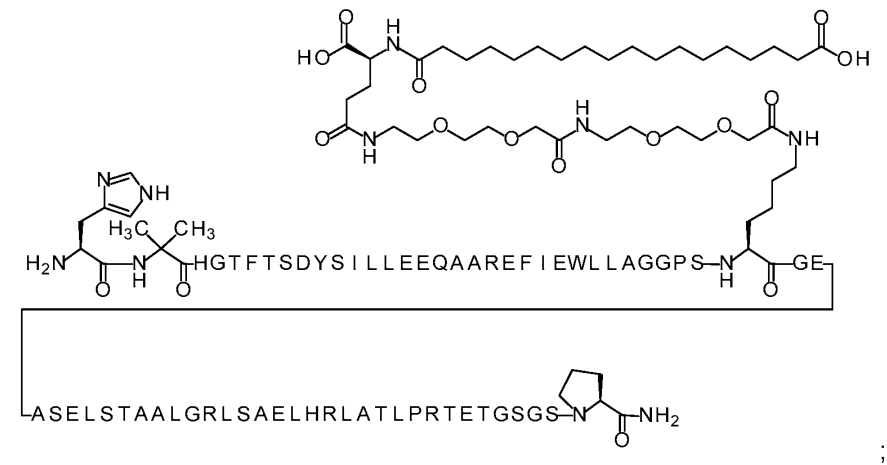


compound 23

compound 24

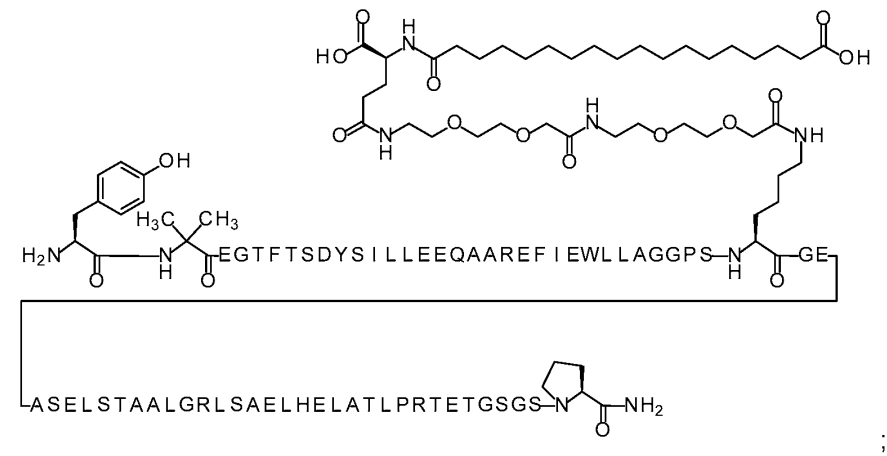


compound 25

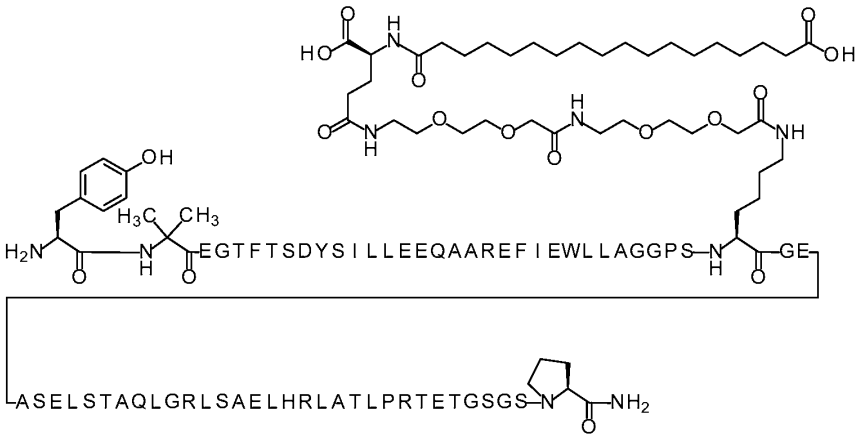


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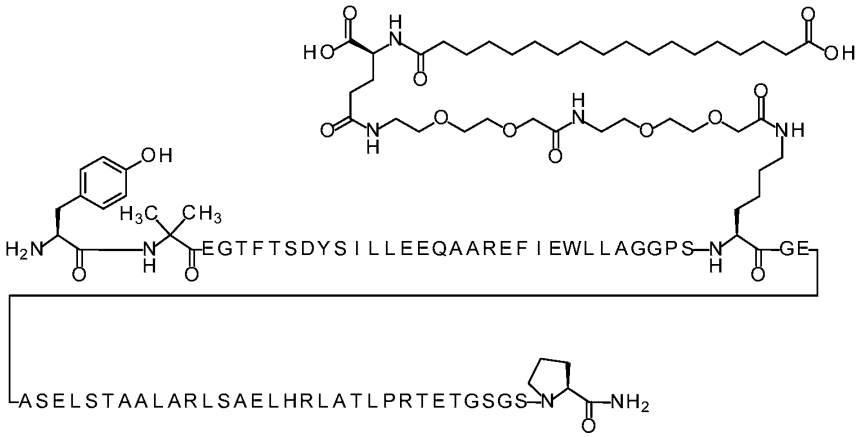
compound 26



compound 27

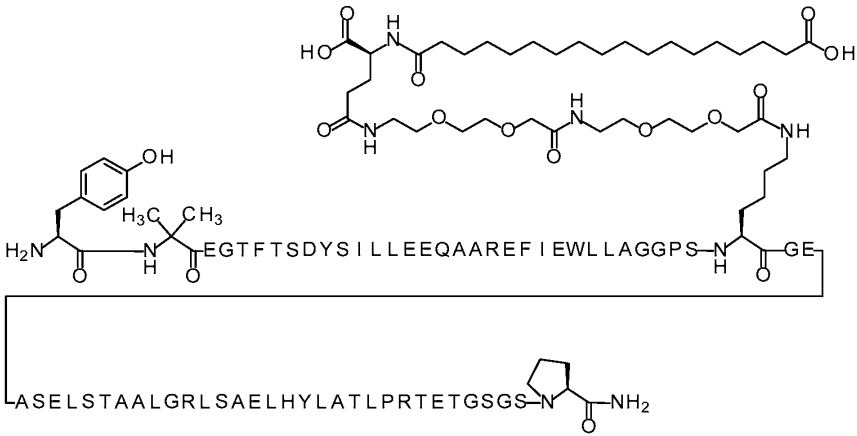


compound 28

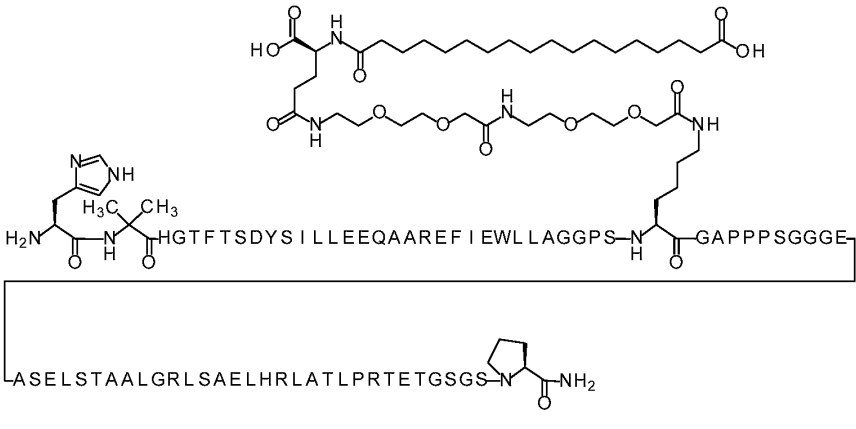


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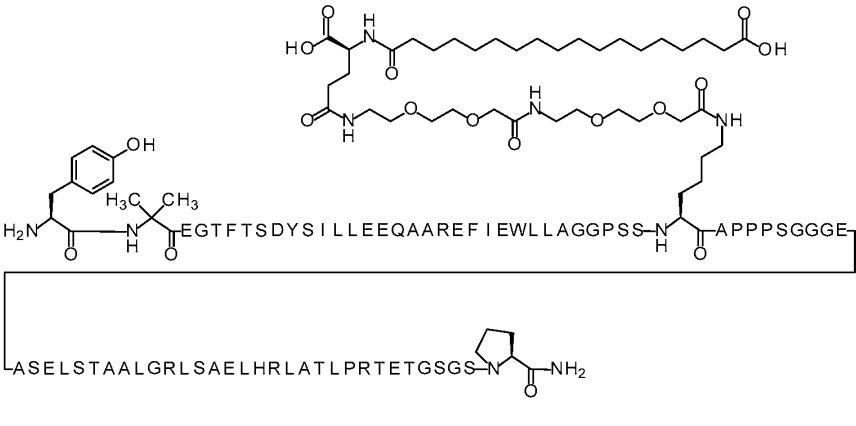
compound 29



compound 30

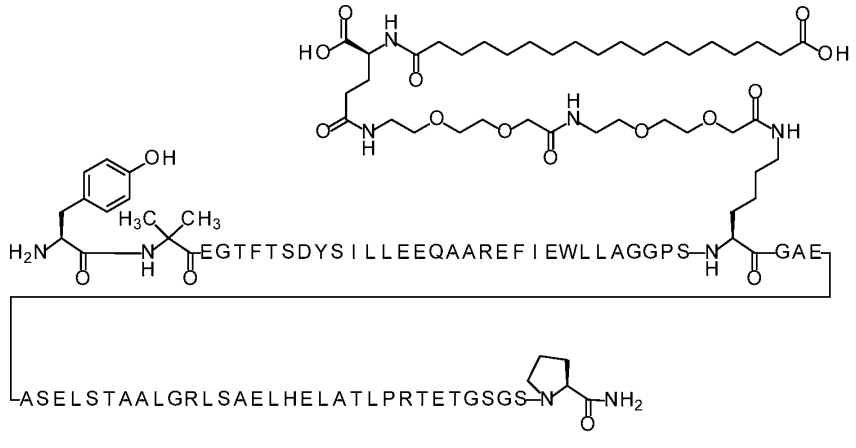


compound 31

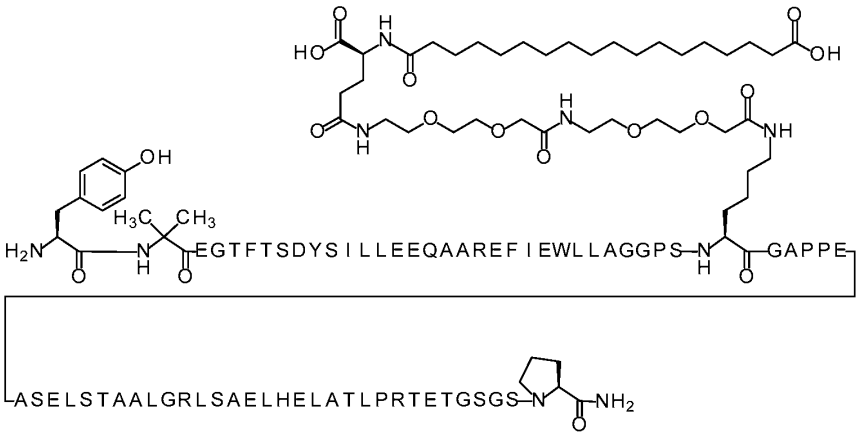


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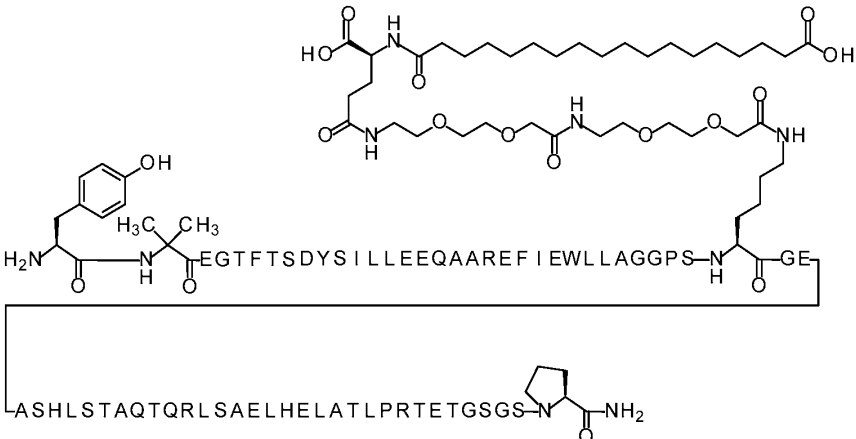
compound 32



compound 33

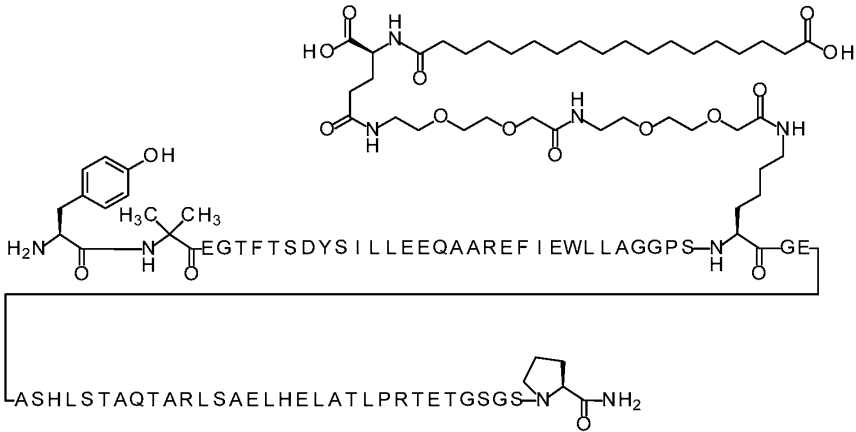


compound 34

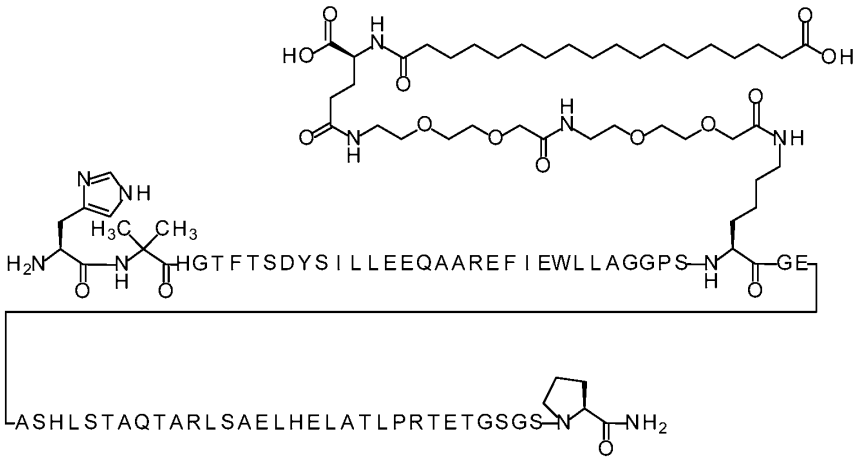


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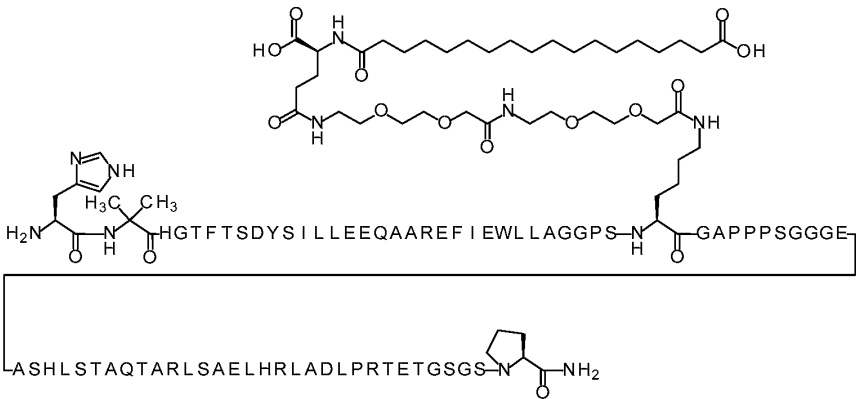
compound 35



compound 36

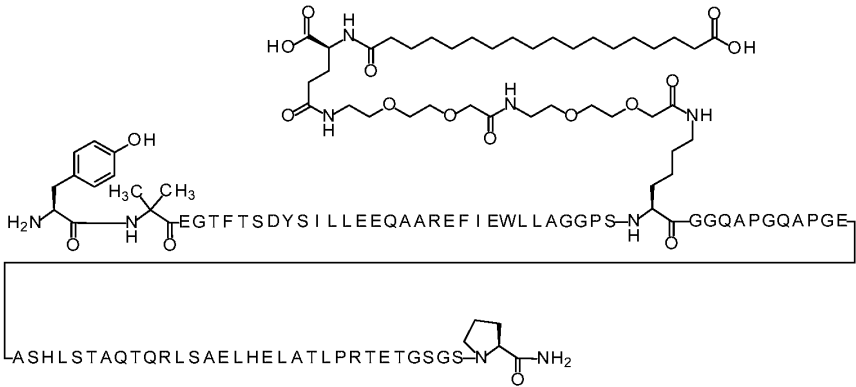


compound 37

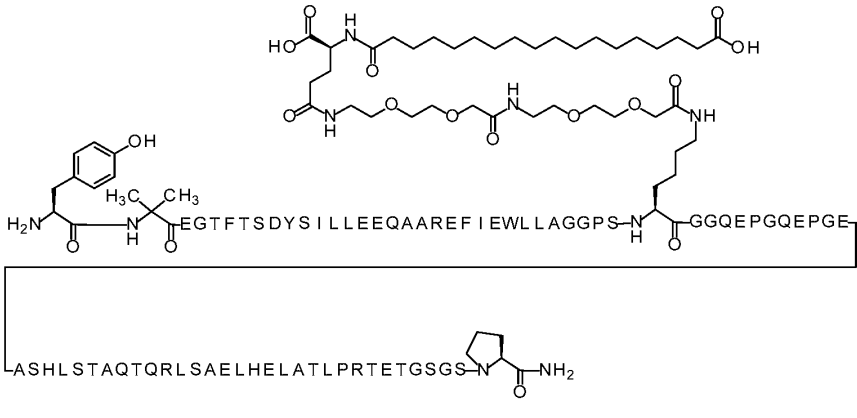


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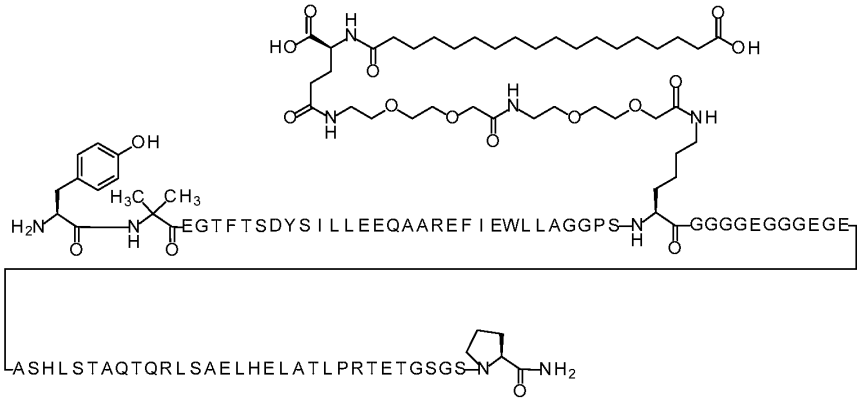
compound 38



compound 39

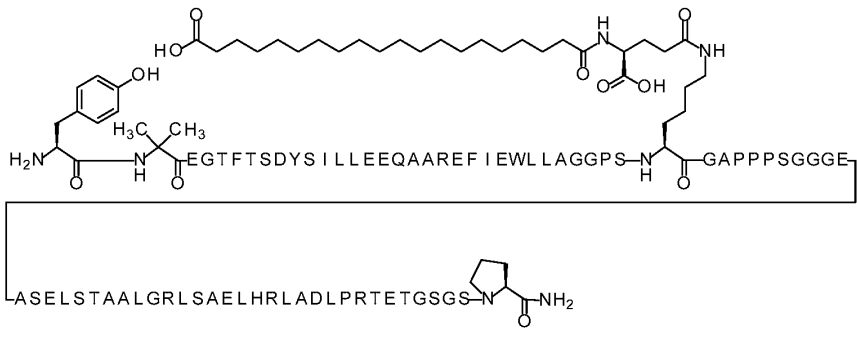


compound 40

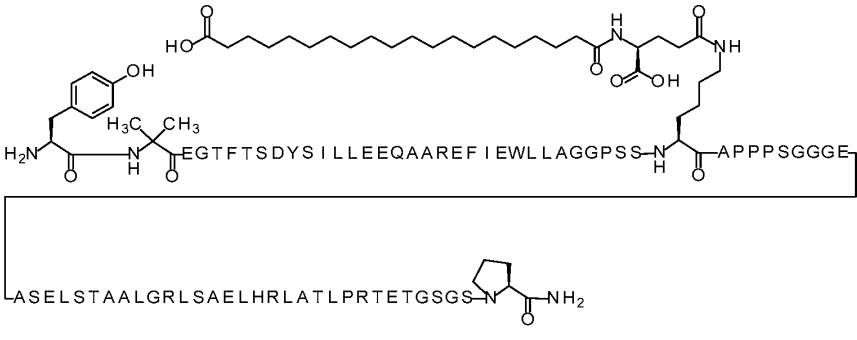


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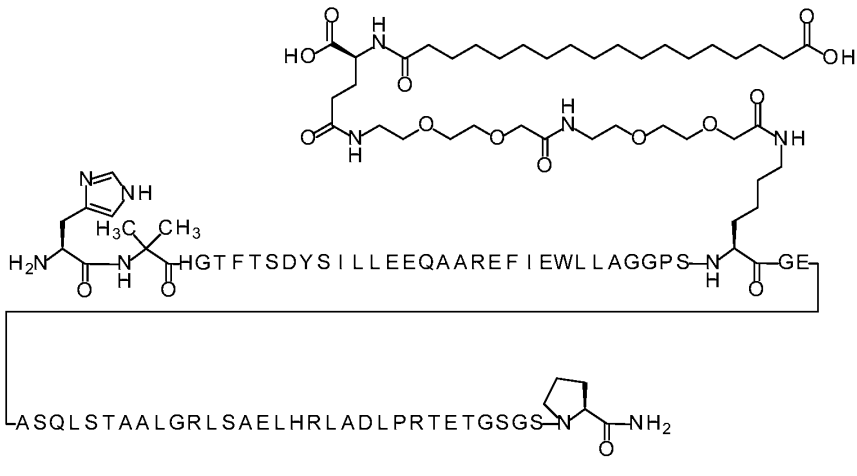
compound 41



compound 42

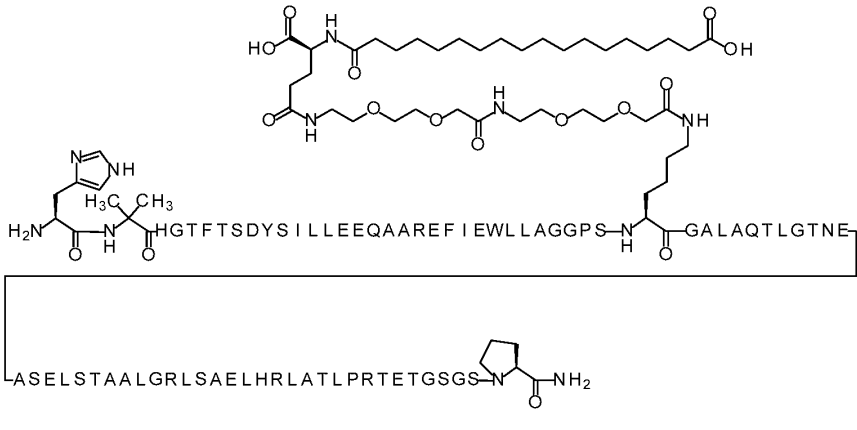


compound 43



5

compound 44



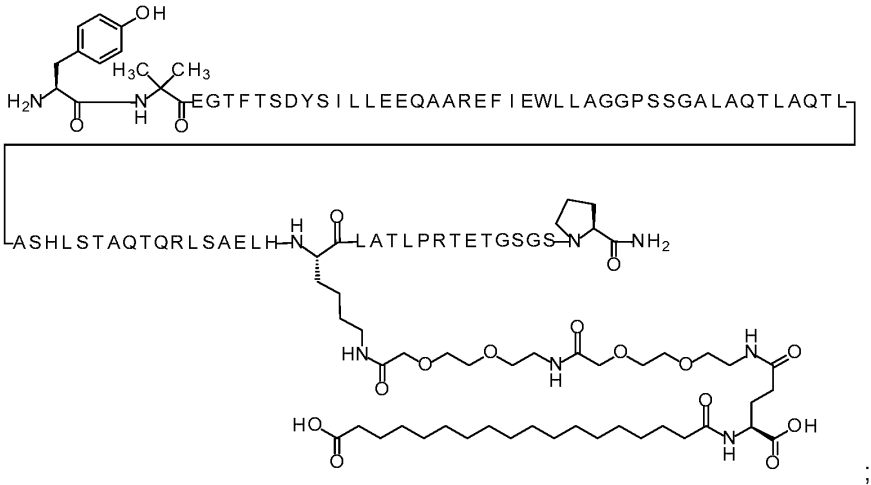


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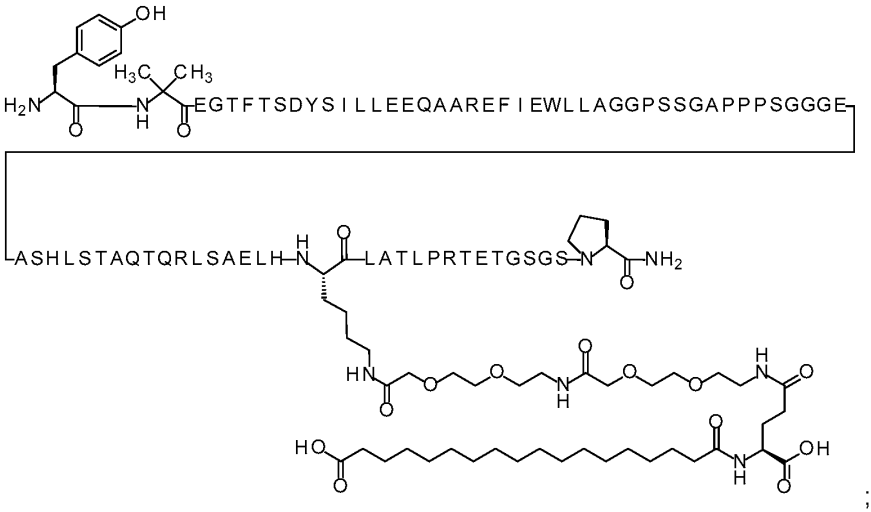
compound 47



compound 48



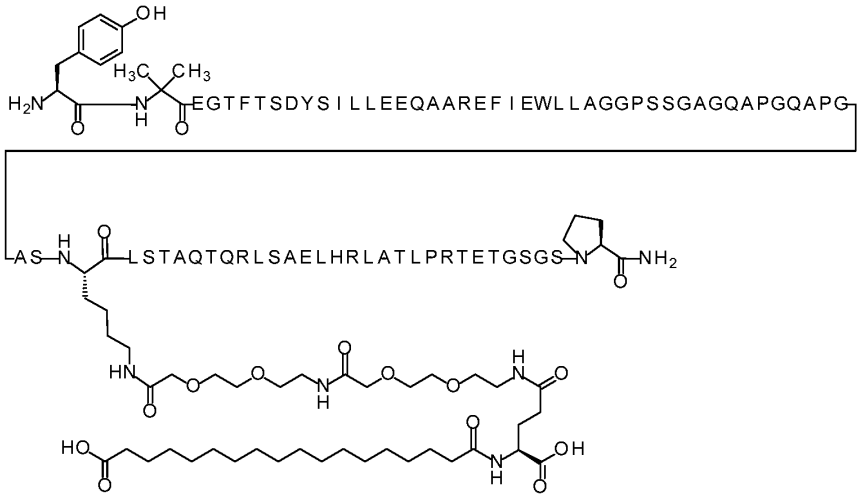
compound 49



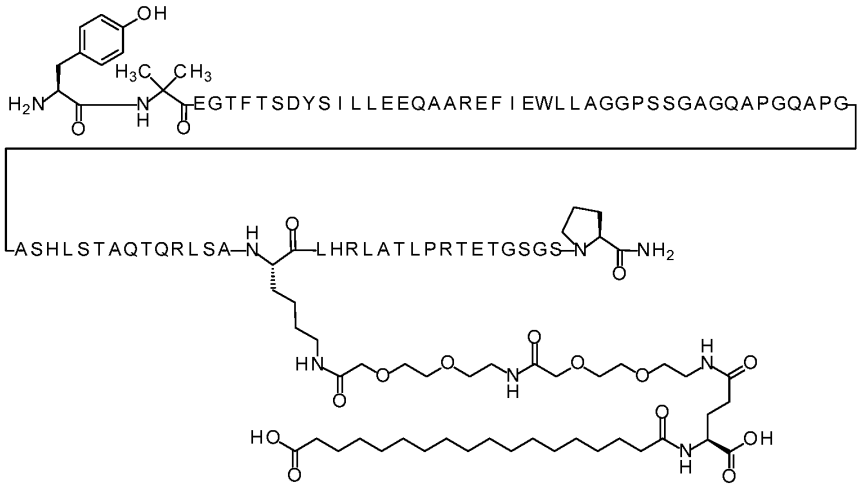
[illegible]

Chemical structure of the protein HGTFTSDYSILLEEQAAREFIEWLLAGGPSSGALAQT LAQTLAHLSTAQ TQRLSAELH. The structure shows a complex fold with a large alpha-helical domain and a smaller beta-sheet domain. The structure is labeled with residue numbers 1 through 100.

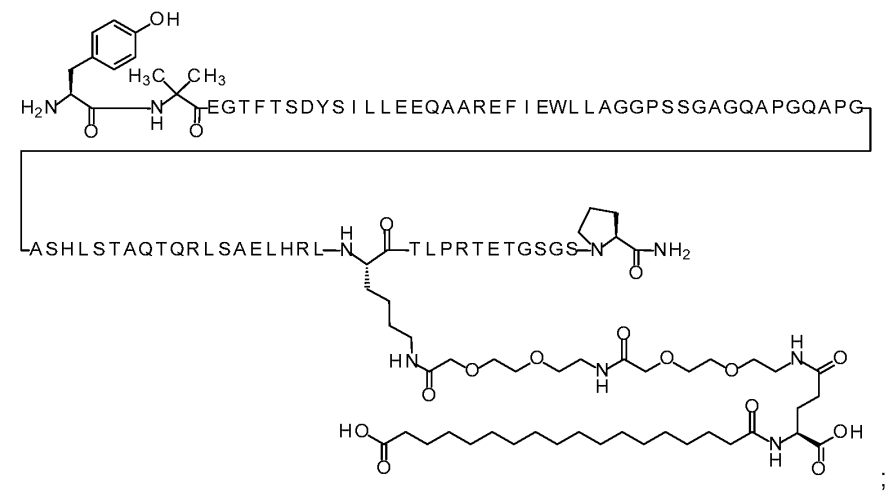
compound 54



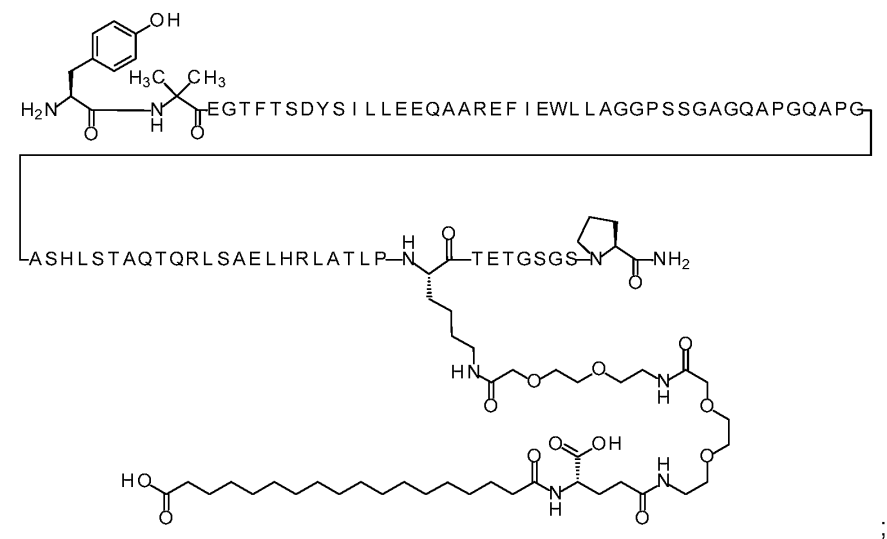
compound 55



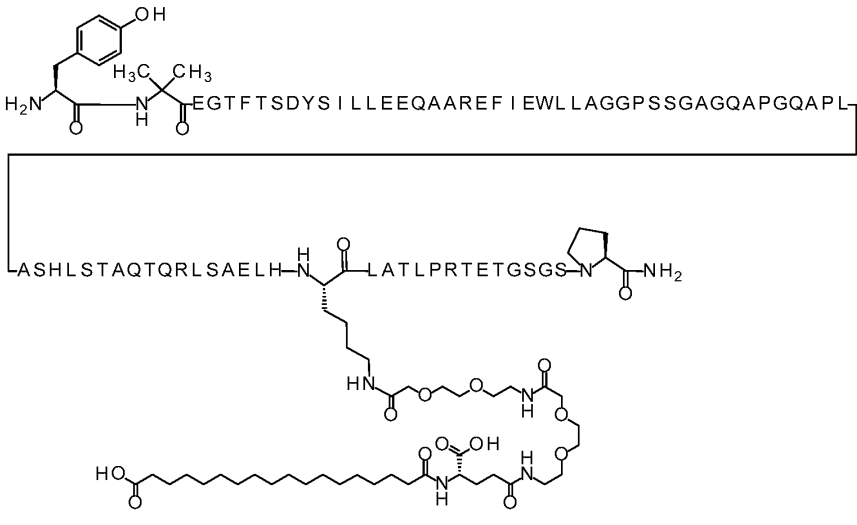
compound 56



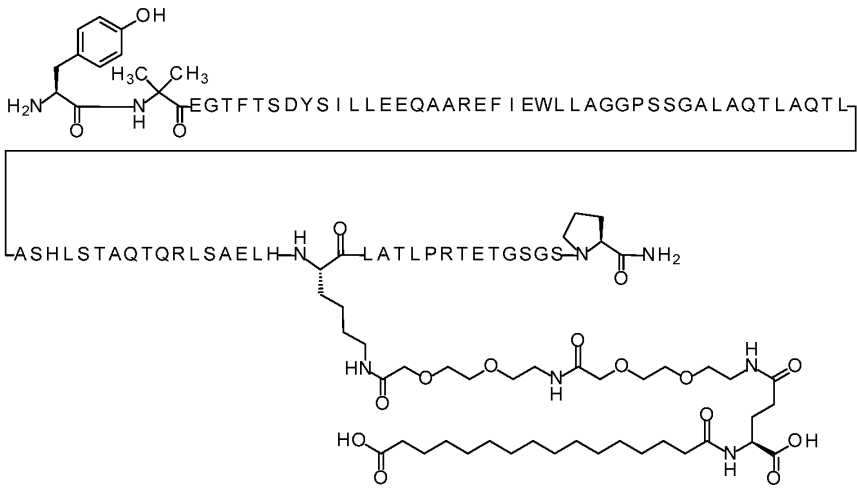
compound 57



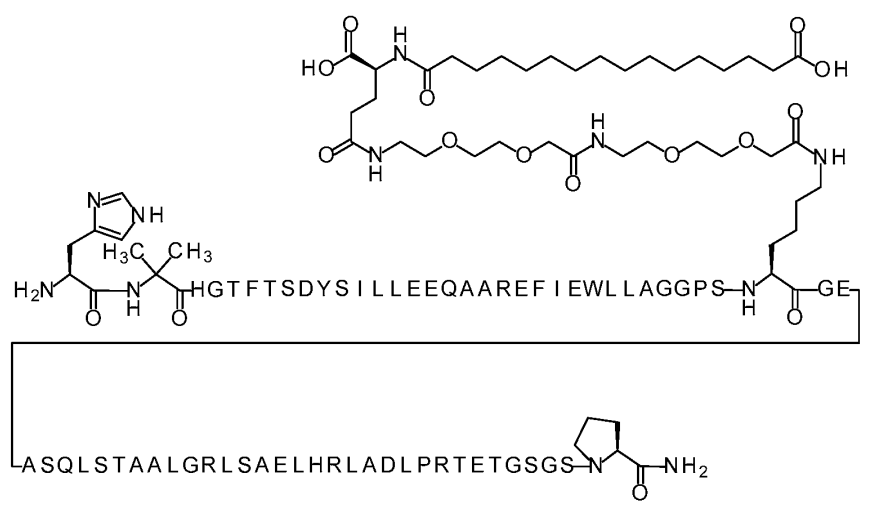
compound 58



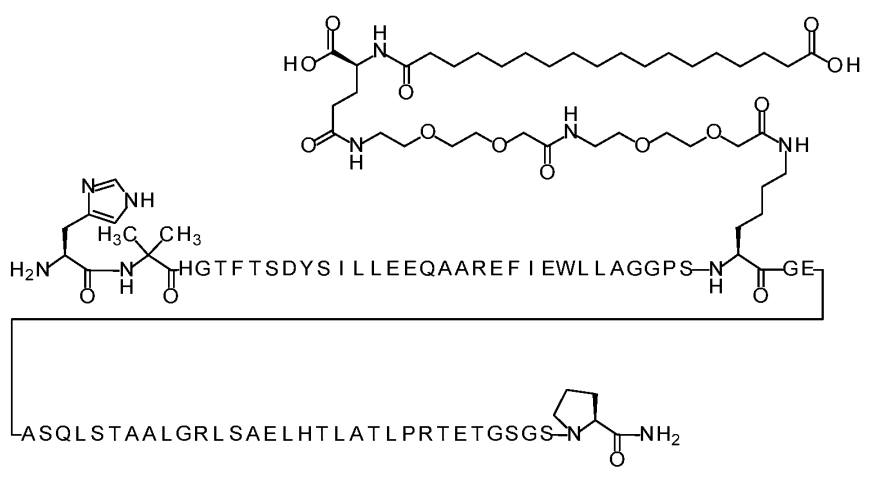
compound 59

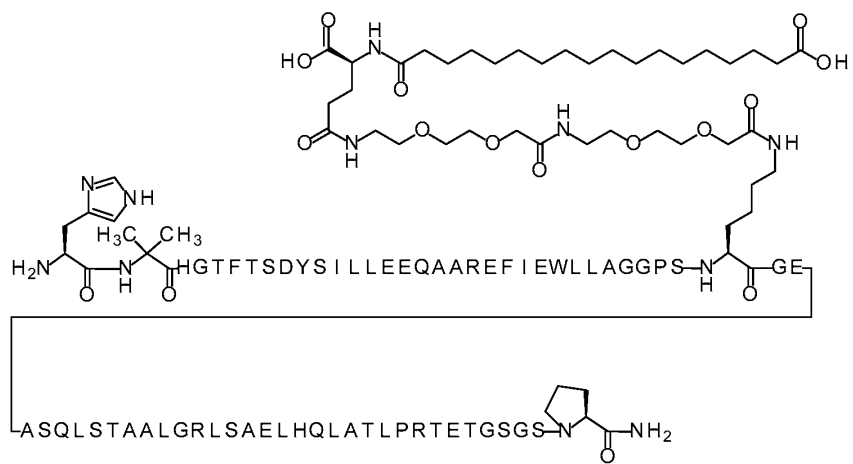


compound 62

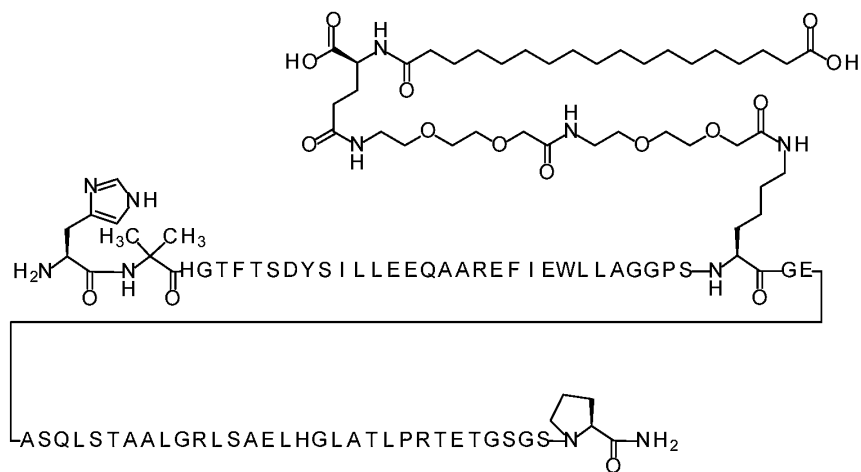


compound 63

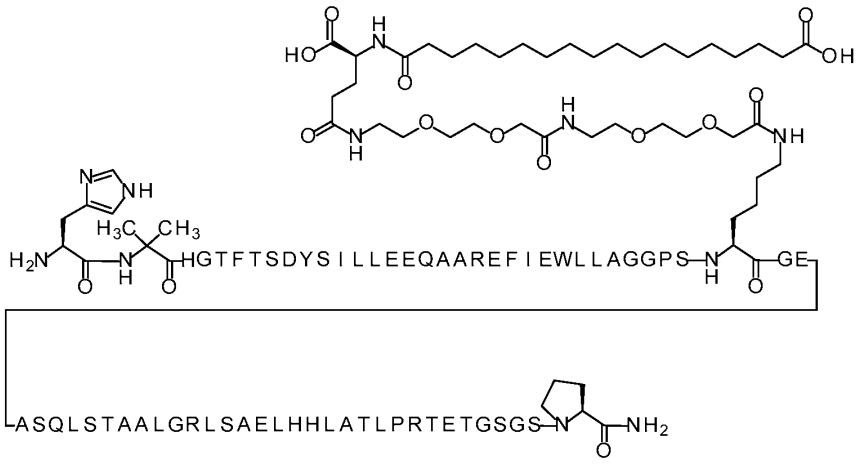




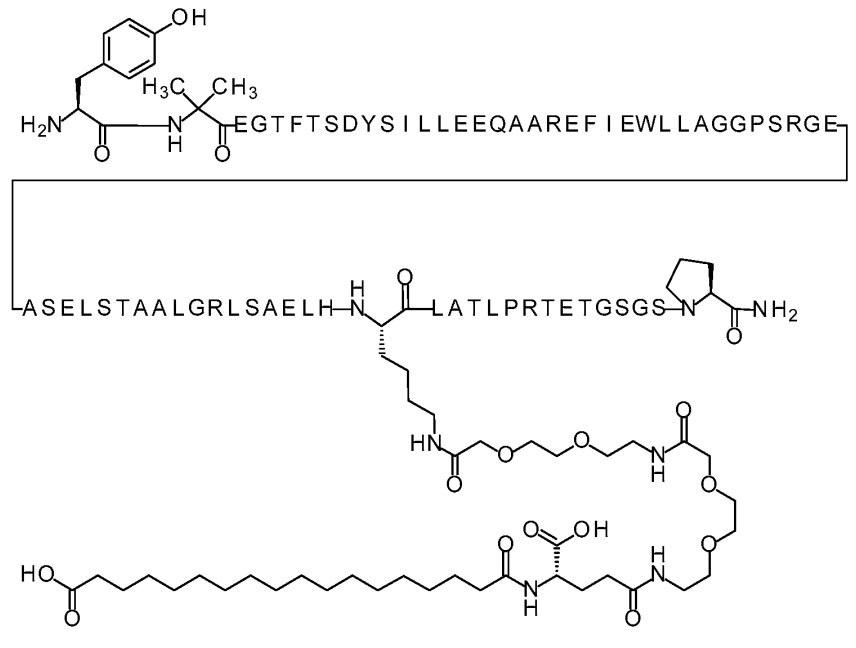
compound 65



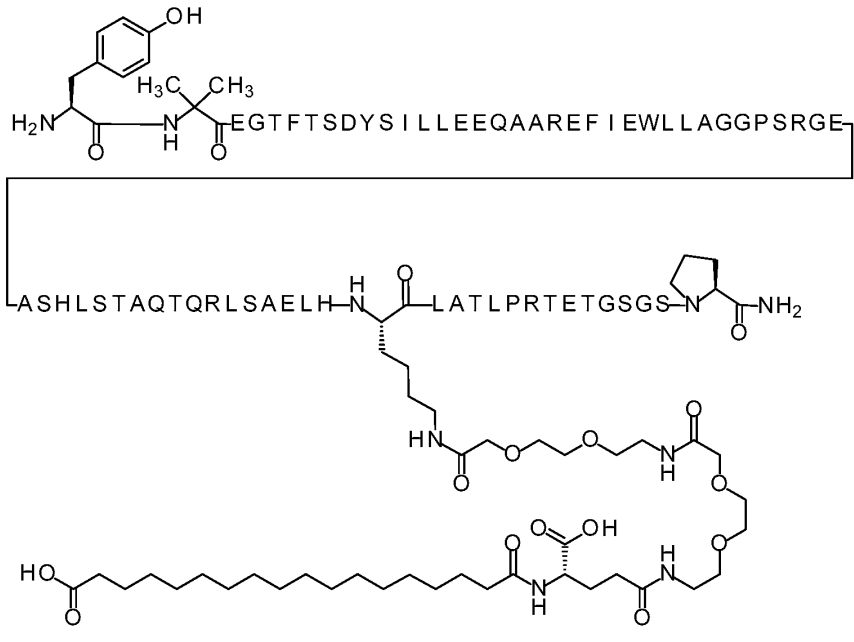
compound 66



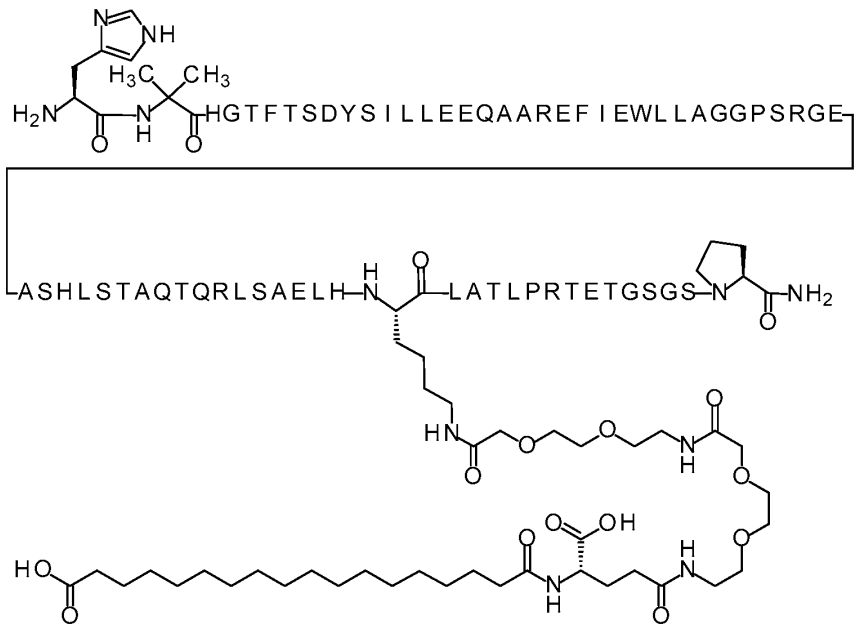
compound 67



compound 68



compound 69

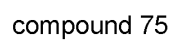


[illegible]

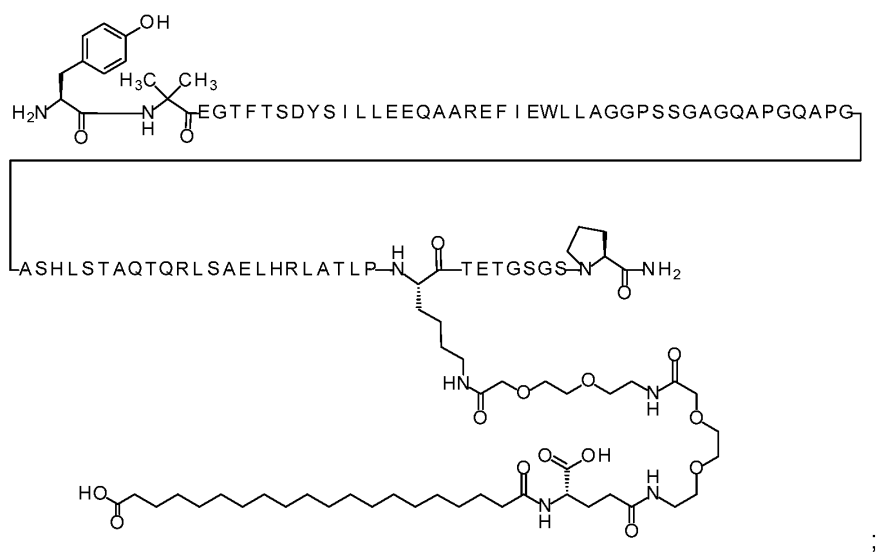
Chemical structure of the protein HGTFTSDYSILLEEQAAREFIEWLLAGGPSRGAPPPSGGGE. The structure is a ribbon diagram showing the protein backbone and side chains. The protein is a long, flexible chain with a complex fold. The N-terminal region (residues 1-10) is a 5-stranded alpha-helical bundle. The C-terminal region (residues 91-100) is a 4-stranded alpha-helical bundle. The two domains are connected by a long, flexible linker region (residues 11-90). The structure is shown in a ribbon representation with a light blue color scheme.

Chemical structure of the 100-residue protein E. coli O157:H7 Shiga toxin B subunit (Stx2B) with a 100-residue alpha-helical coiled-coil stalk. The structure shows the N-terminal domain with a 4-hydroxyphenyl group, the stalk region with a disulfide bond, and the C-terminal domain with a 100-residue alpha-helical coiled-coil stalk. The stalk is composed of two alpha-helices, one from each monomer, which are intertwined. The C-terminal domain is a beta-barrel structure. The structure is shown in a ribbon representation with a stick model for the N-terminal domain and a ribbon model for the stalk and C-terminal domain.

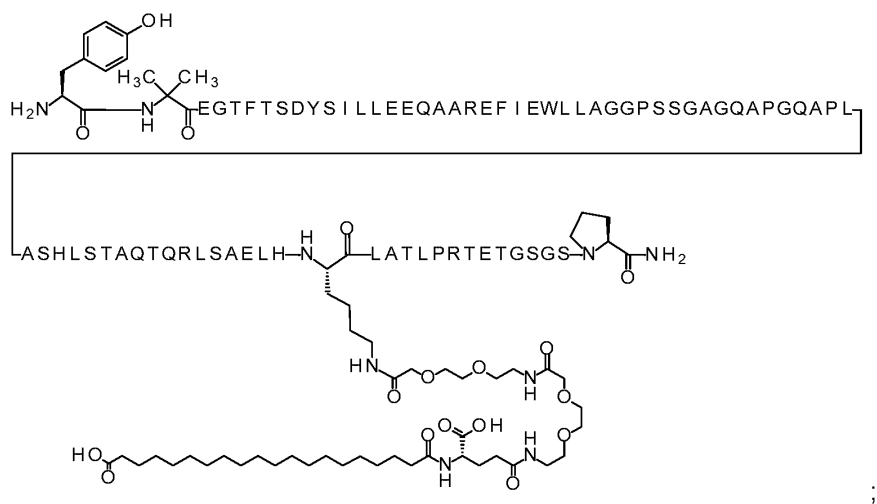
C[C@H](Cc1c[nH]cn1)C(=O)NCC(=O)NC(C)[C@@H](CCCCNC(=O)NCCOCOCOC(=O)NCCOCOC(=O)NCCC[C@H](C)C(=O)NCC(=O)O)C(=O)O



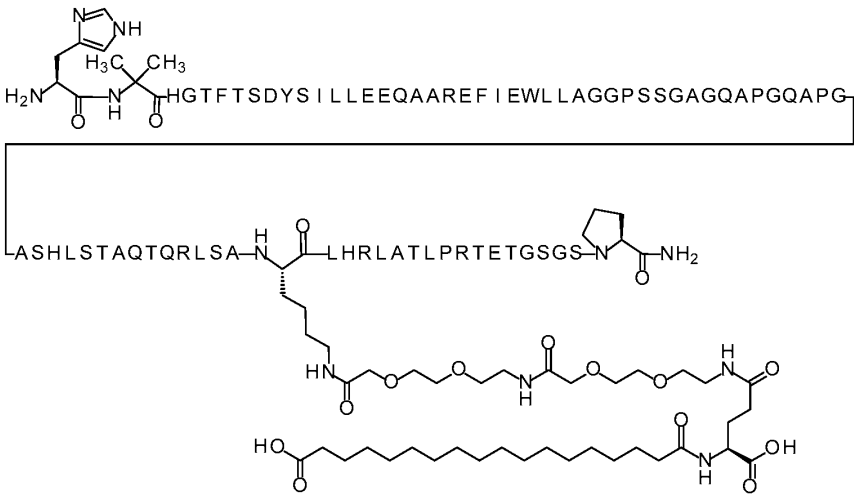
compound 76



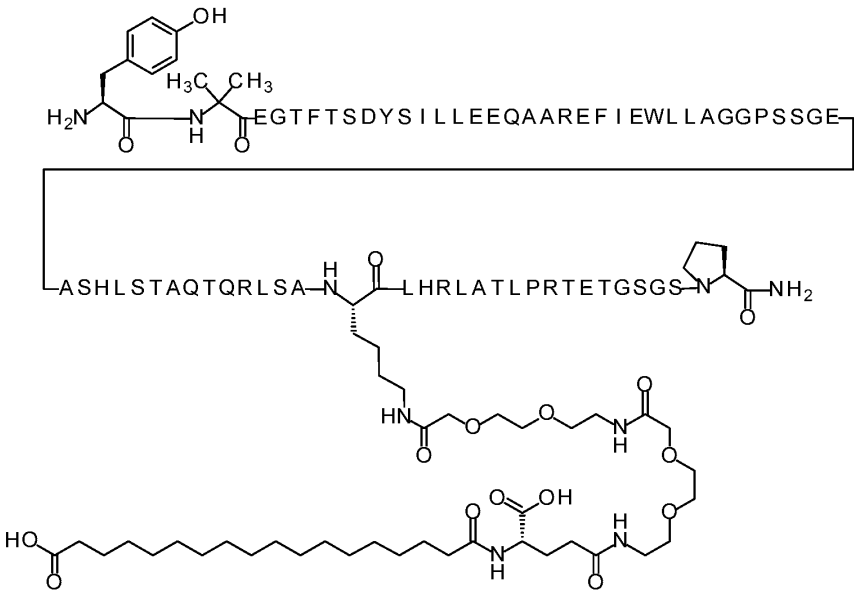
compound 77



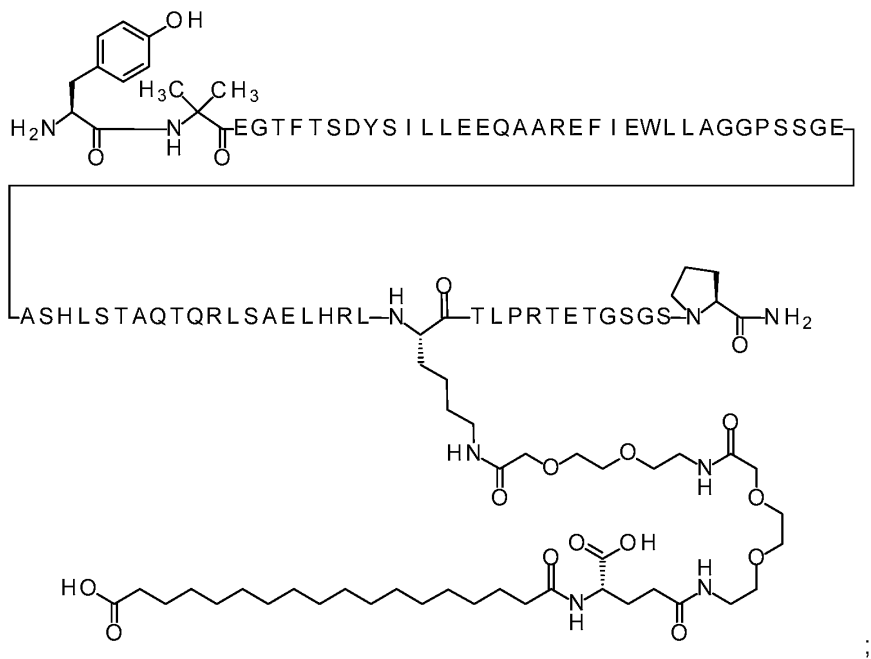
compound 78



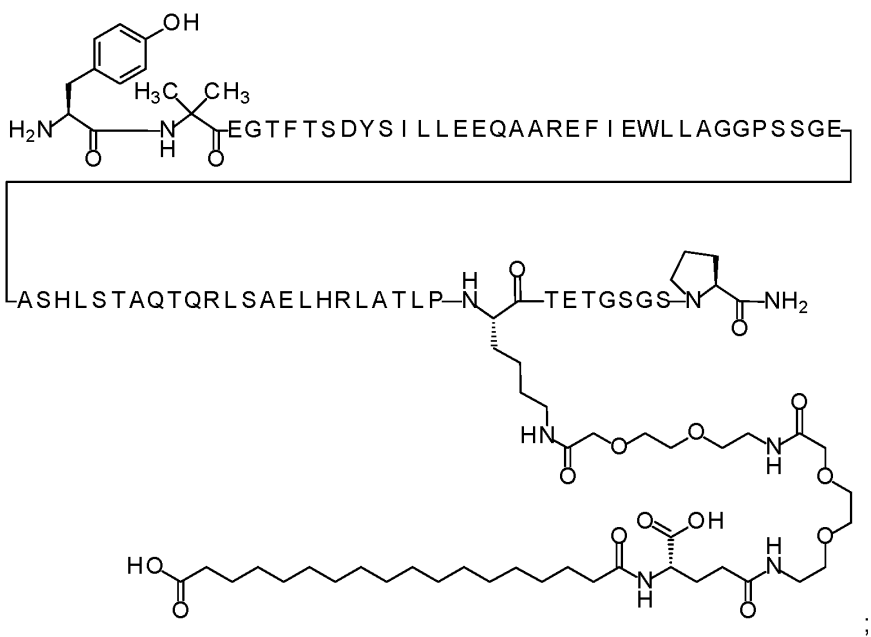
compound 79

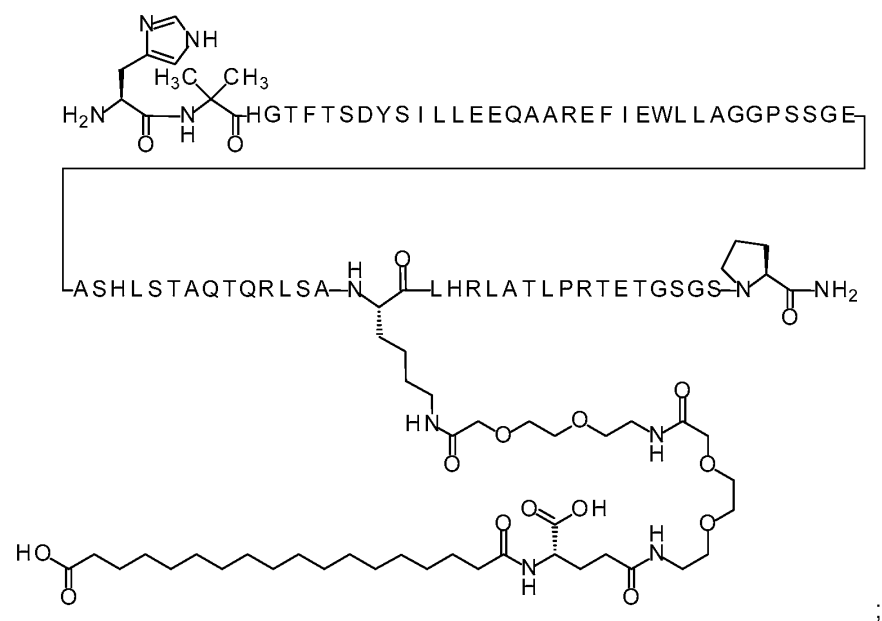


compound 80

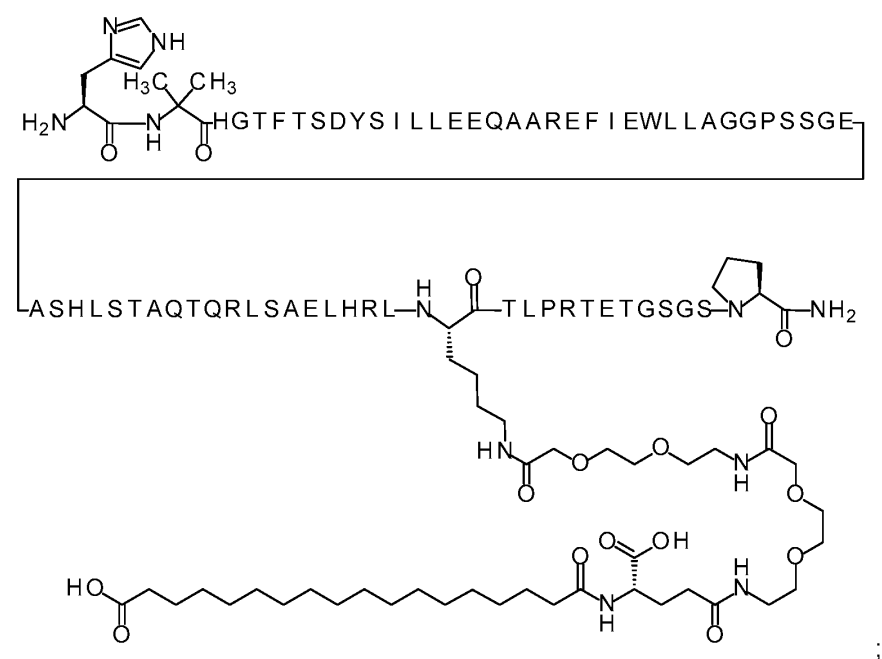


compound 81





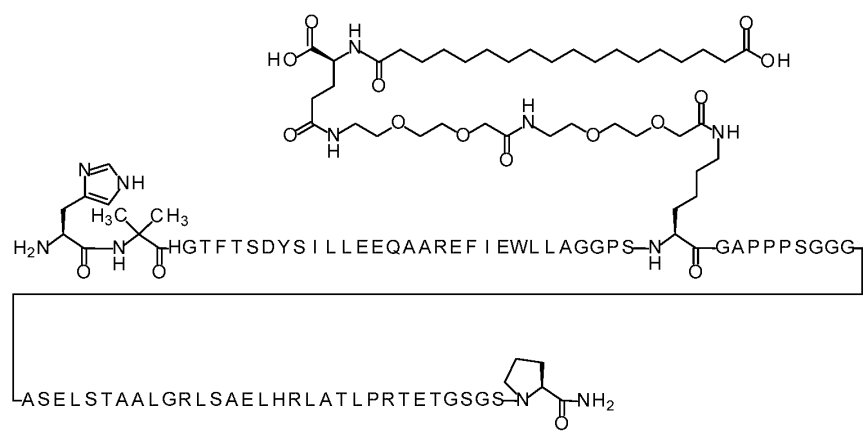
compound 83



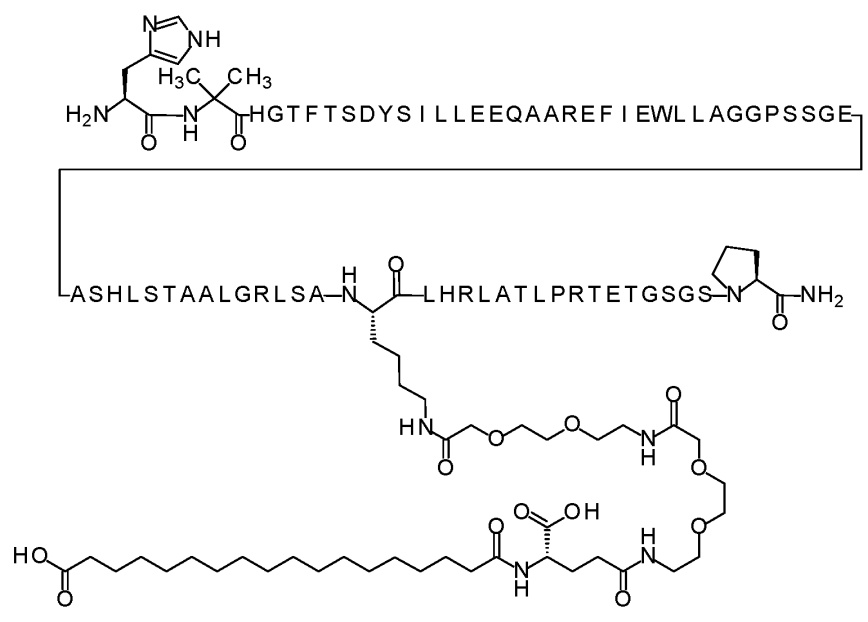
Chemical structure of the protein HGTFTSDYSILLEEQAAREFIEWLLAGGPSSGEASHLSTAQTQRLSAELHRLATLP. The structure shows a long alpha-helical domain followed by a beta-sheet domain. A disulfide bond is formed between the Cys100 and Cys101 residues. The N-terminus is modified with a 2-mercaptoethylamine group. The C-terminus is modified with a 2-mercaptoethylamine group. The structure is shown in a ribbon representation with side chains.

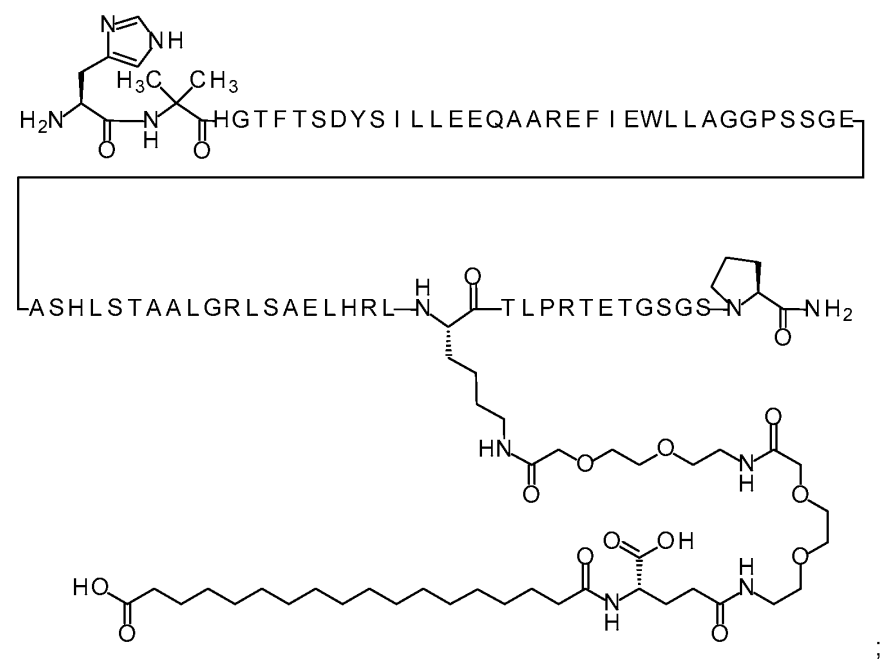
The chemical structure of the protein HGTFTSDYSILLEEQAAREFIEWLLAGGPSSGE is shown. The protein is a 120-residue protein. The N-terminal region (residues 1-10) is a long alpha-helical domain. The C-terminal region (residues 111-120) is a short alpha-helical domain. The protein is shown in a ribbon representation, with the N-terminal region in blue and the C-terminal region in red. The protein is shown in a side view, with the N-terminal region on the left and the C-terminal region on the right. The protein is shown in a ribbon representation, with the N-terminal region in blue and the C-terminal region in red. The protein is shown in a side view, with the N-terminal region on the left and the C-terminal region on the right.

compound 86

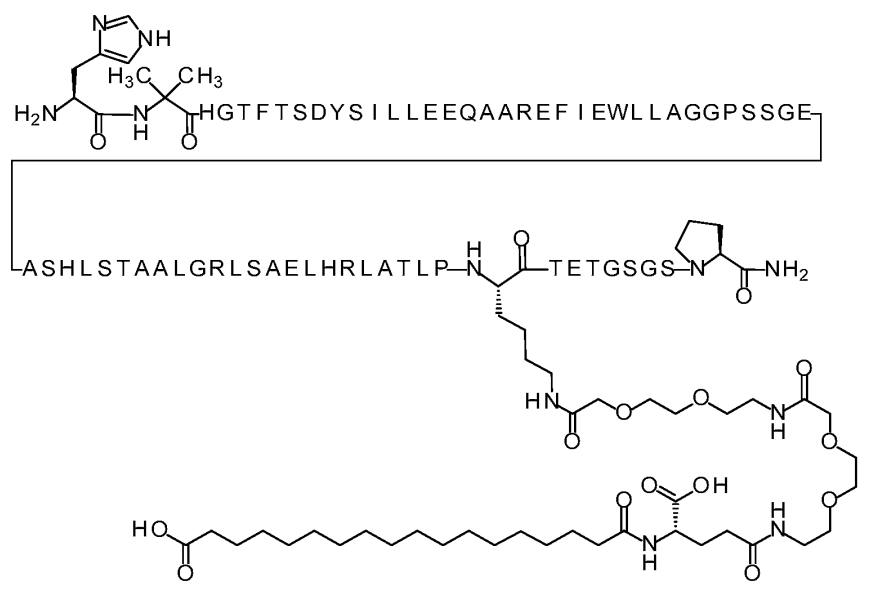


compound 87

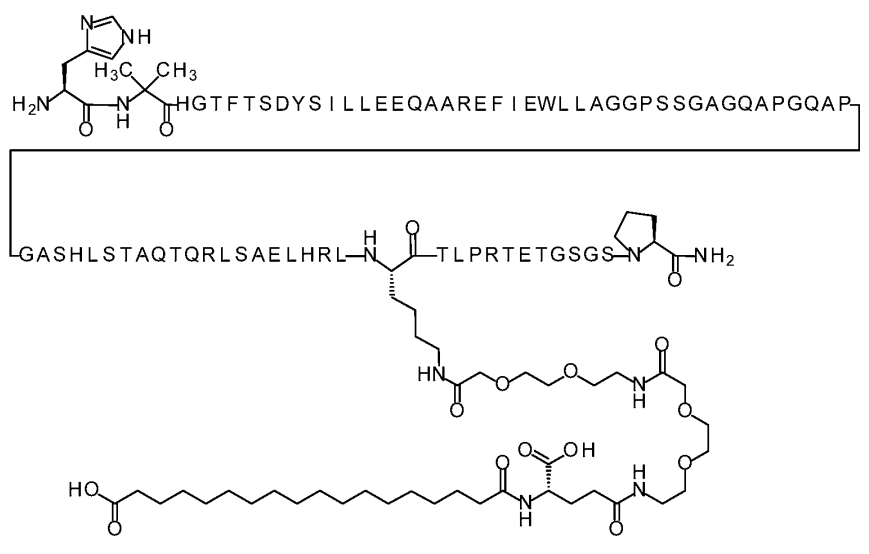




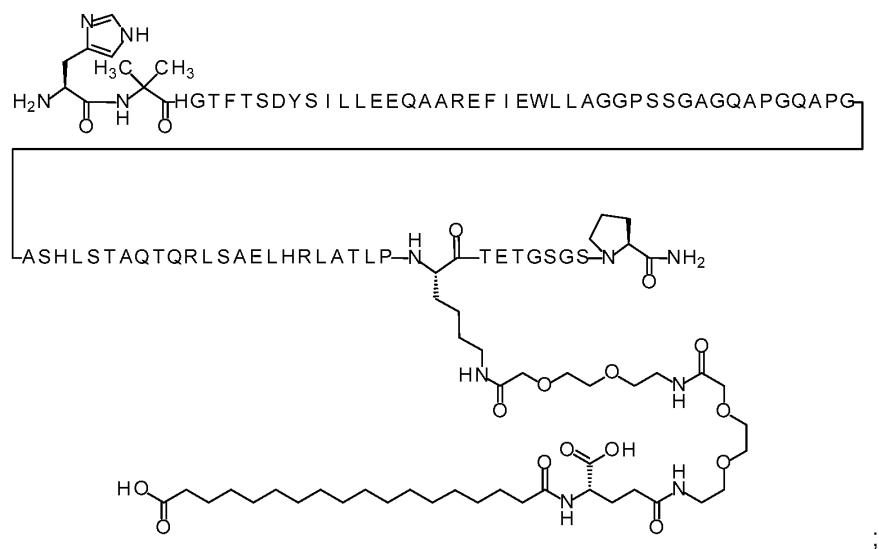
compound 90



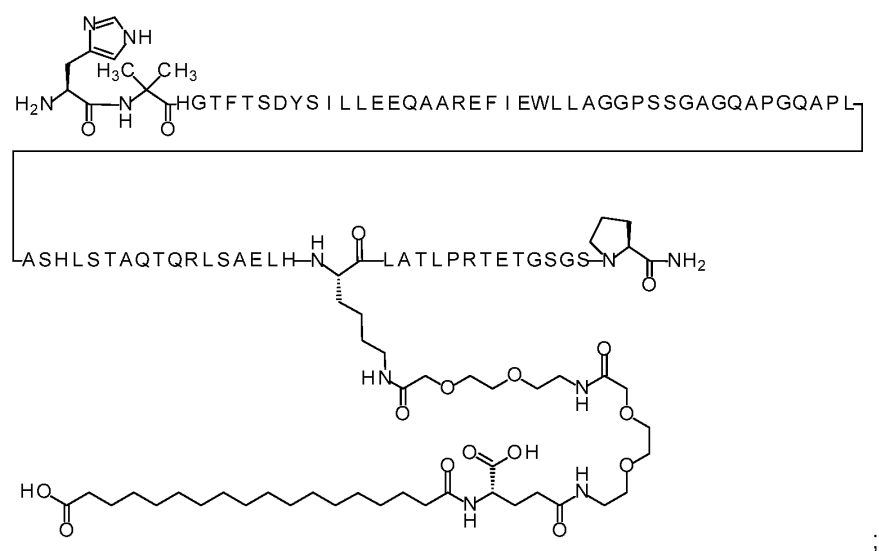
compound 91



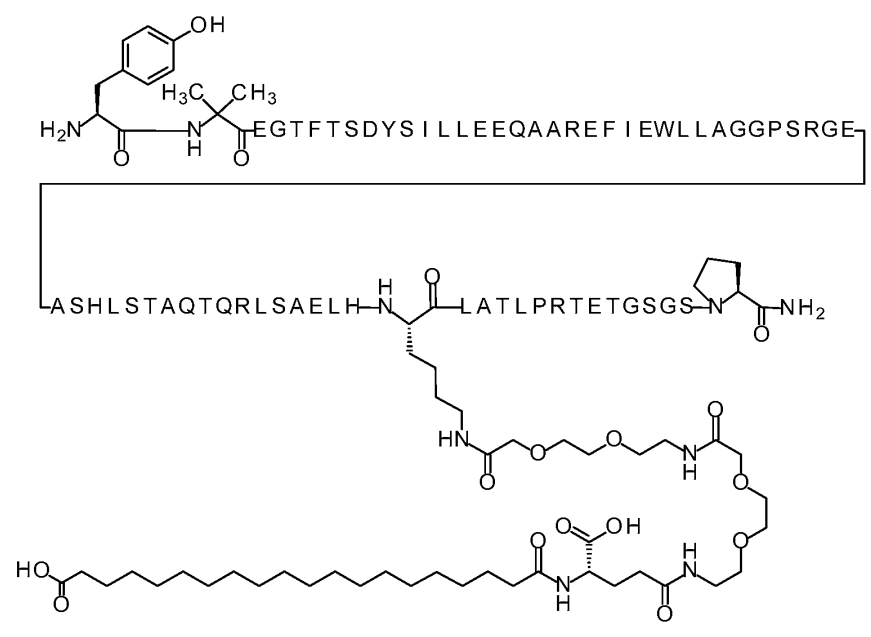
compound 92



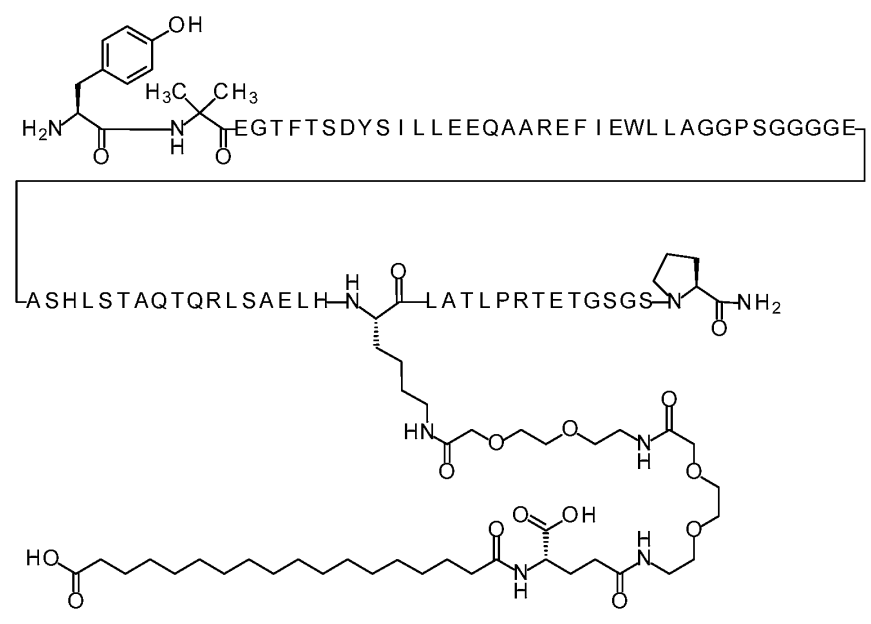
compound 93



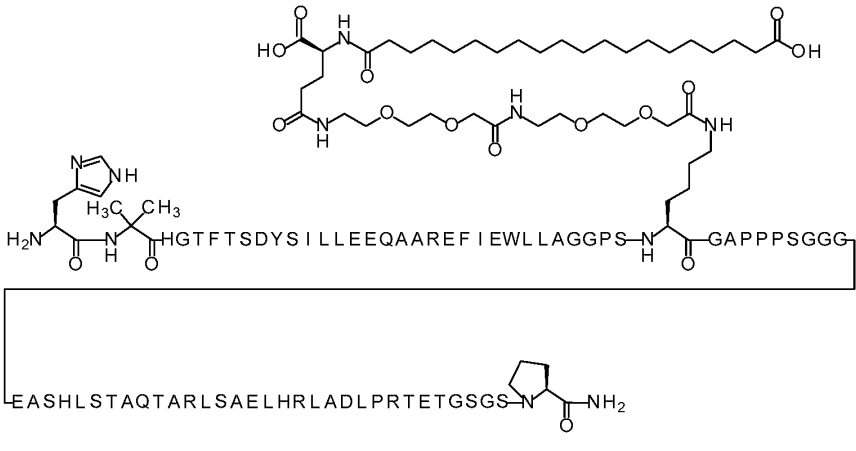
compound 94



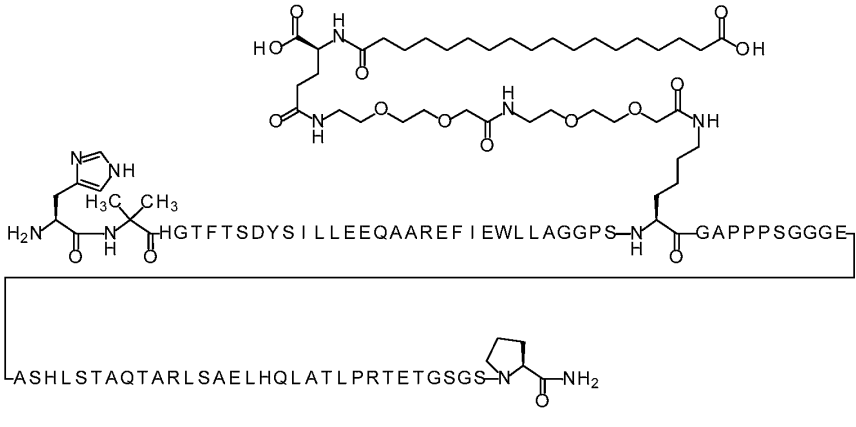
compound 95



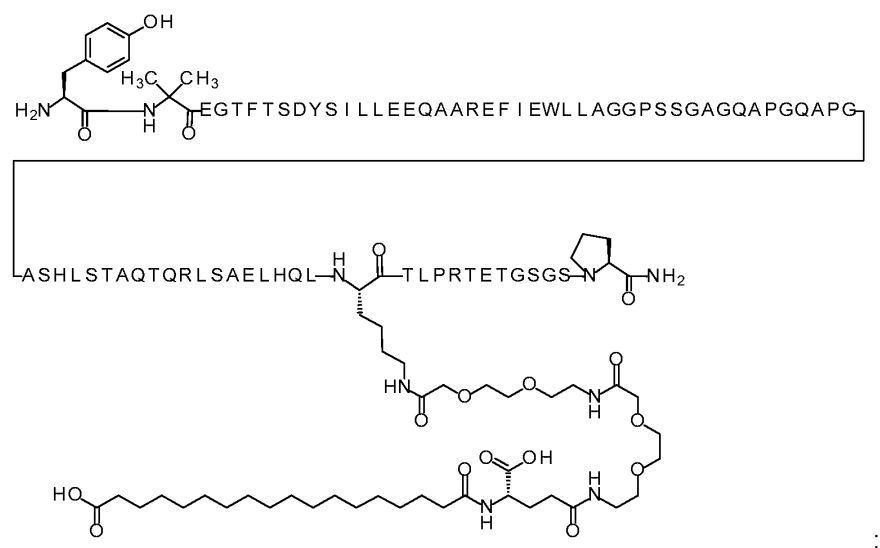
compound 100



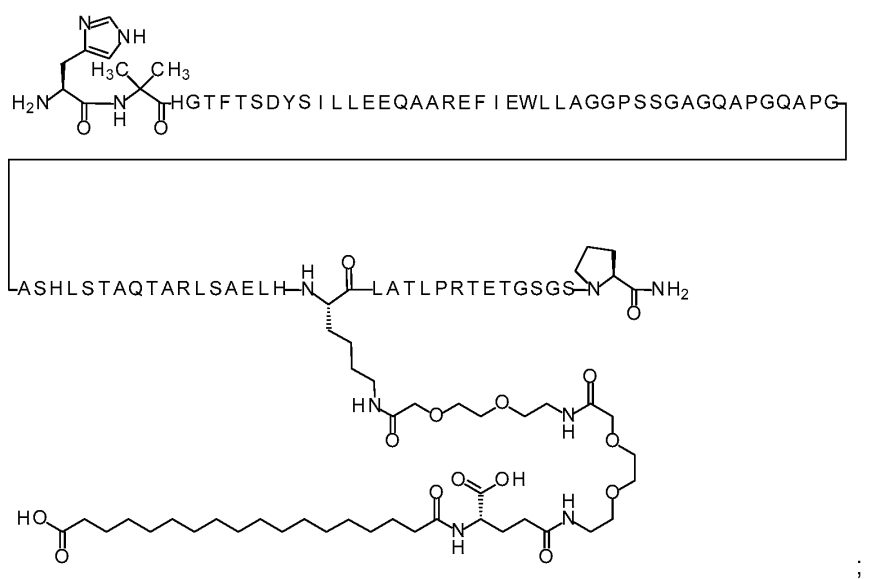
compound 101



compound 102



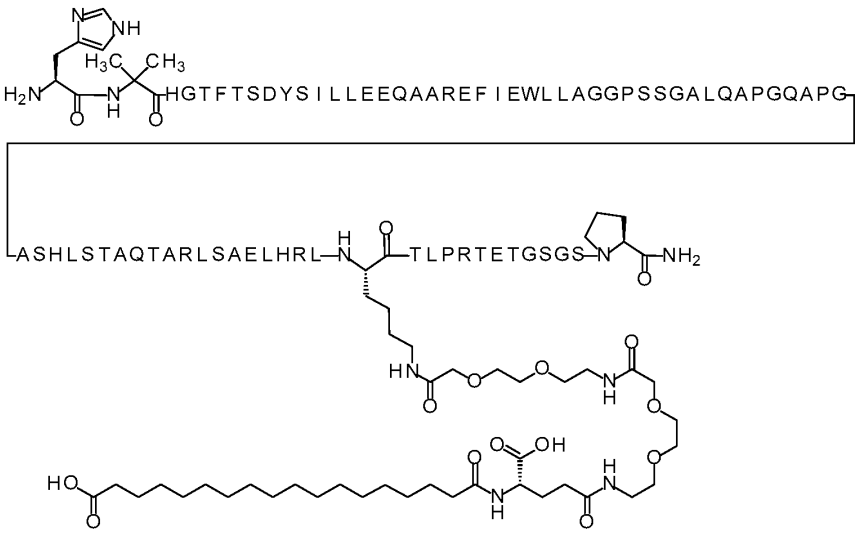
compound 103



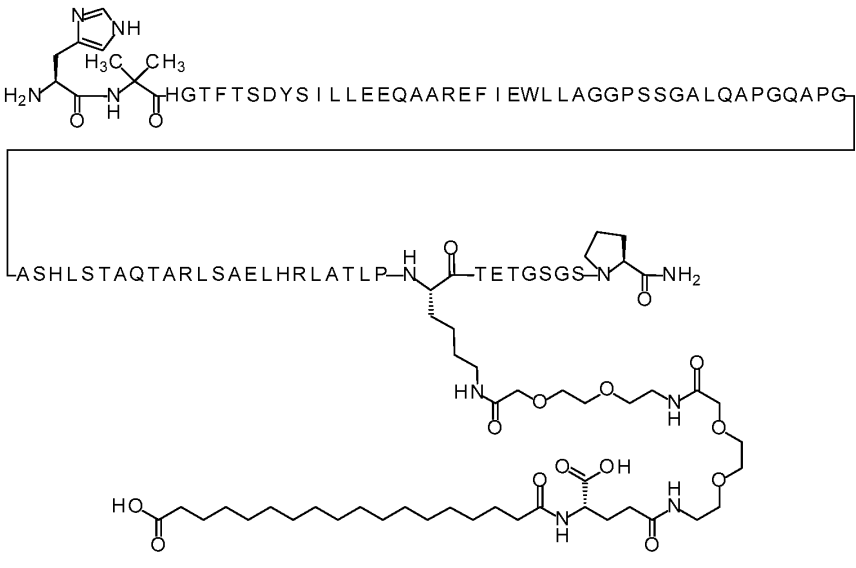
Chemical structure of the 100-residue protein HGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPG. The structure shows a long alpha-helical bundle with a disulfide bond between Cys1 and Cys100. The N-terminus is modified with a 2-methyl-5-imidazolylpropanoyl group. The C-terminus is a long, flexible loop containing a hydrophobic core and a disulfide bond between Cys98 and Cys99.

The image displays the chemical structure of the 120-residue protein E. coli Lactococcus lactis protein. The top part shows the full sequence: H₂N-EGTFTSDYSILLEEQAAREFIEWLLAGGPSSGALQAPGQAPL- (with a line indicating the continuation of the sequence). The bottom part is a detailed view of the C-terminal region, showing the sequence ASHLSTAQTARLSAELH- followed by a complex structure including a long aliphatic chain, a hydroxyl group, and a terminal amide group.

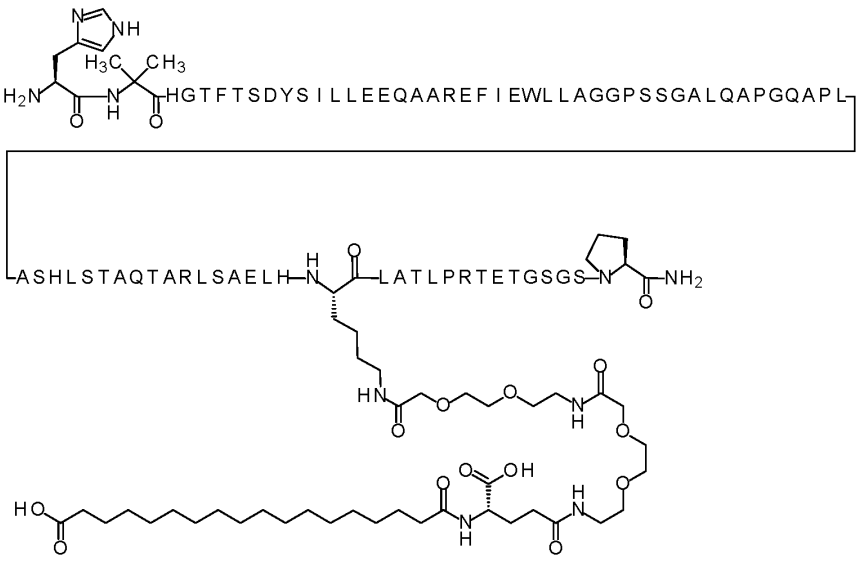
compound 106



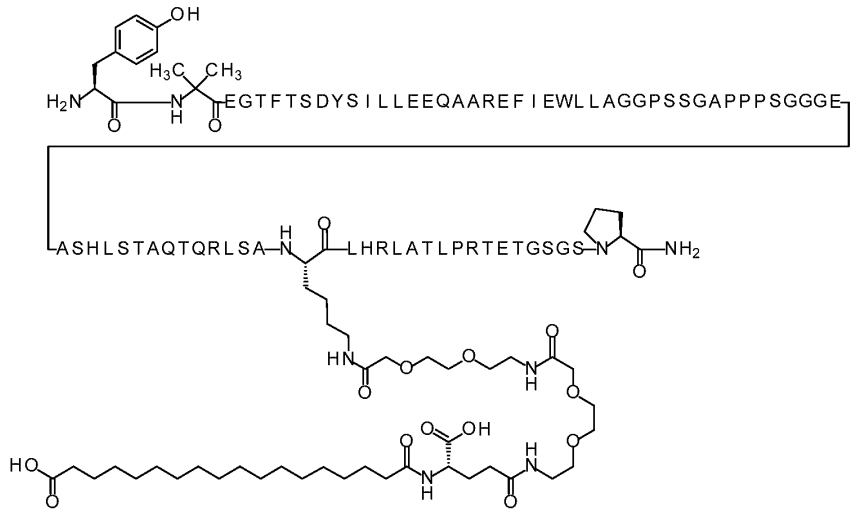
compound 107



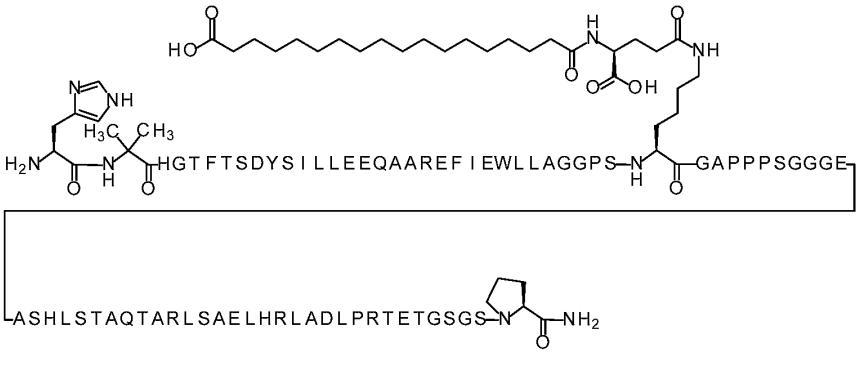
compound 108



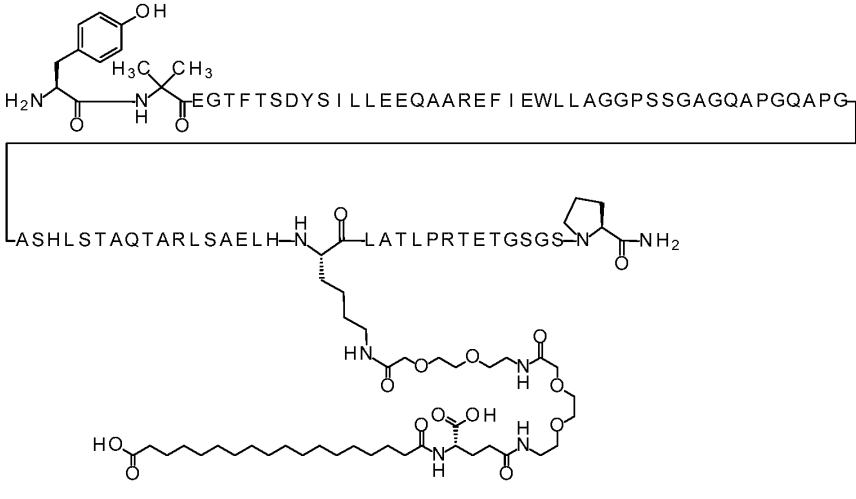
compound 109



compound 112

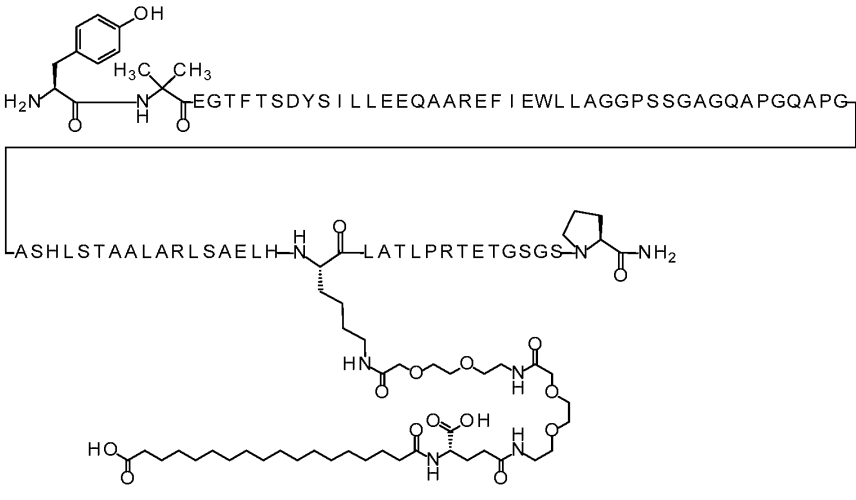


compound 113

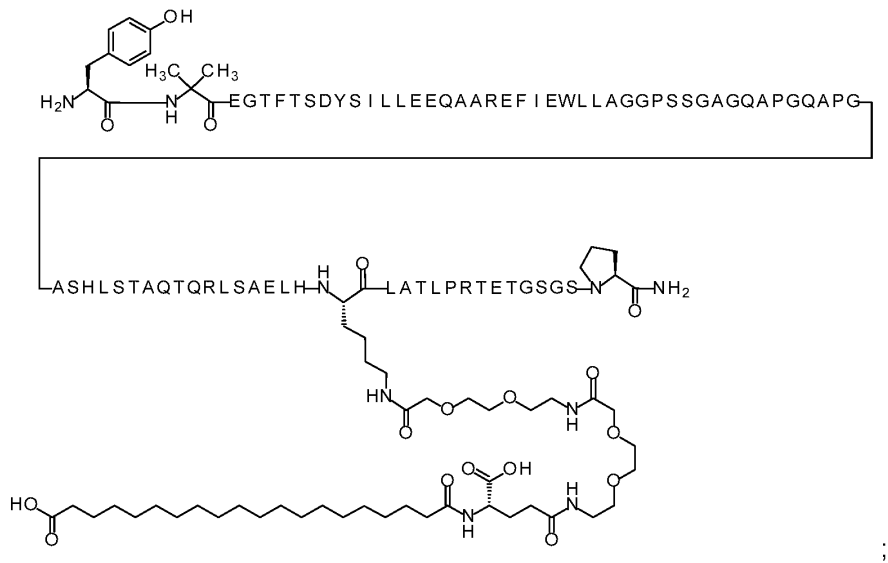


5

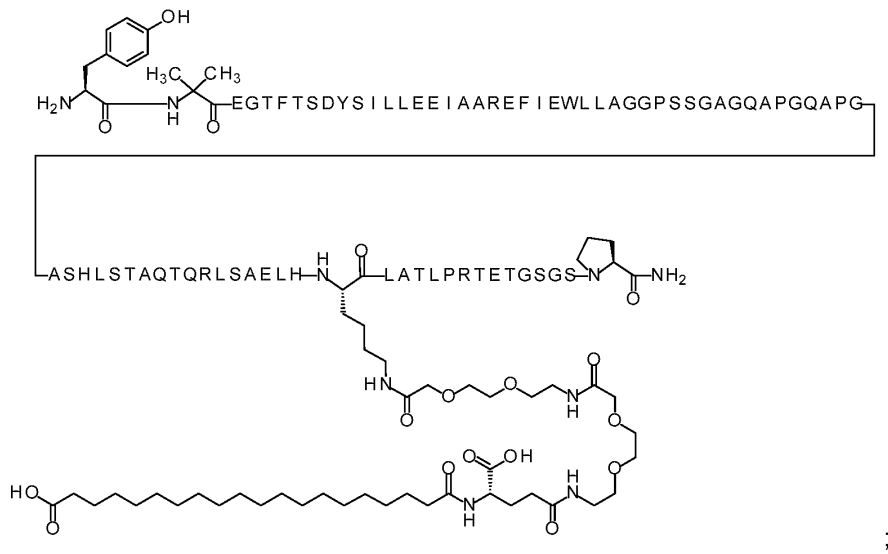
compound 114



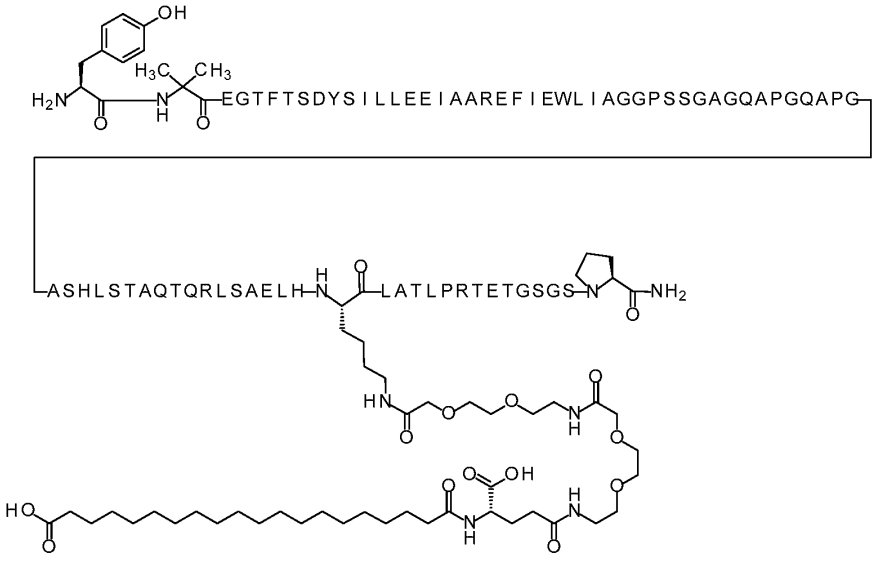
Compound 115



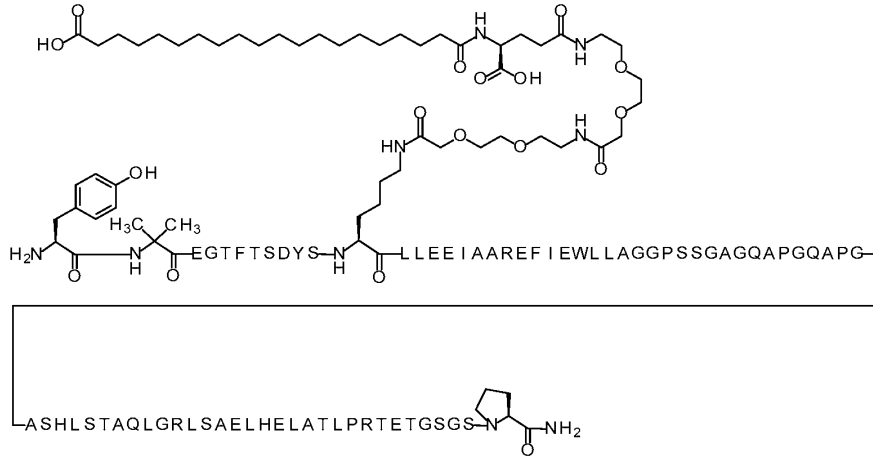
Compound 120



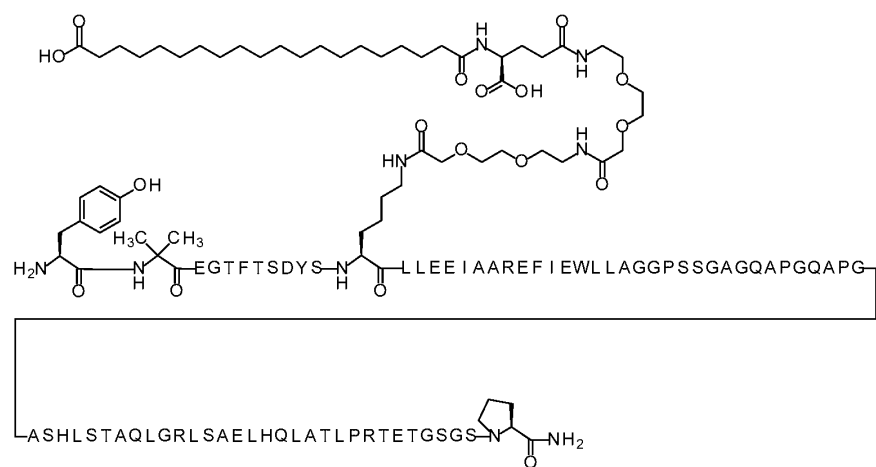
Compound 121



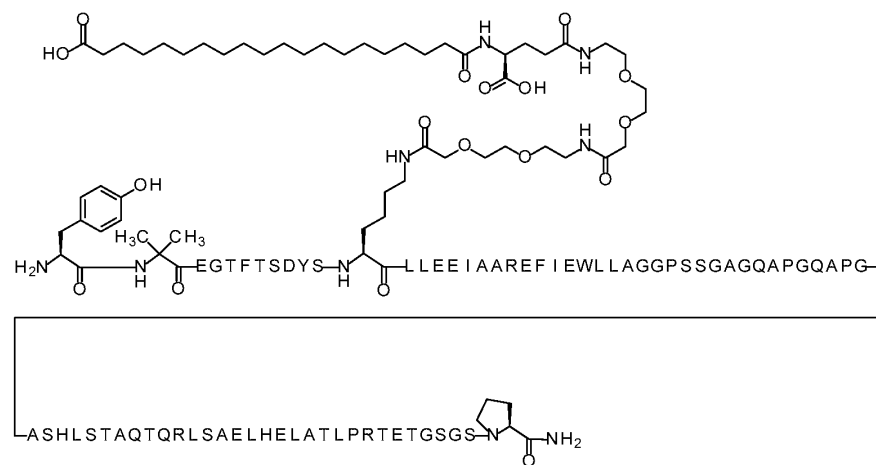
Compound 122



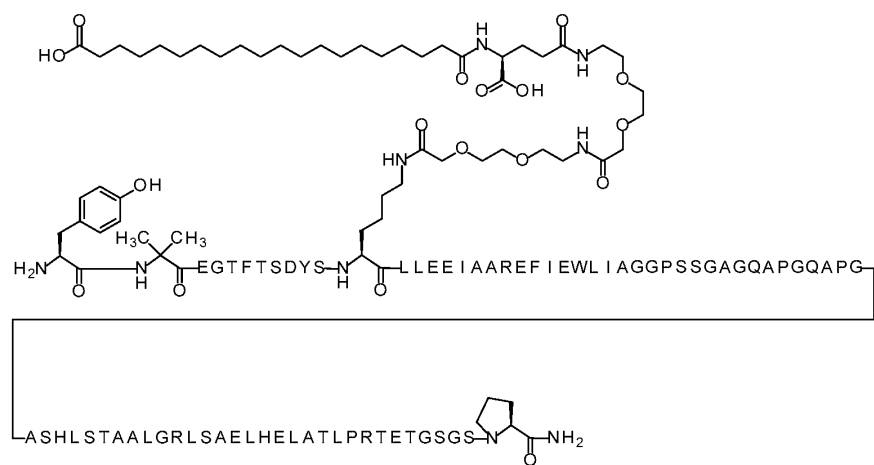
Compound 123



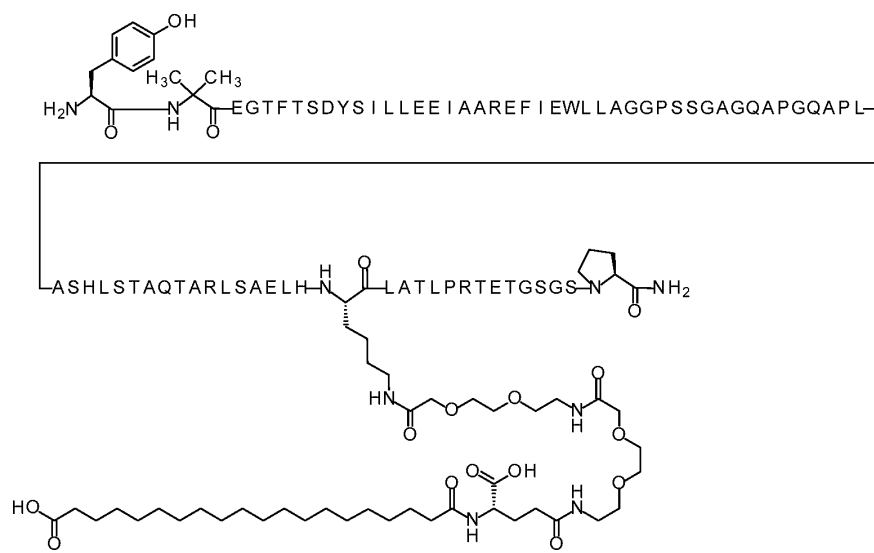
Compound 124



Compound 127



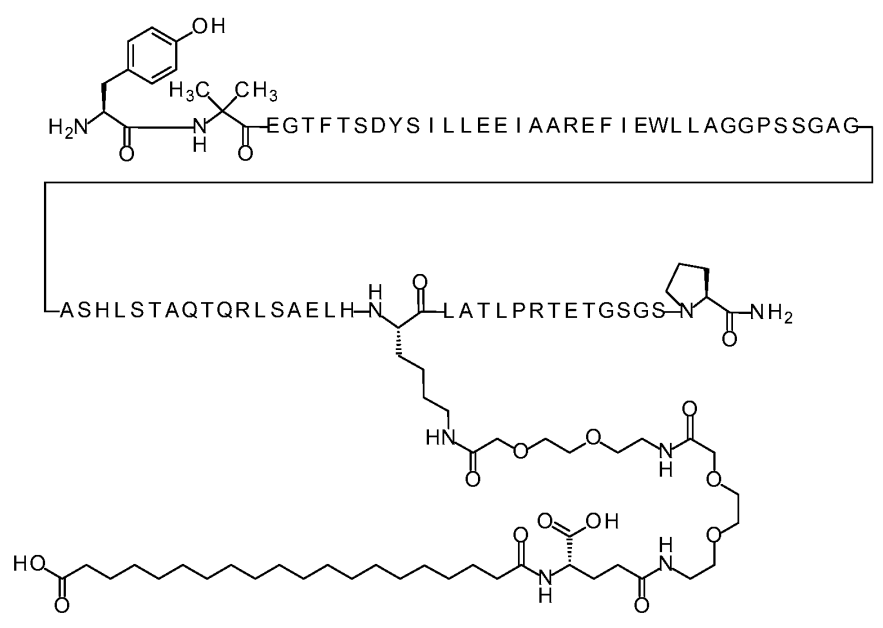
Compound 128



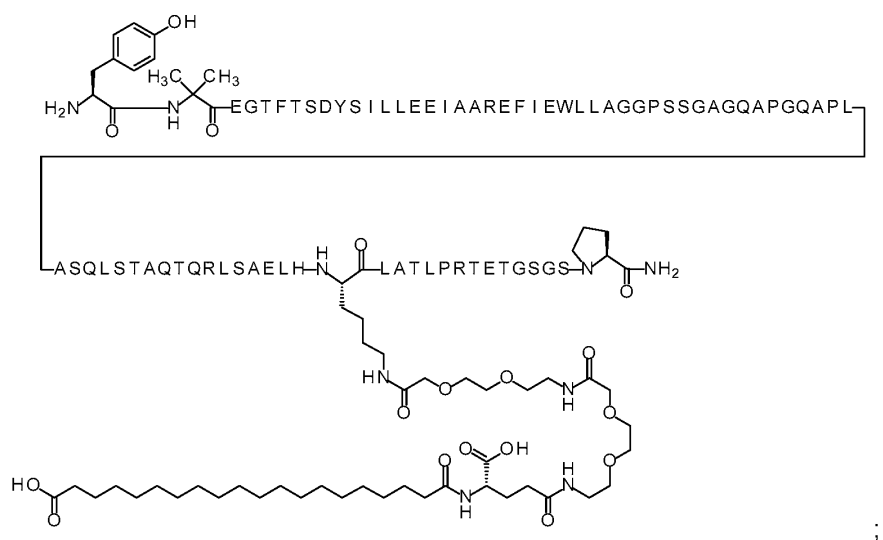
[illegible]

Chemical structure of the 120-residue protein, showing the full sequence and the cyclization of the C-terminal residue (Glycine) to the N-terminal residue (Asparagine). The structure includes a long alpha-helix, a beta-sheet, and a gamma-turn. The cyclization is indicated by a line connecting the C-terminal Glycine to the N-terminal Asparagine.

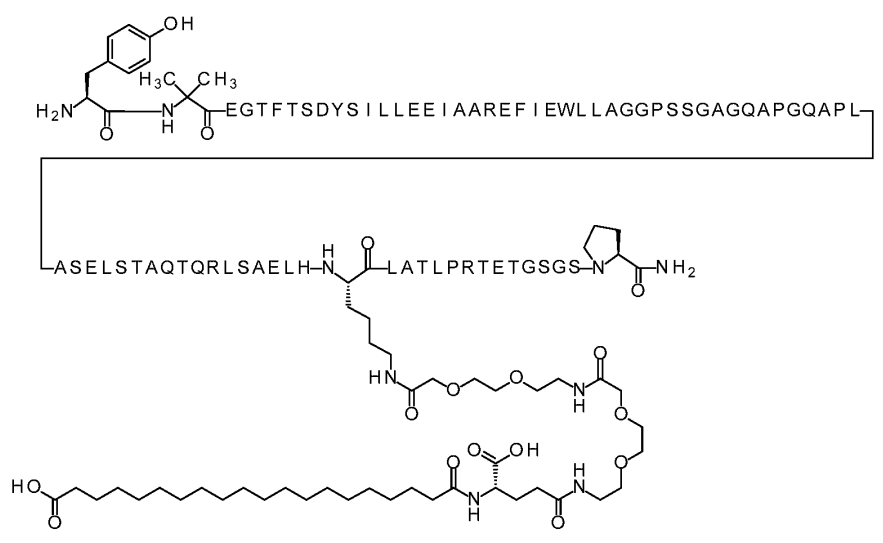
Compound 131



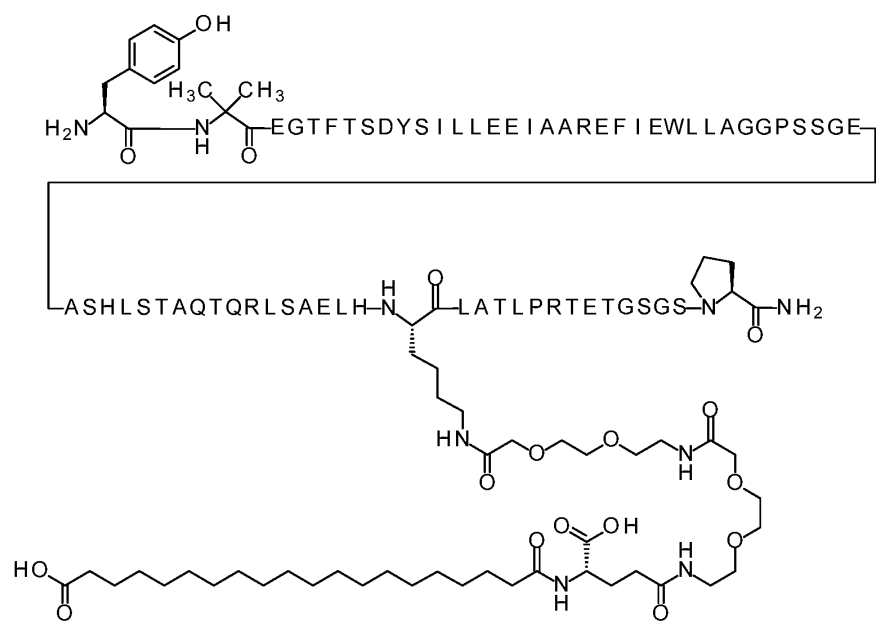
Compound 132



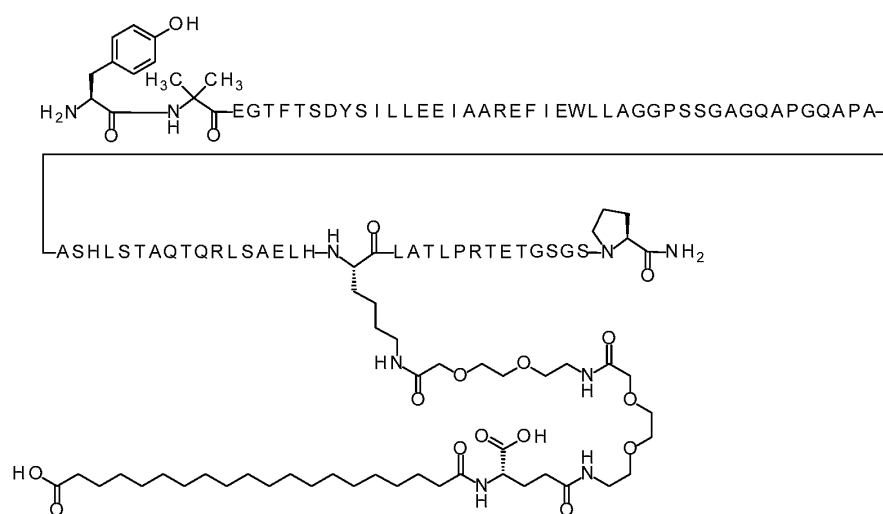
Compound133



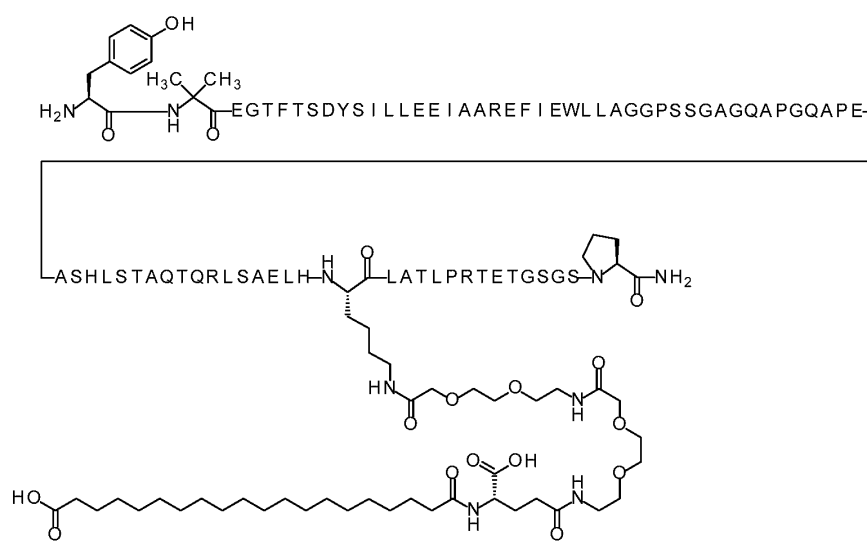
Compound 134



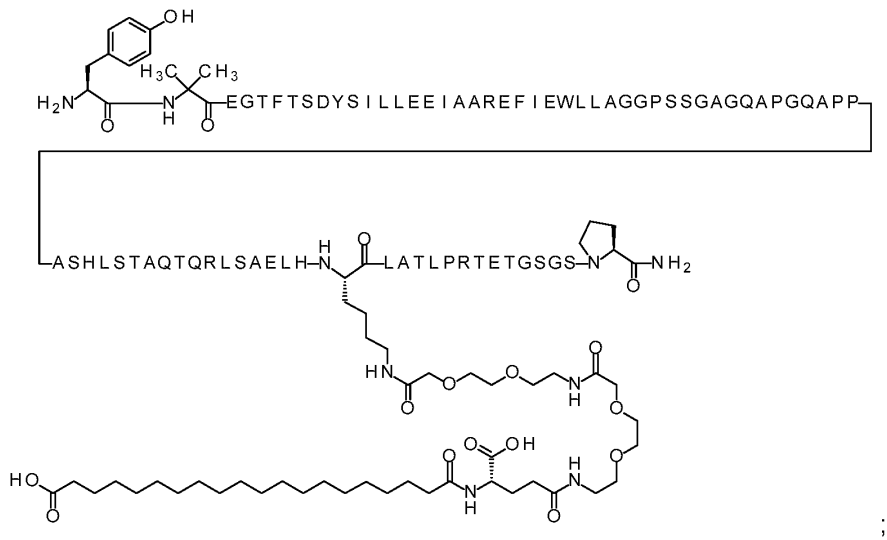
Compound 135



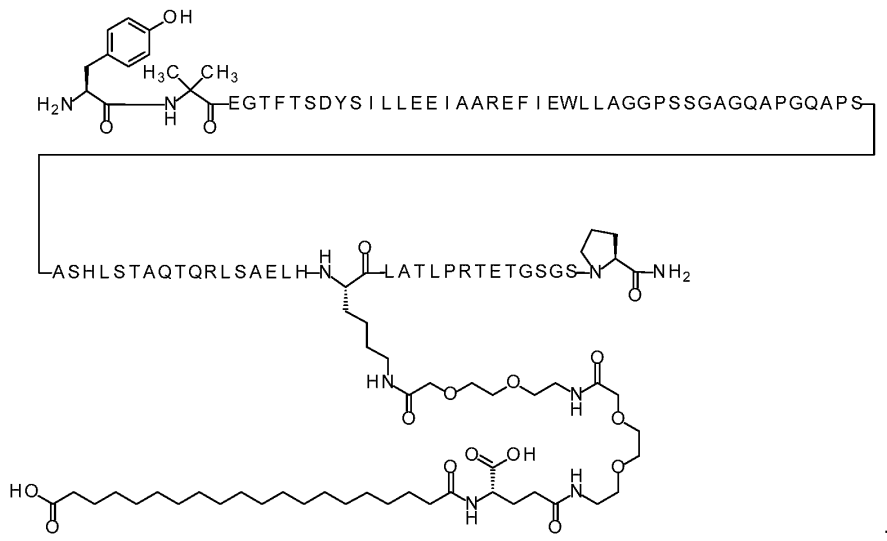
Compound 136



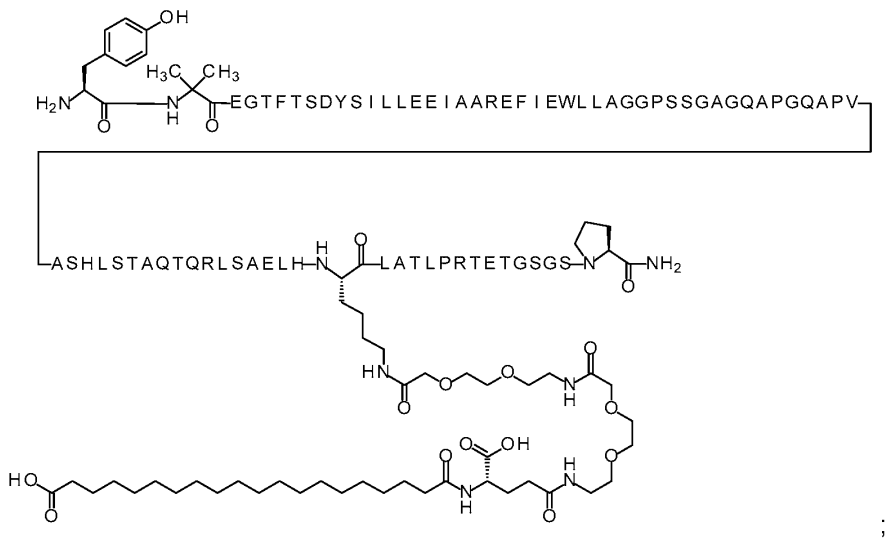
Compound 137



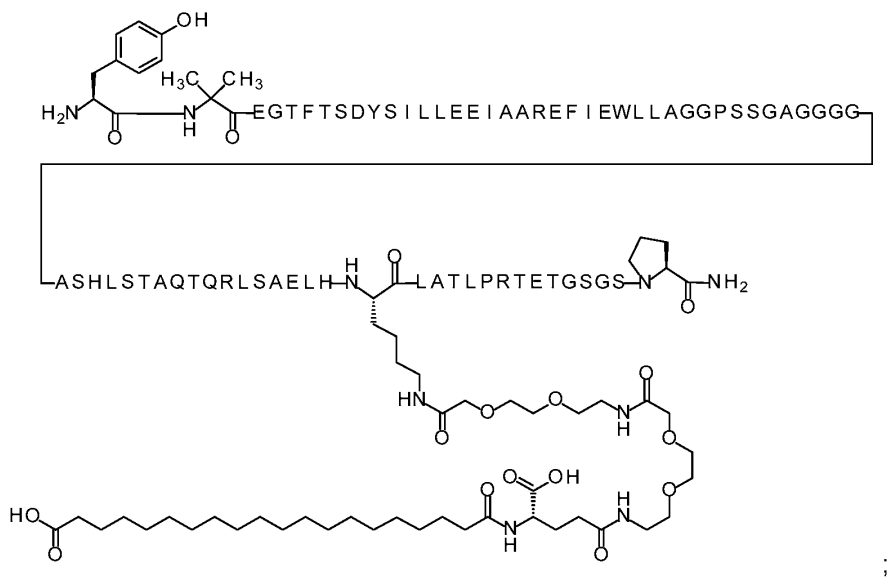
Compound 138



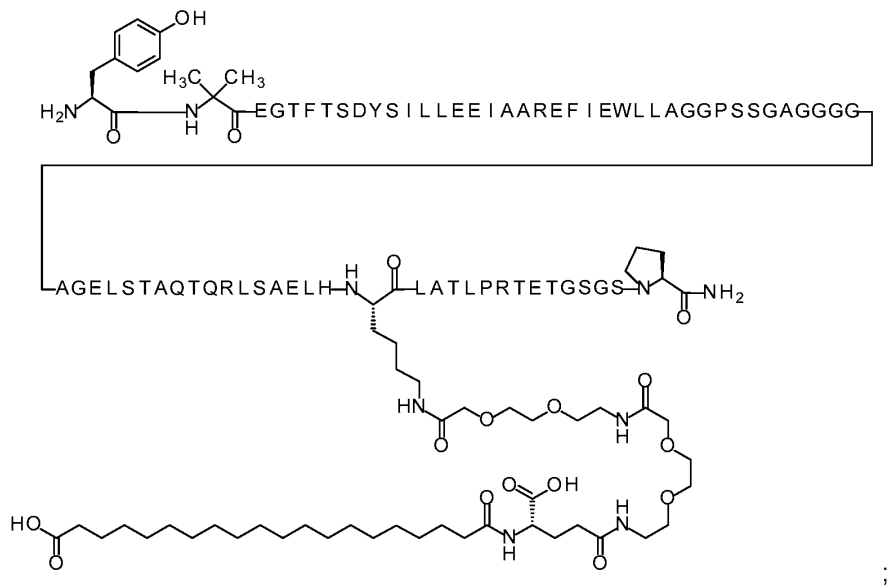
Compound 139



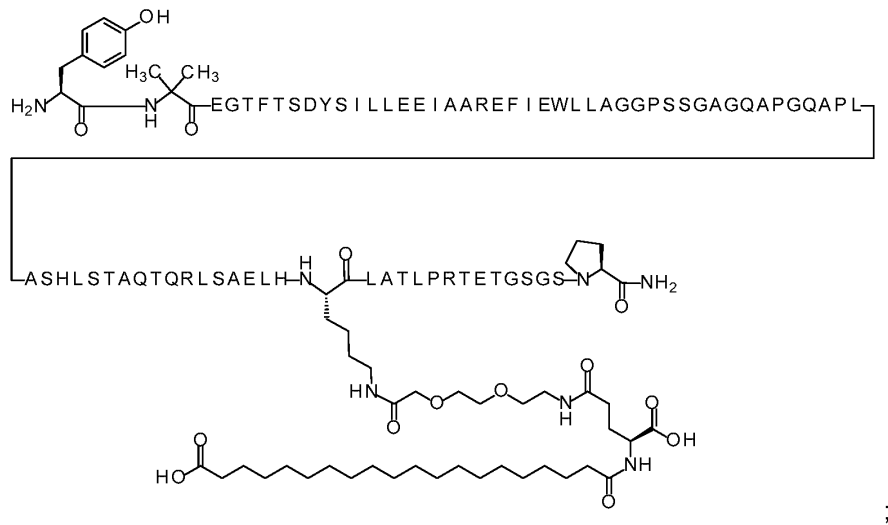
Compound 140



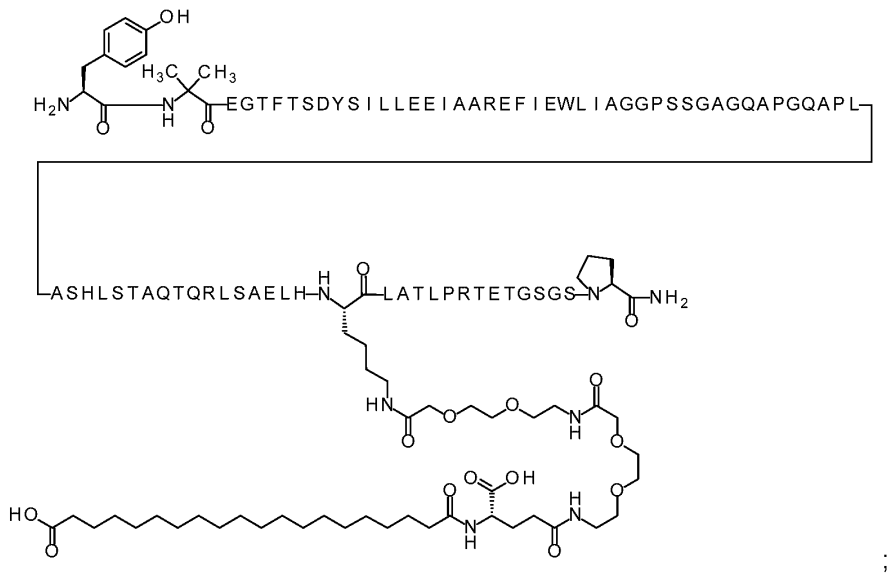
Compound 141



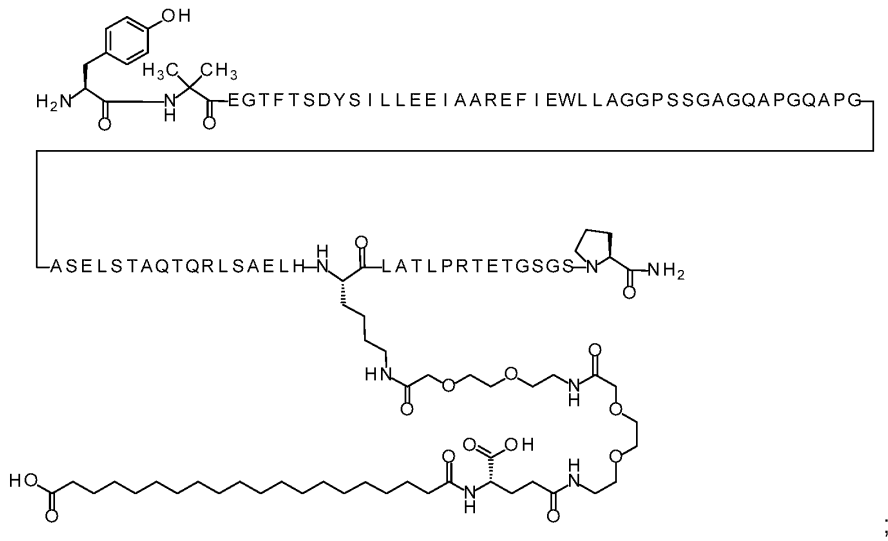
Compound 142



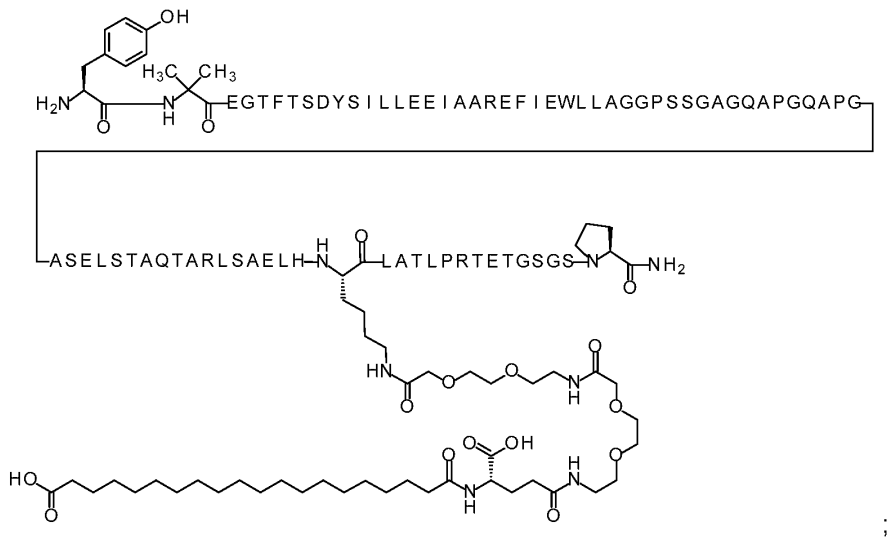
Compound 143



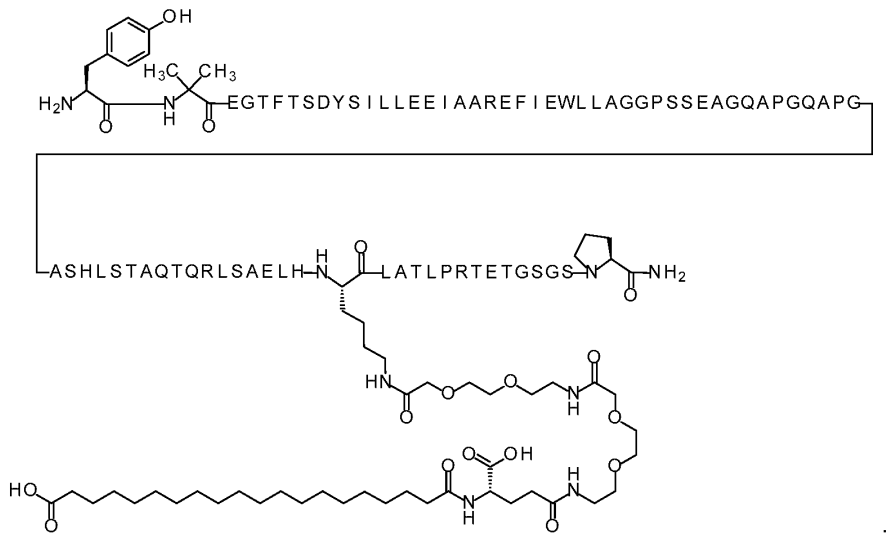
Compound 144



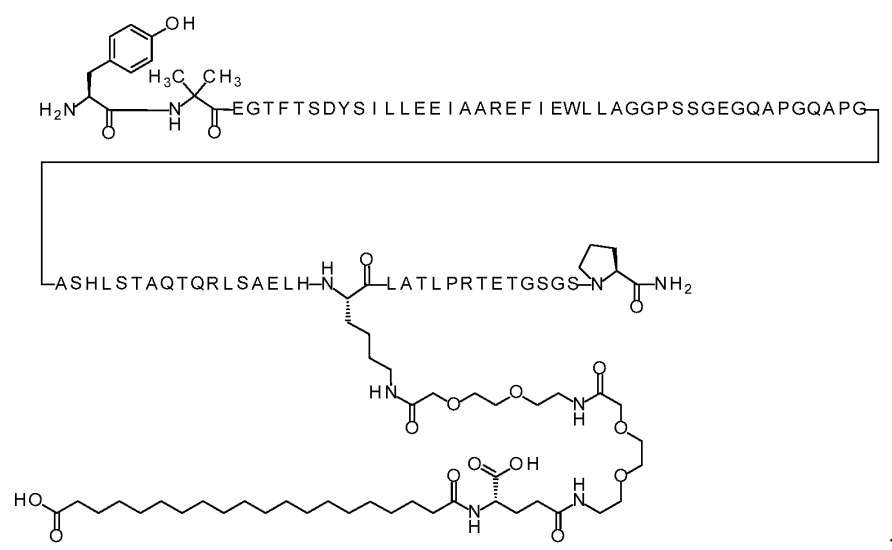
Compound 145



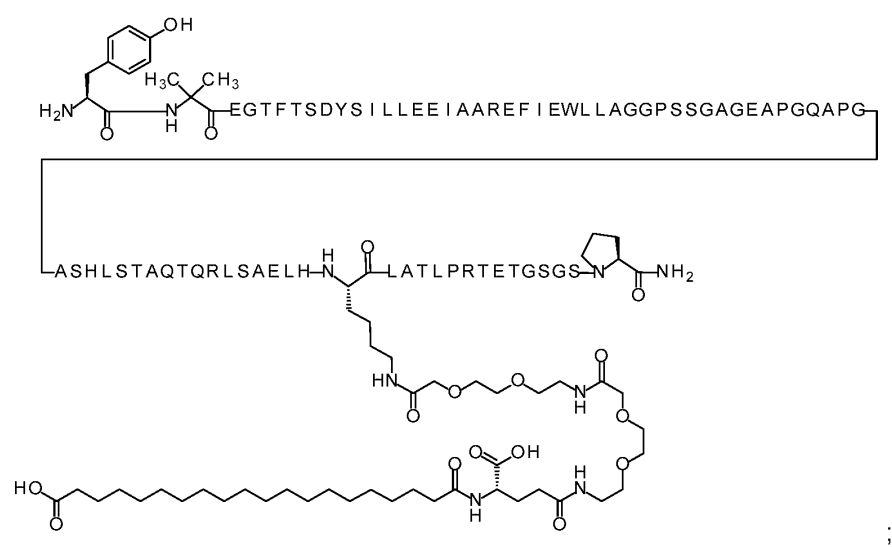
Compound 146



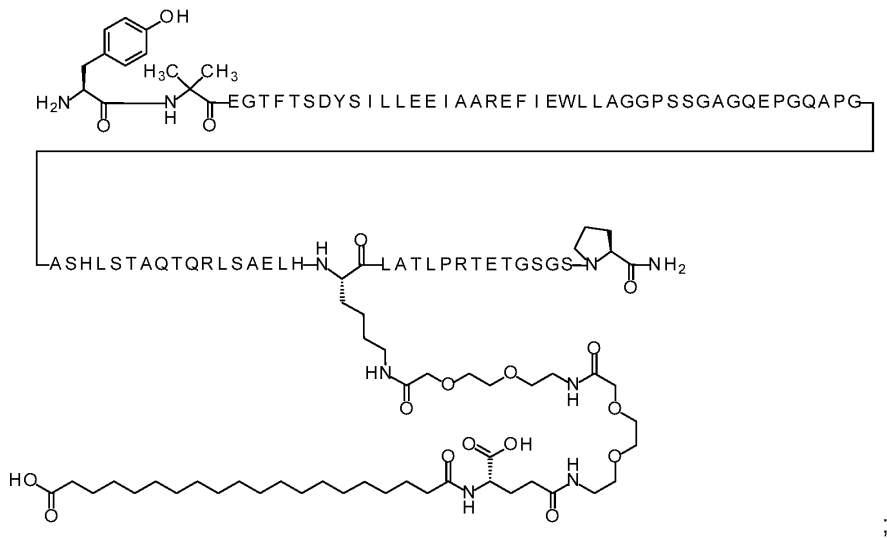
Compound 147



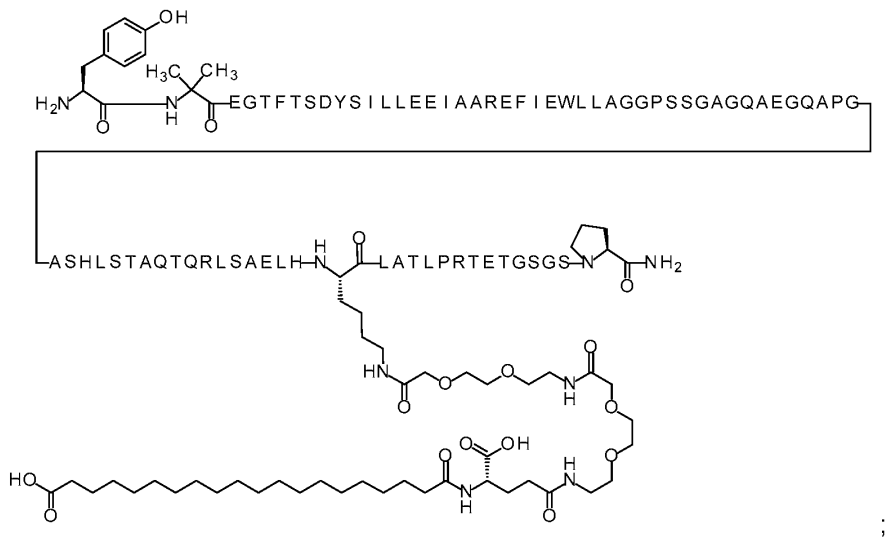
Compound 148



Compound 149



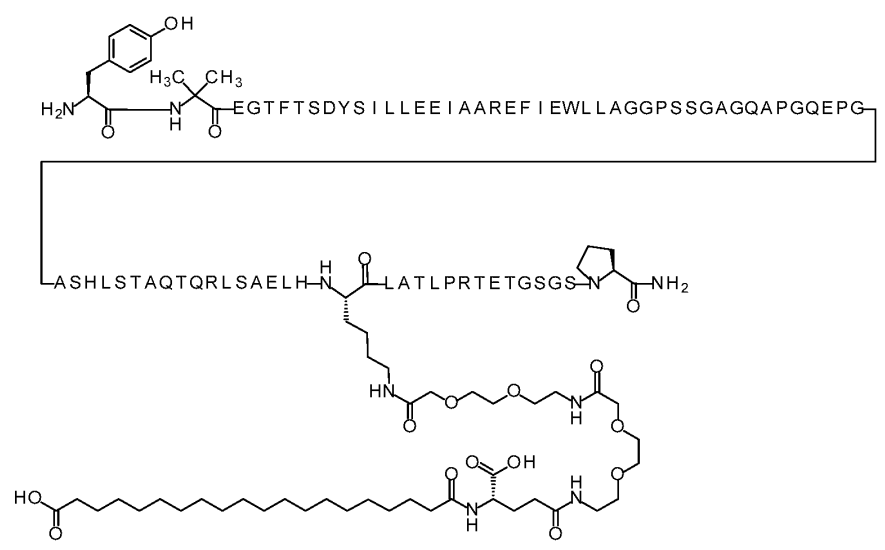
Compound 150



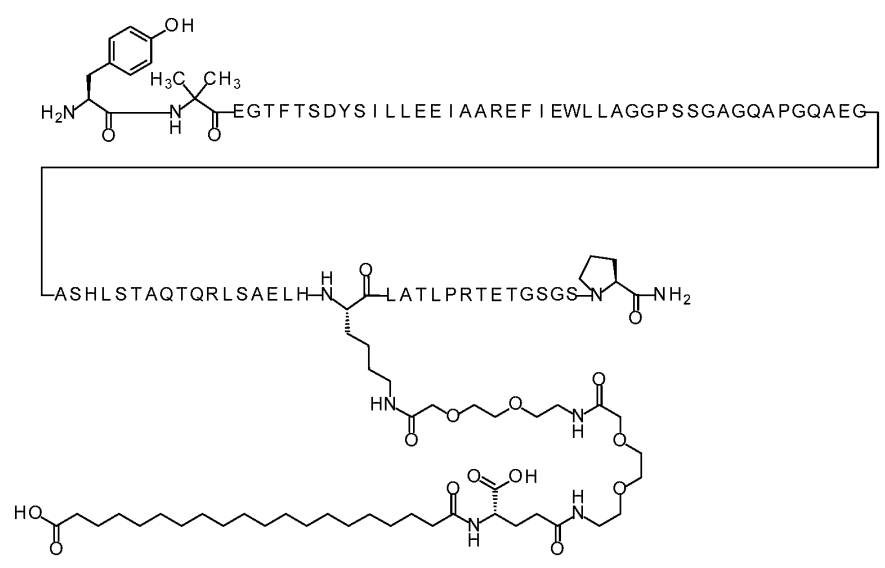
[illegible]

The chemical structure shows the full 100-residue protein sequence: **EGTFTSDYSILLEEIAAREFIEWLLAGGPSSGAGQAPGEAPGASHLSTAQTRLSAELH-N**. The N-terminus (residues 1-10) is shown in detail, including a 4-hydroxyphenyl group and a 2-methylpropanoyl group. The C-terminus (residues 91-100) is also shown in detail, including a long-chain fatty acid and a hydroxamic acid group. The structure illustrates the formation of a large macrocyclic ring structure, which is a characteristic feature of this protein.

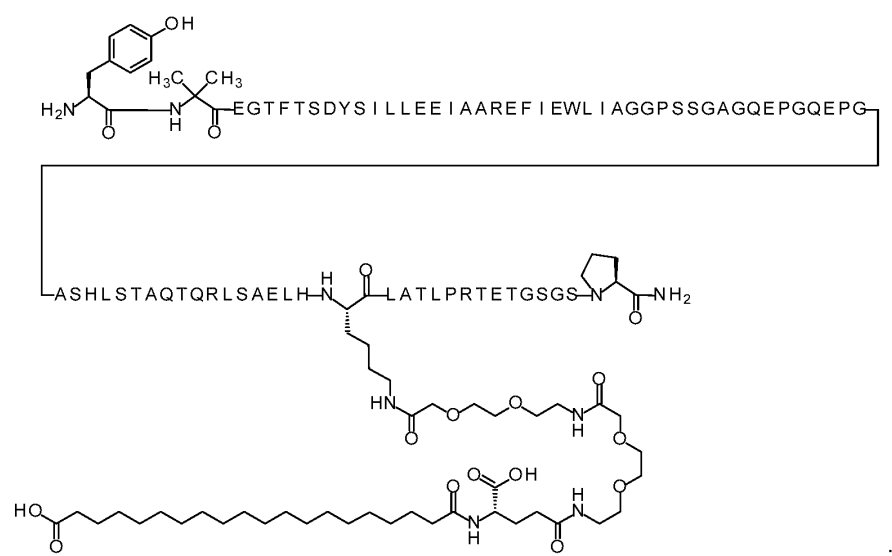
Compound 153



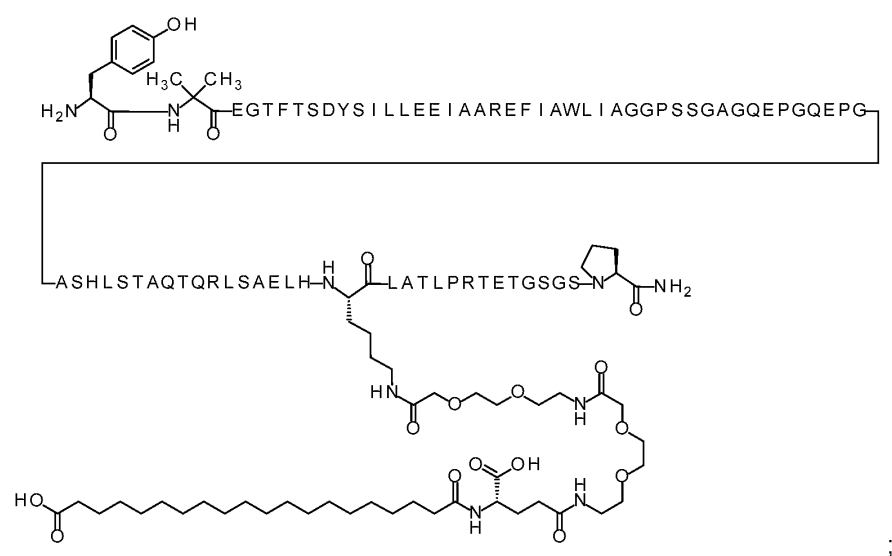
Compound 154



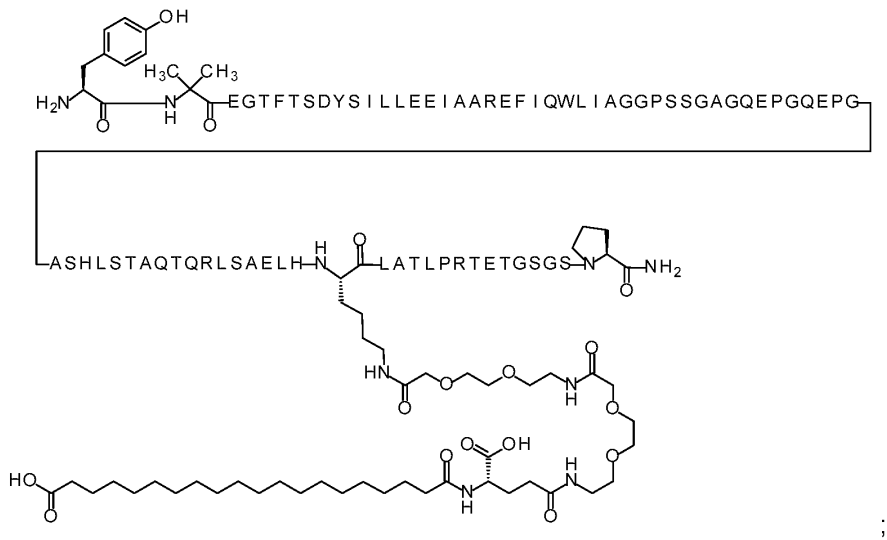
Compound 155



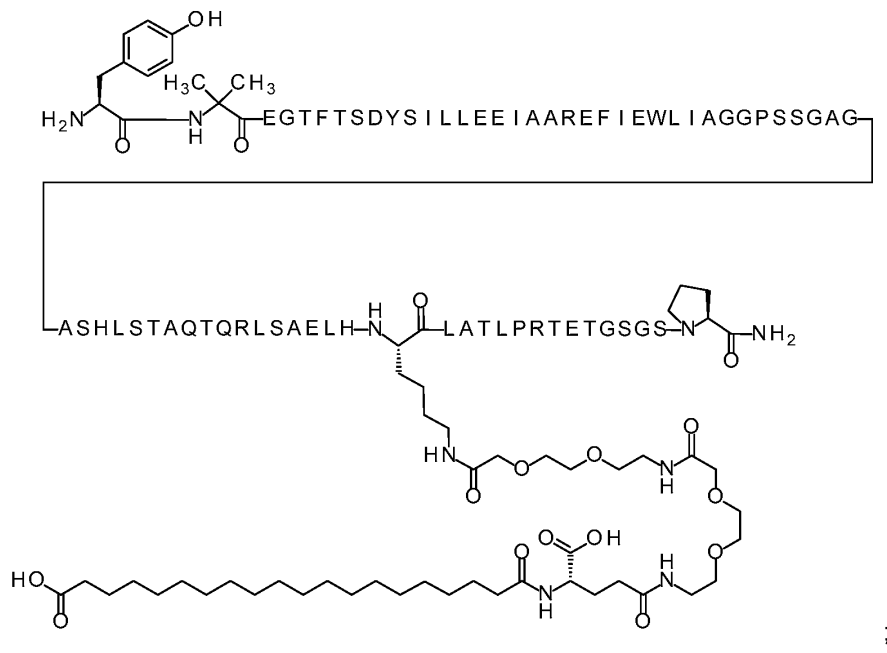
Compound 156



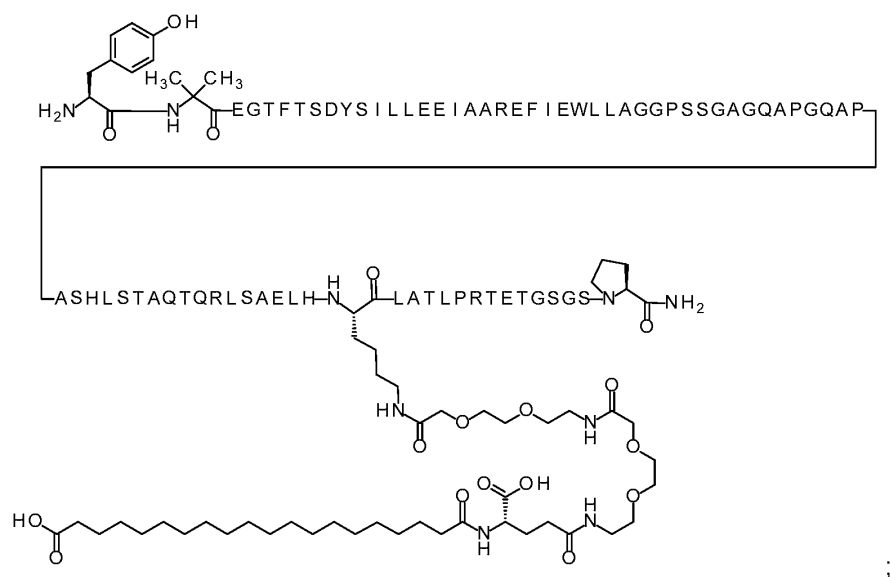
Compound 157



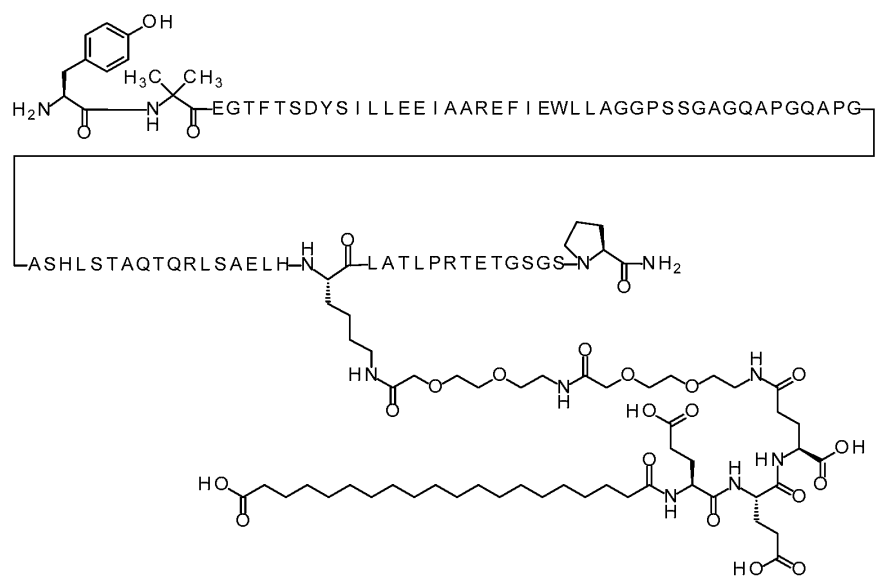
Compound 158



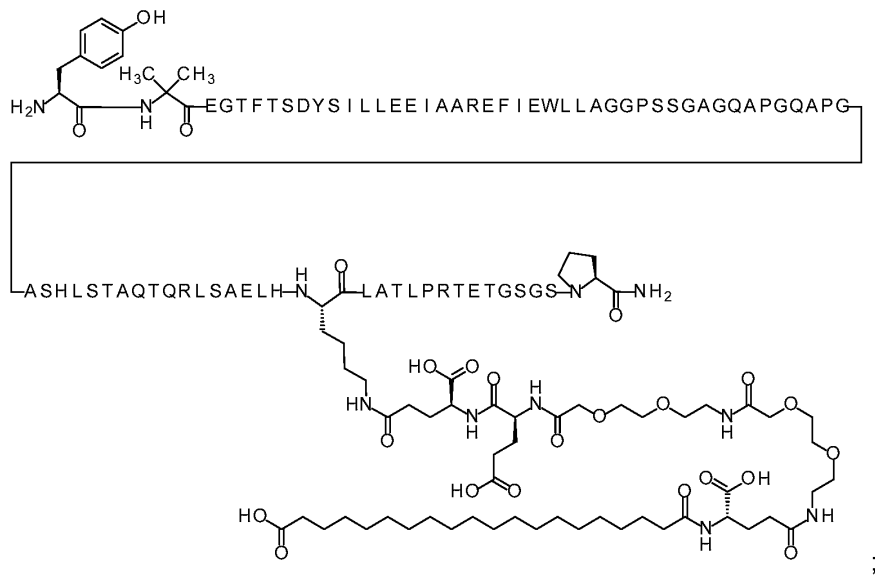
Compound 159



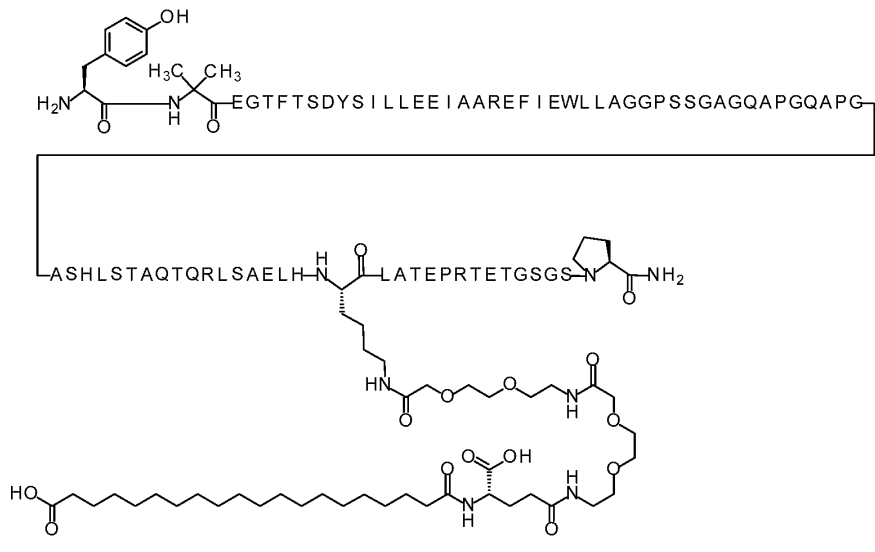
Compound 160



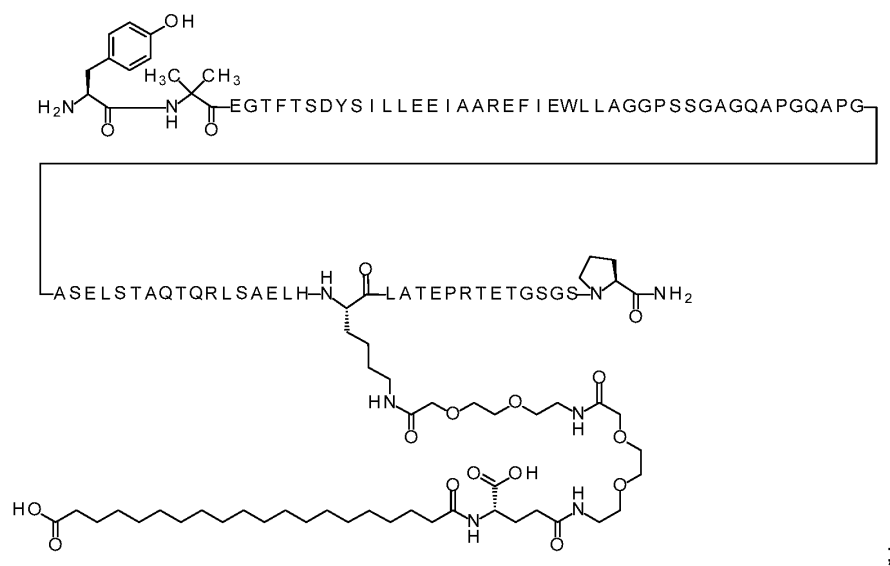
Compound 161



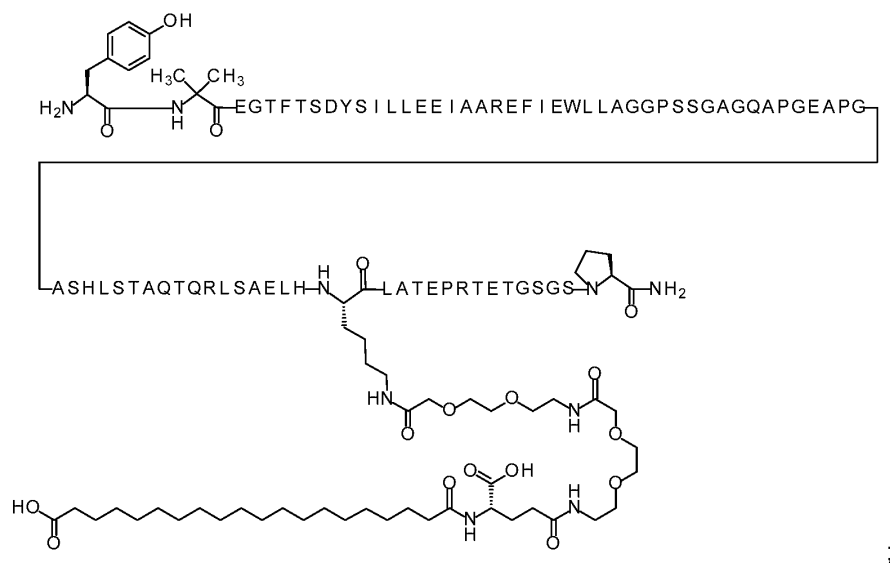
Compound 162



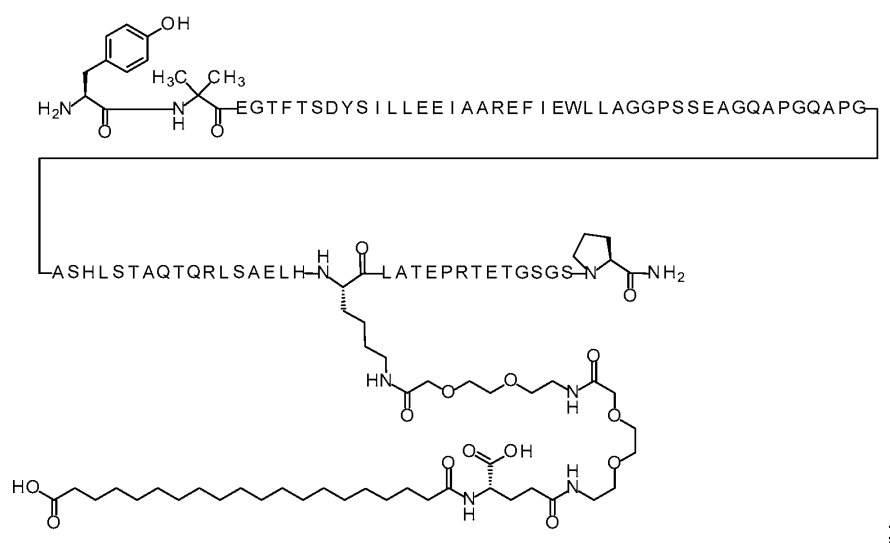
Compound 163



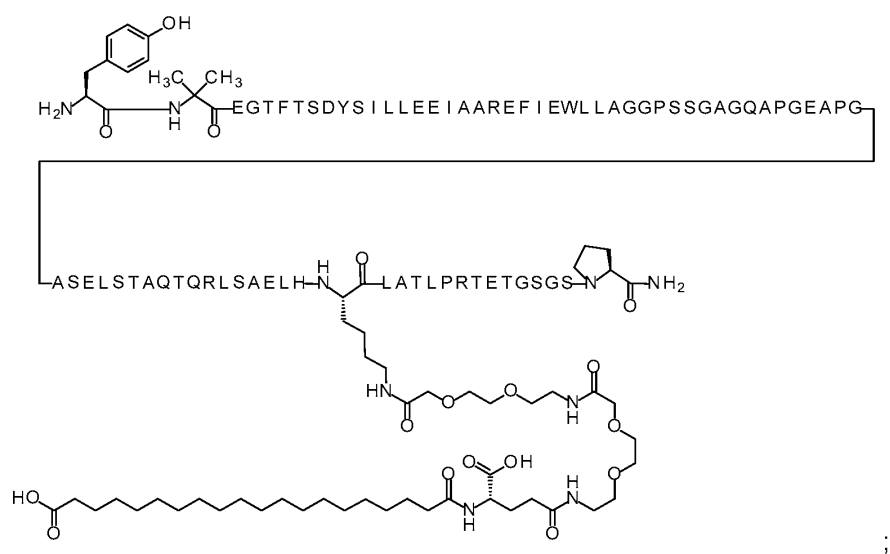
Compound 164



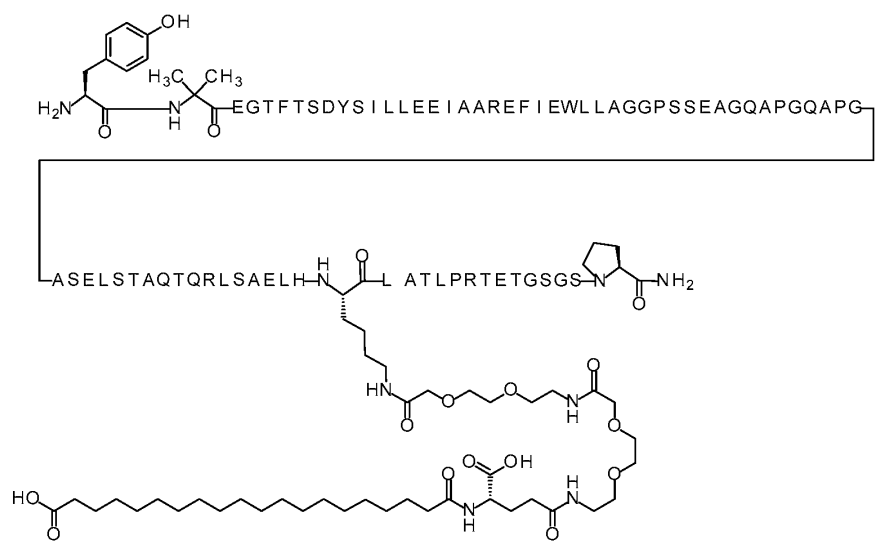
Compound 165



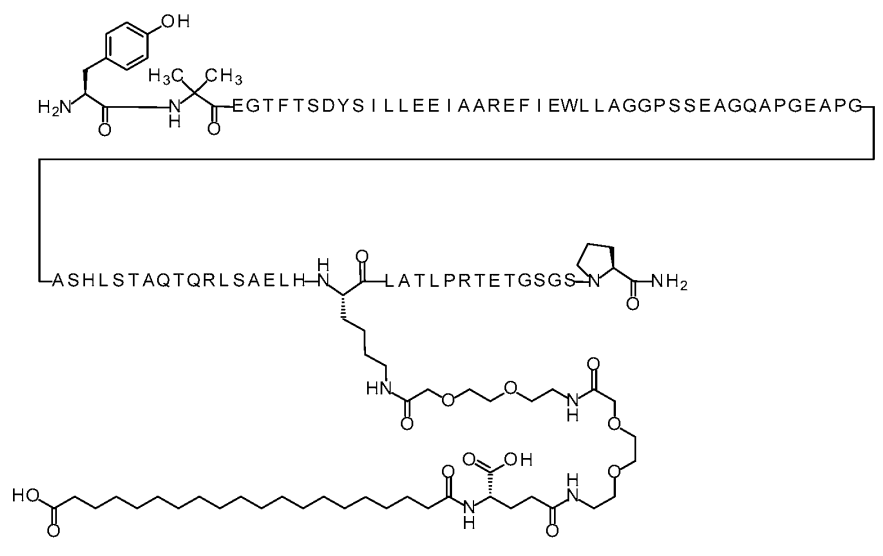
Compound 166



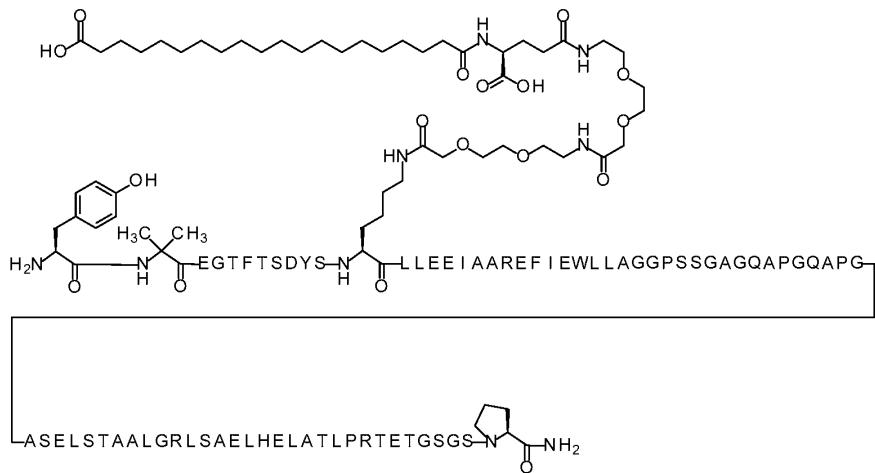
Compound 167



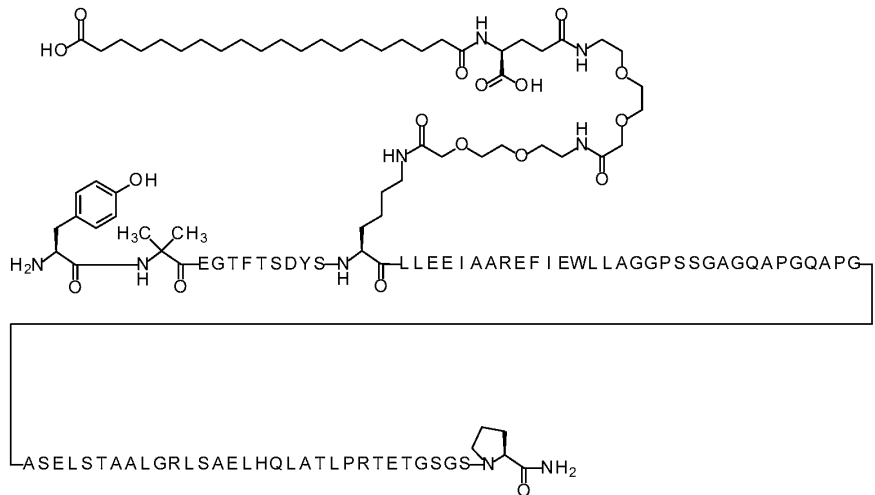
Compound 168



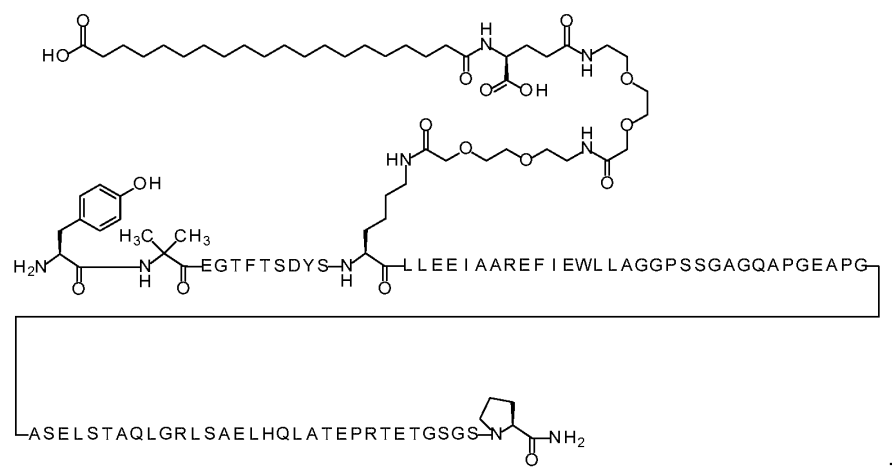
Compound 169



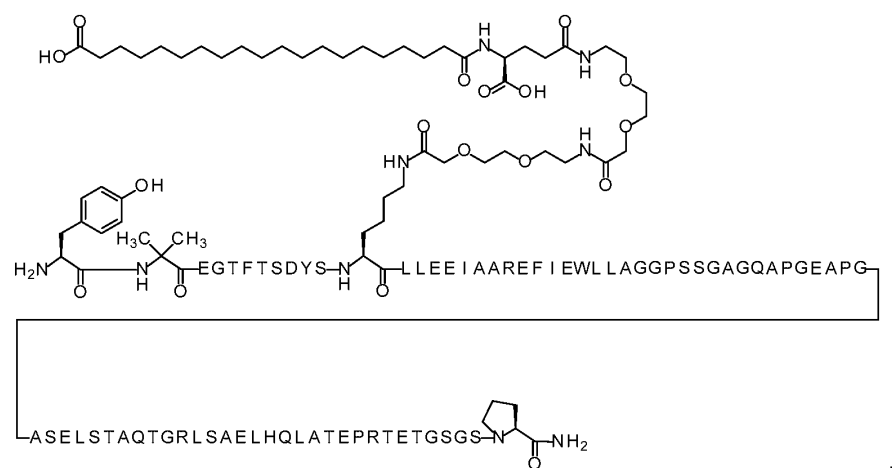
Compound 170



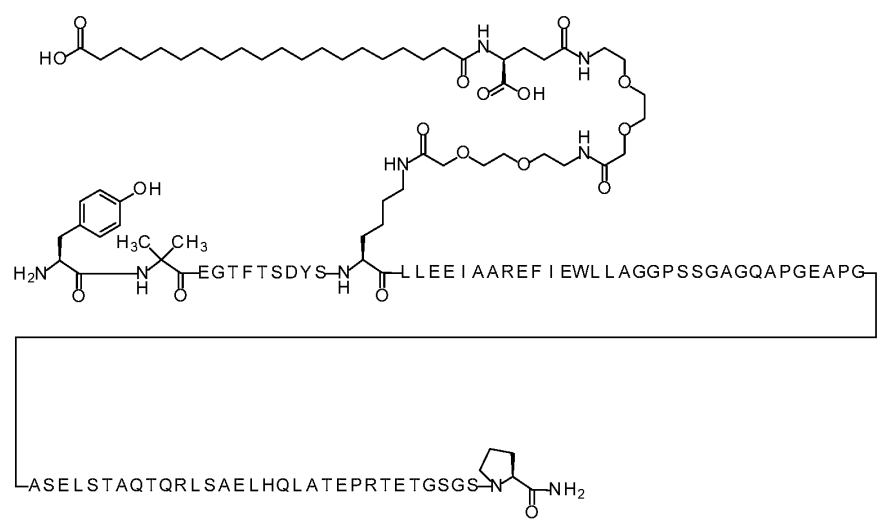
Compound 173



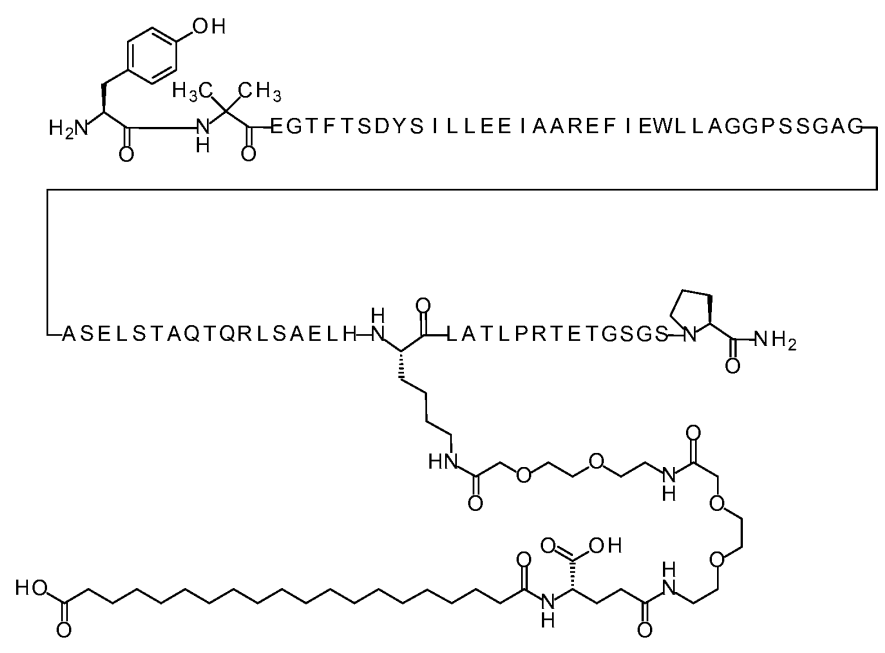
Compound 174



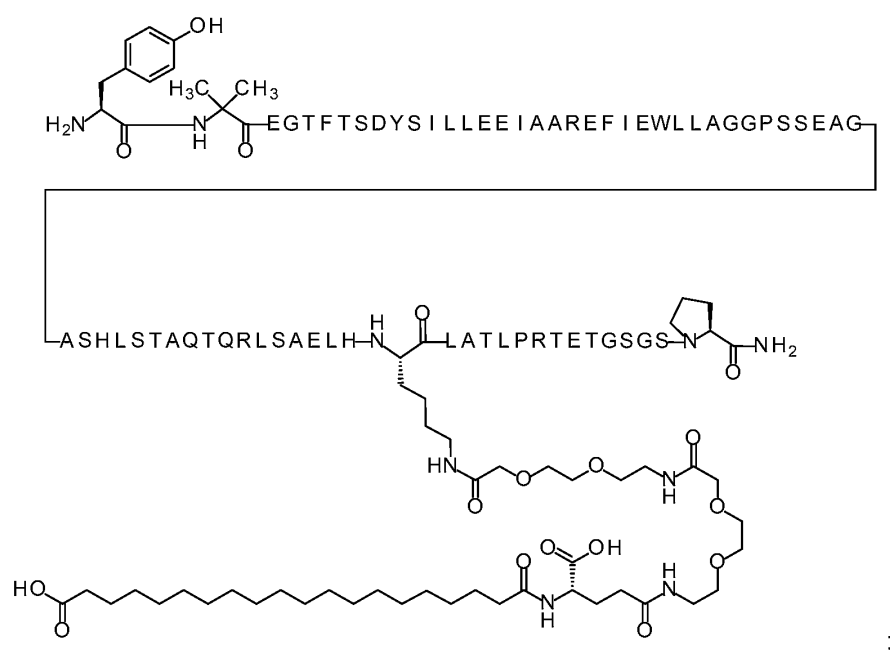
Compound 175



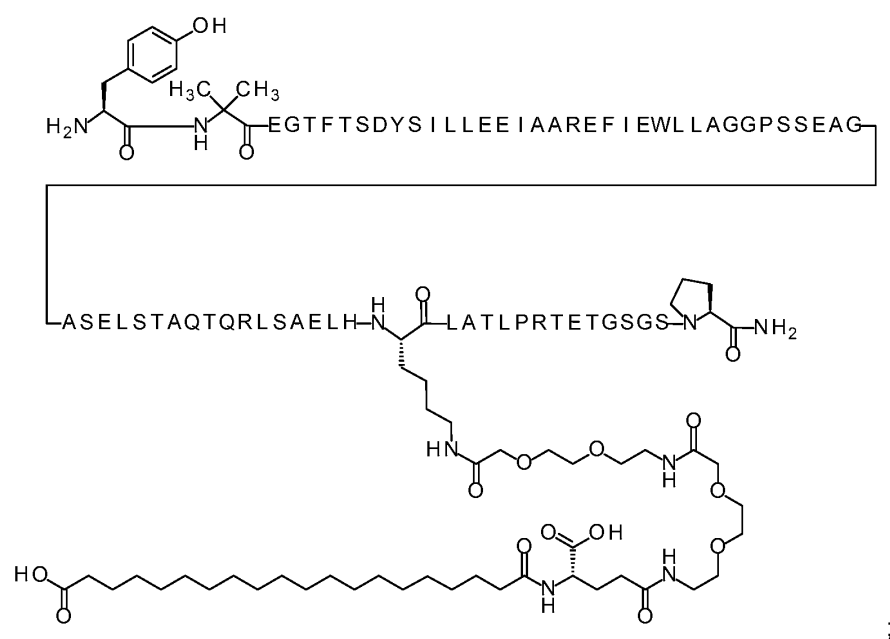
Compound 176



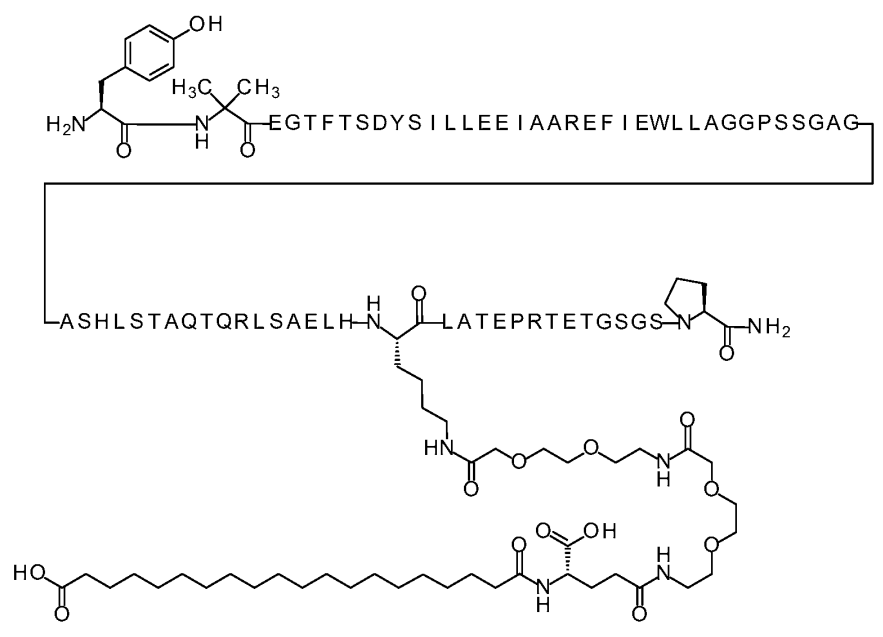
Compound 177



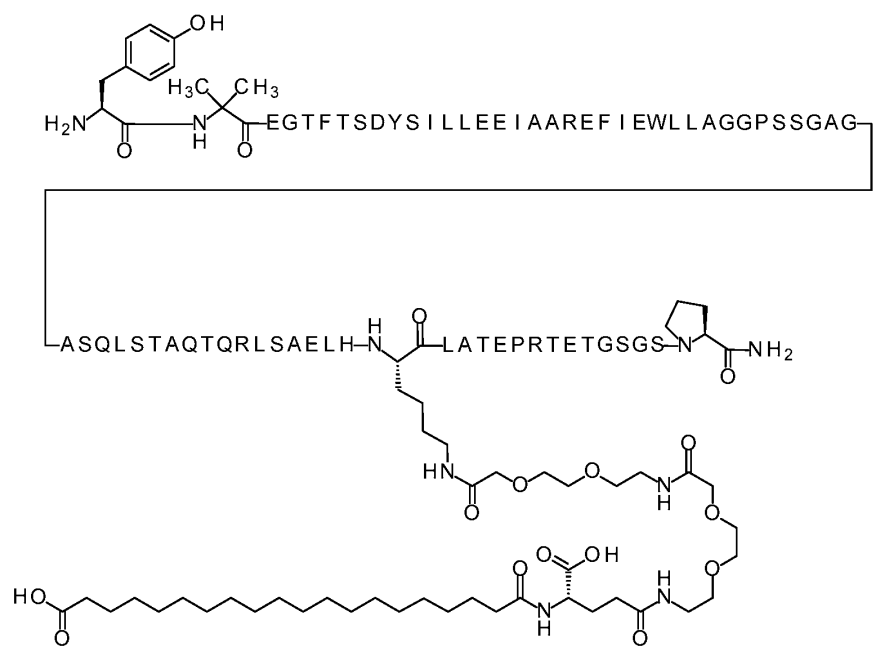
Compound 178



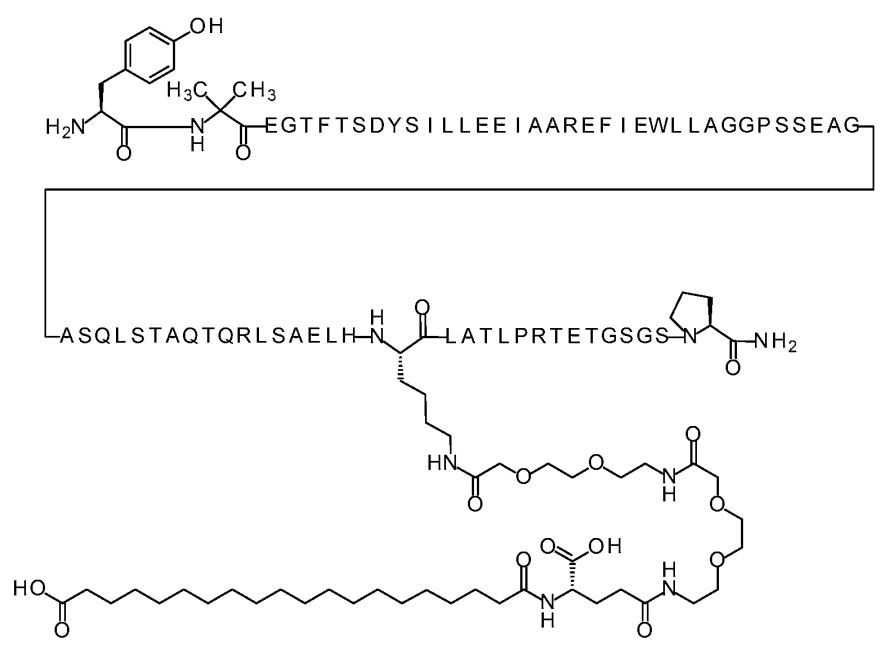
Compound 179



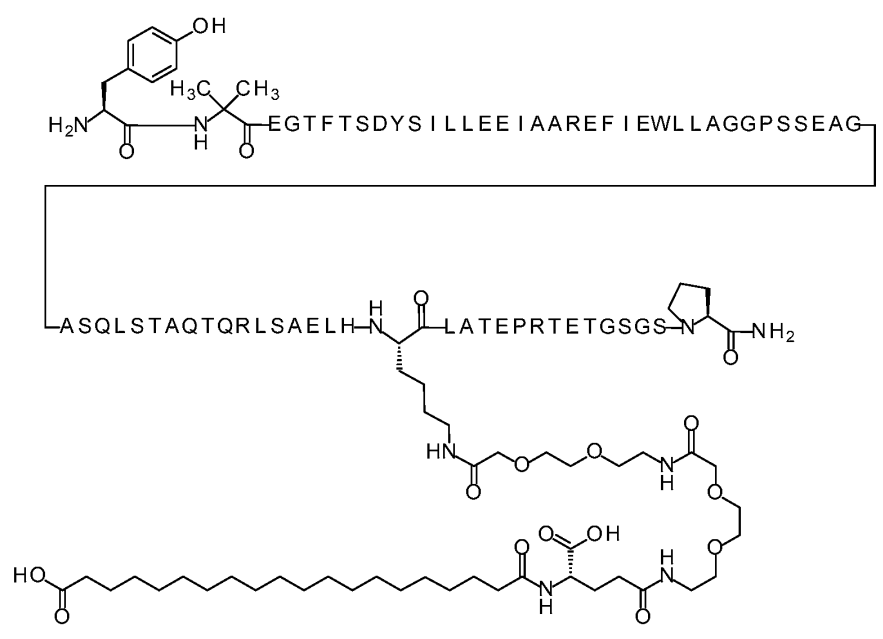
Compound 180



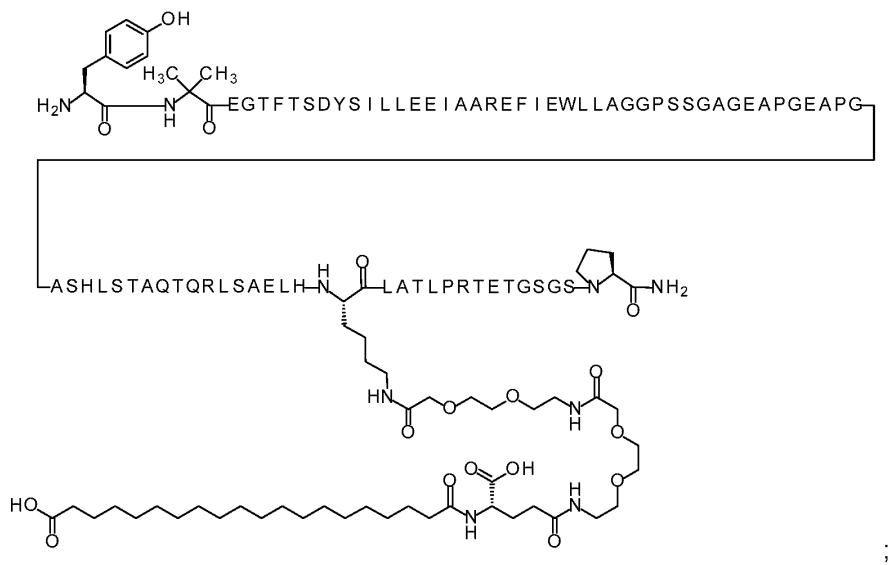
Compound 181



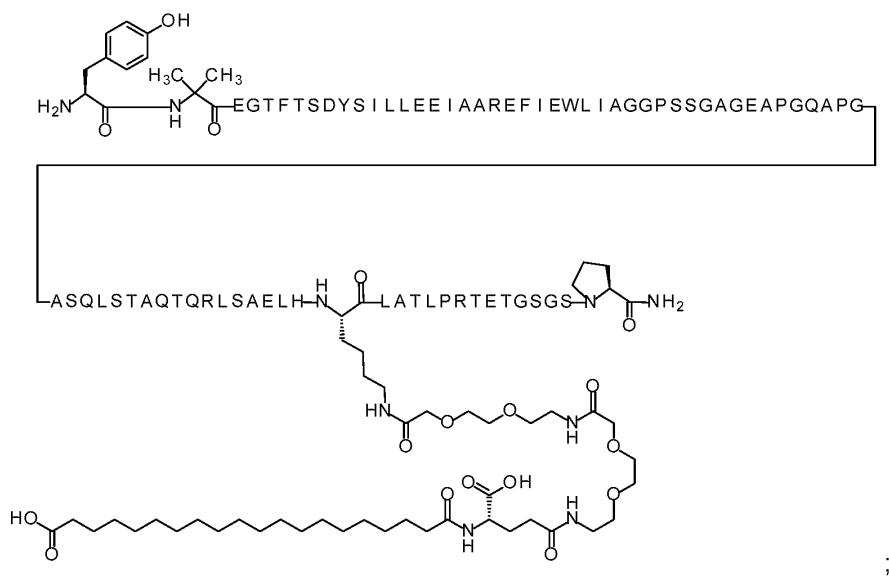
Compound 182



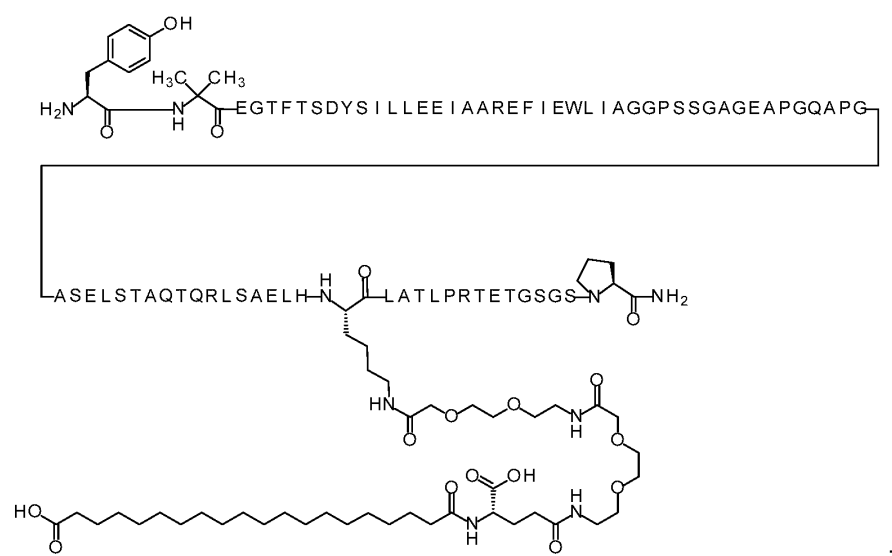
Compound 189



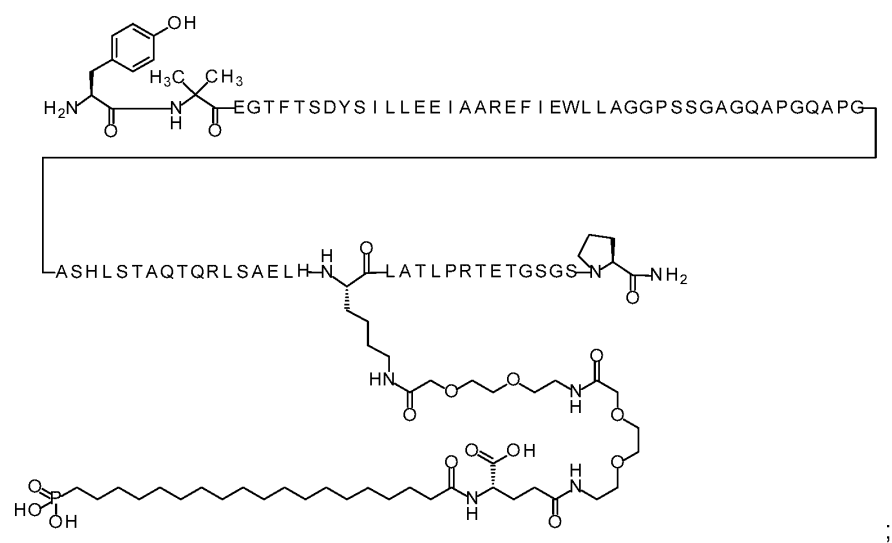
Compound 190



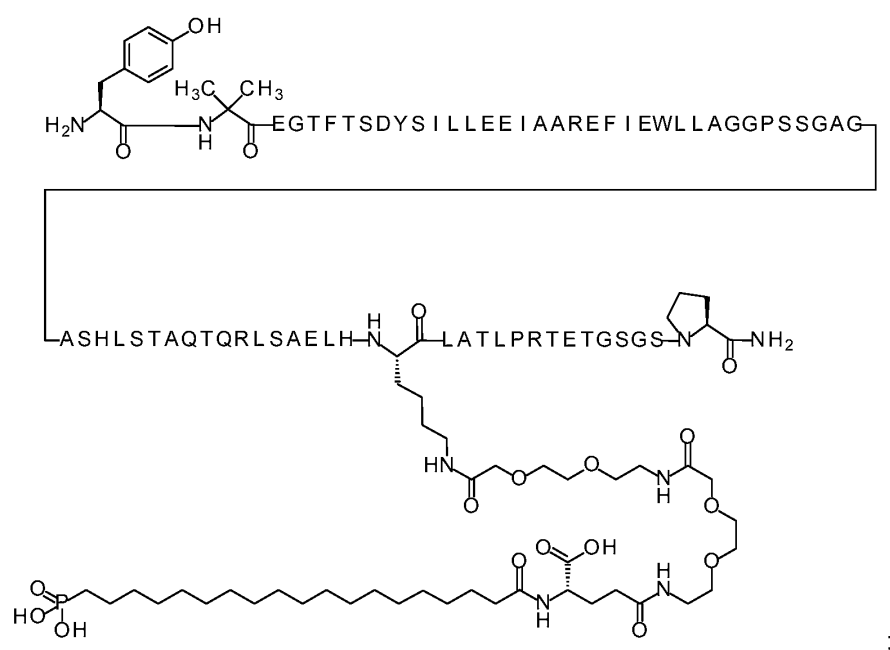
Compound 191



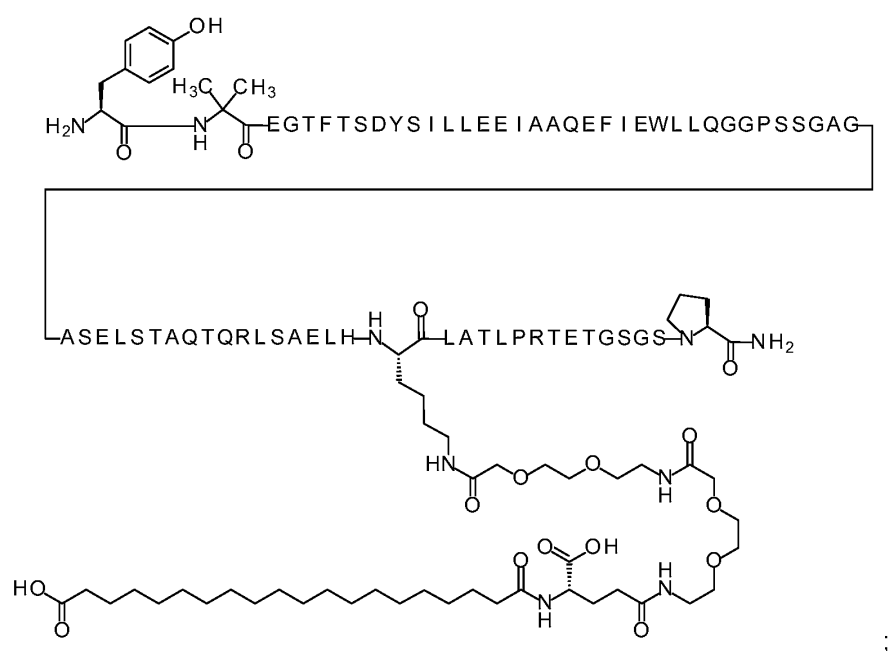
Compound 192



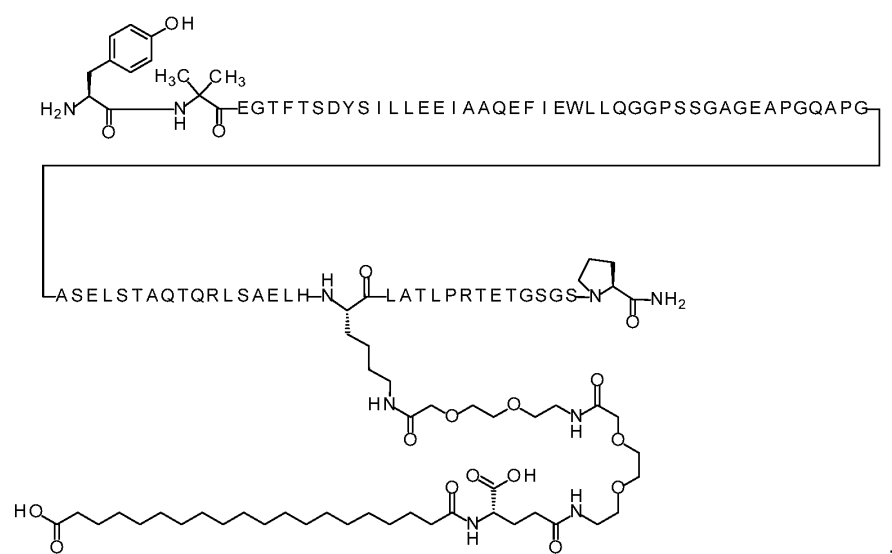
Compound 193



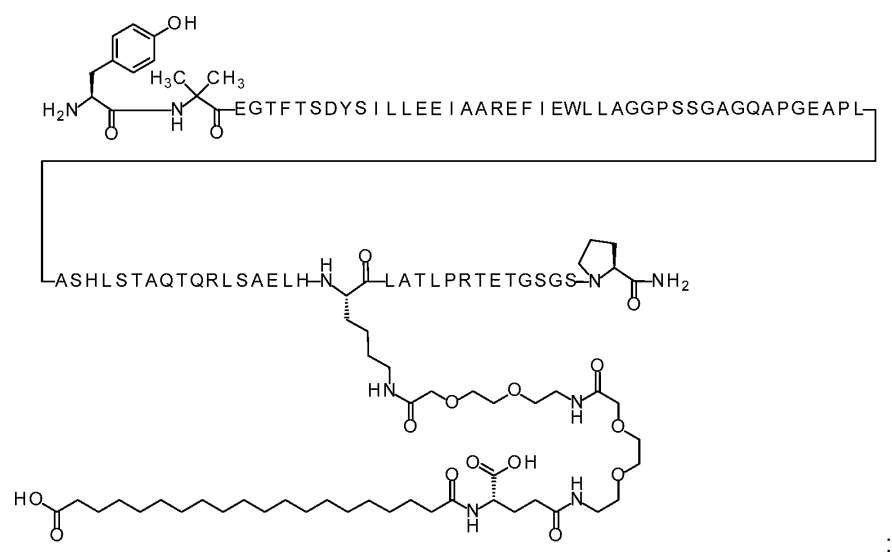
Compound 194



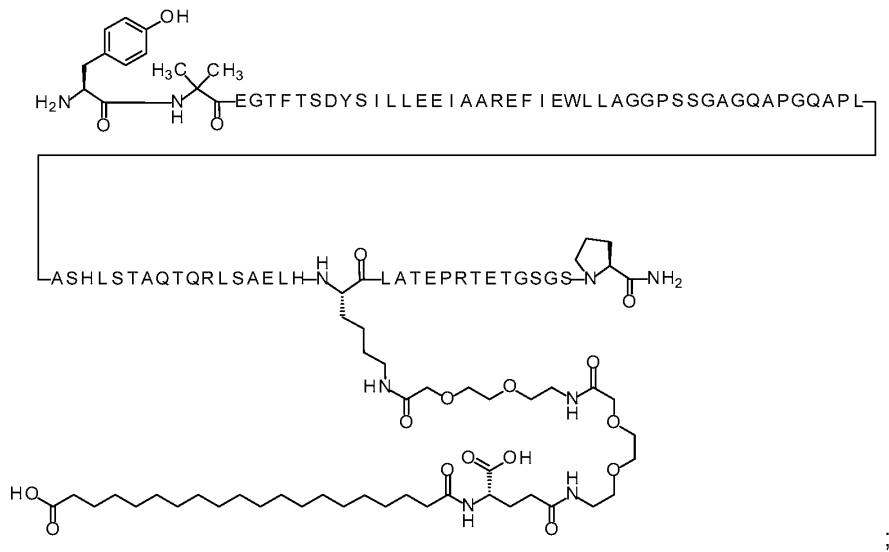
Compound 195



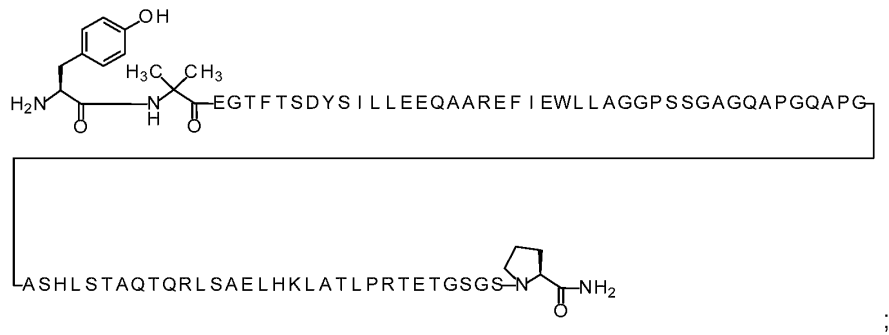
Compound 196



Compound 197

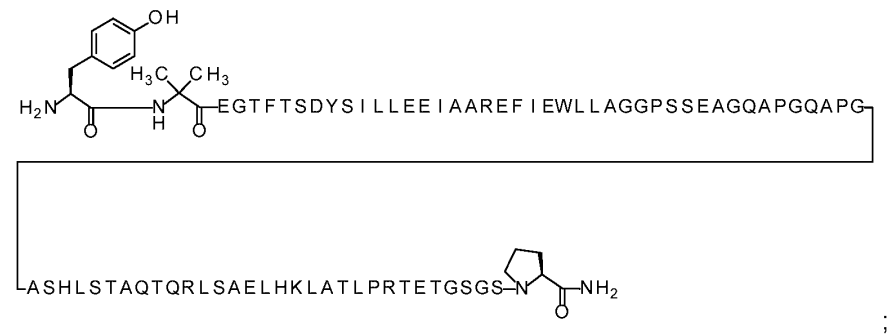


Compound 210

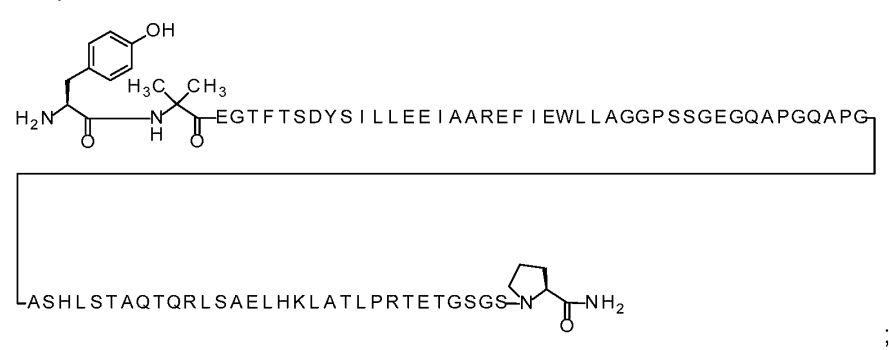


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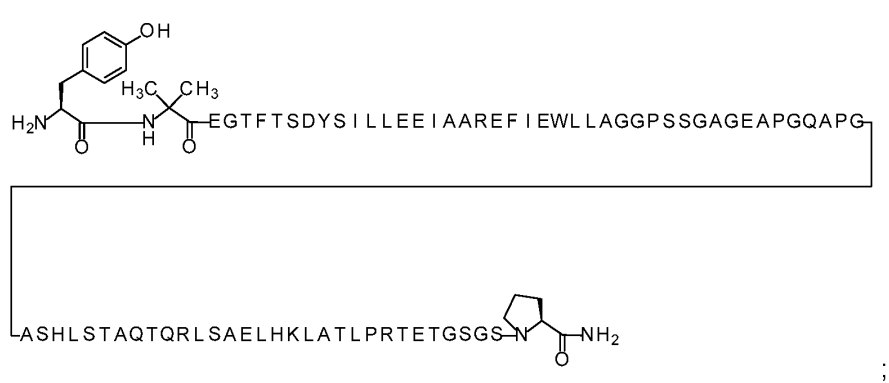
Compound 211



Compound 212

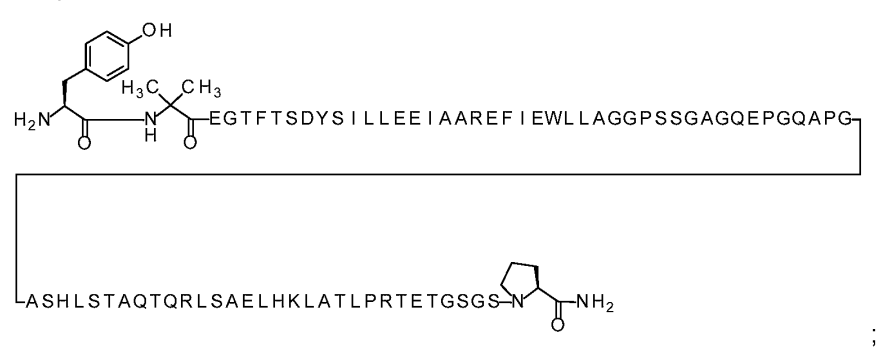


Compound 213

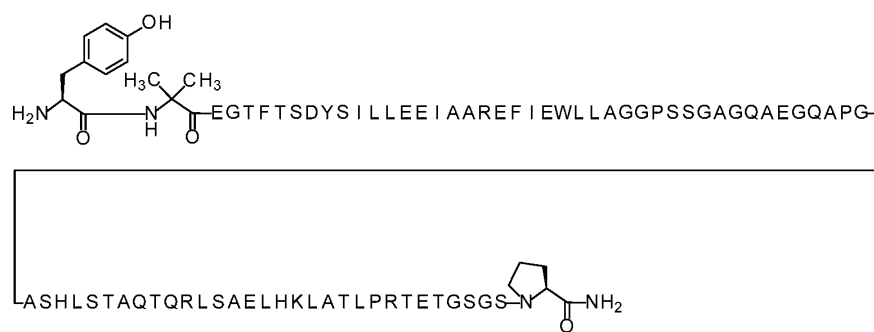


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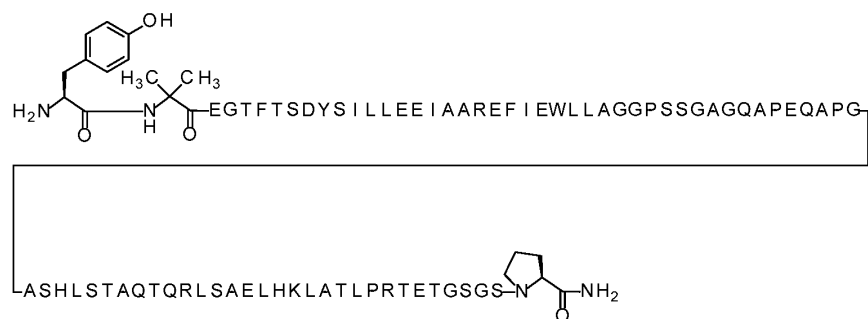
Compound 214



Compound 215

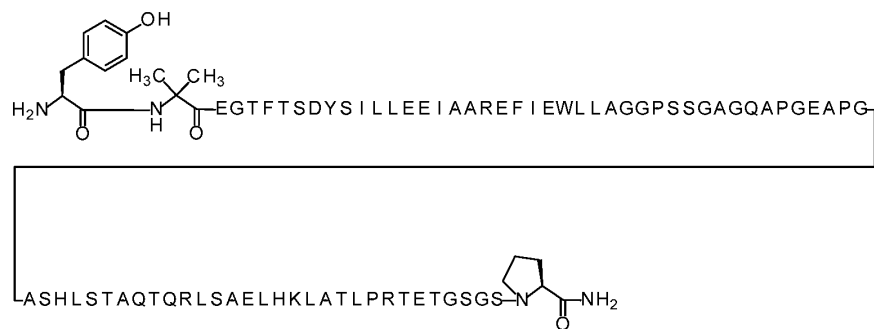


Compound 216

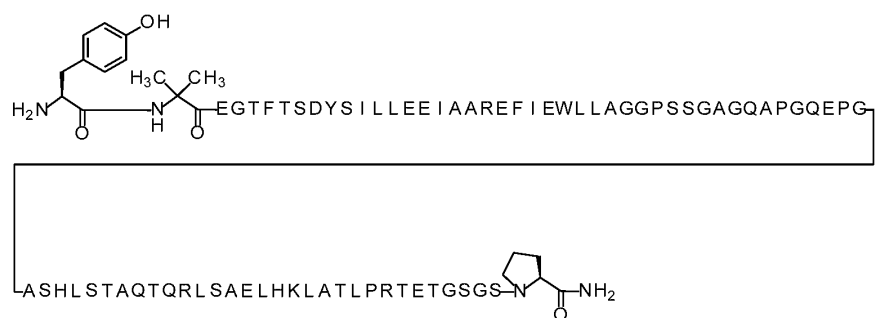


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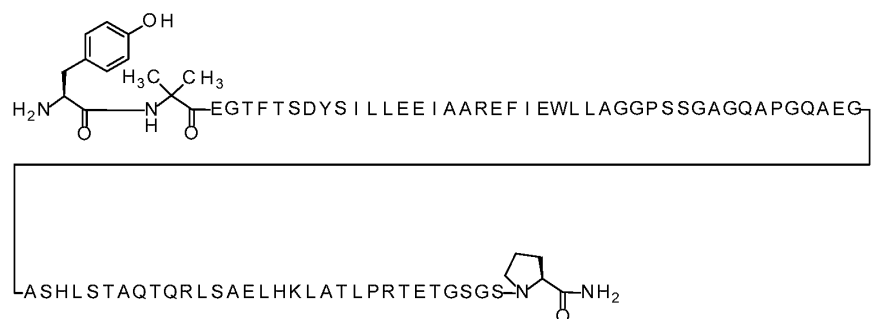
Compound 217



Compound 218

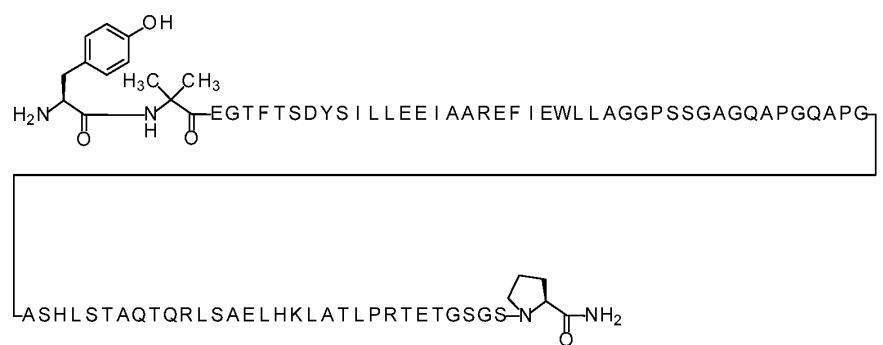


Compound 219

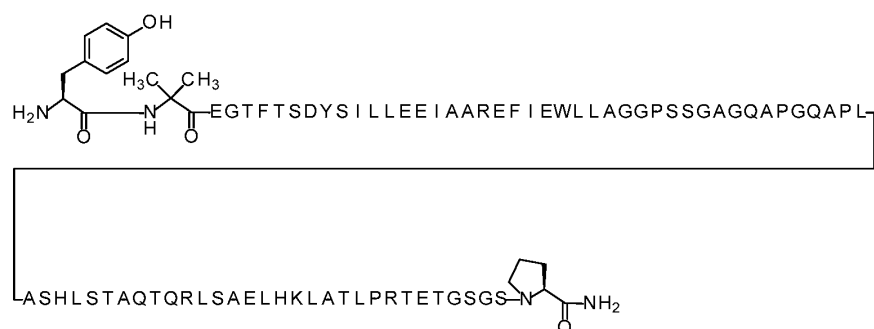


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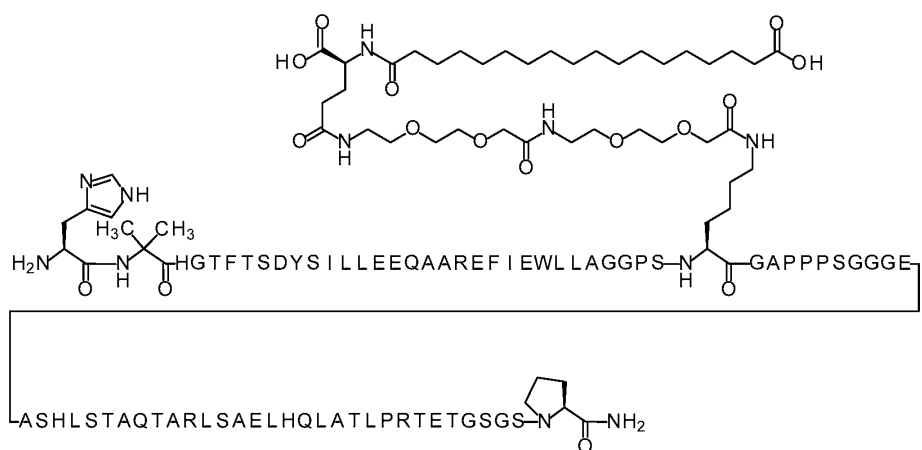
Compound 220



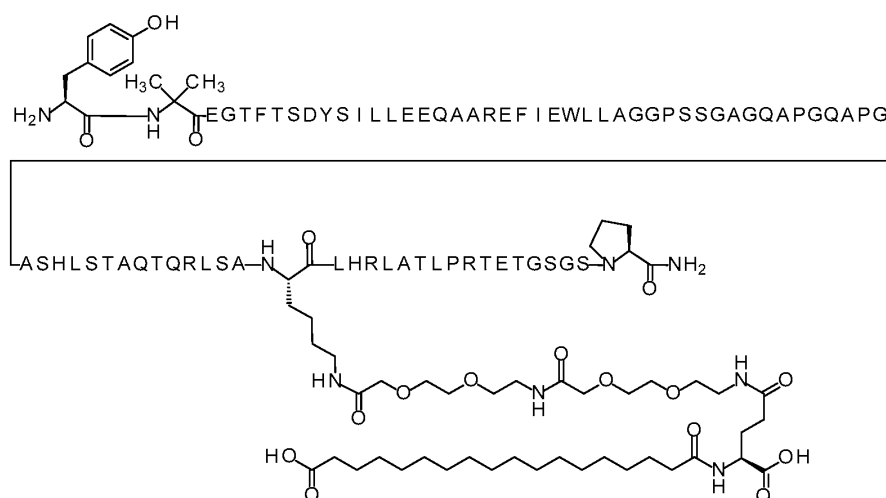
Compound 221



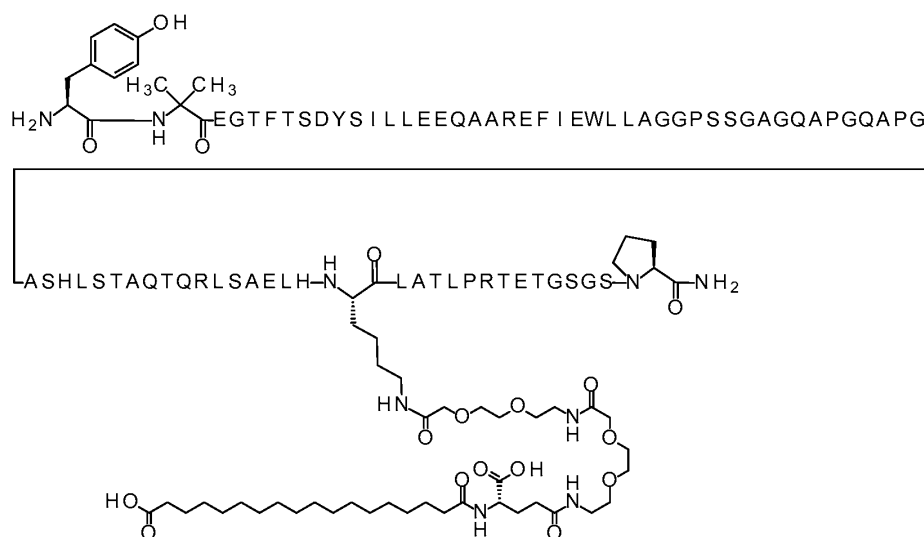
108. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is compound 101



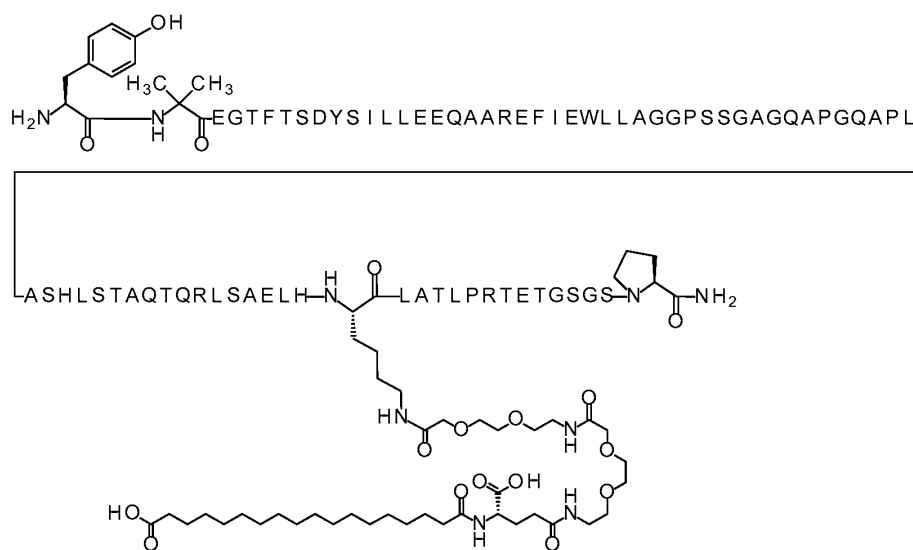
109. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is compound 55



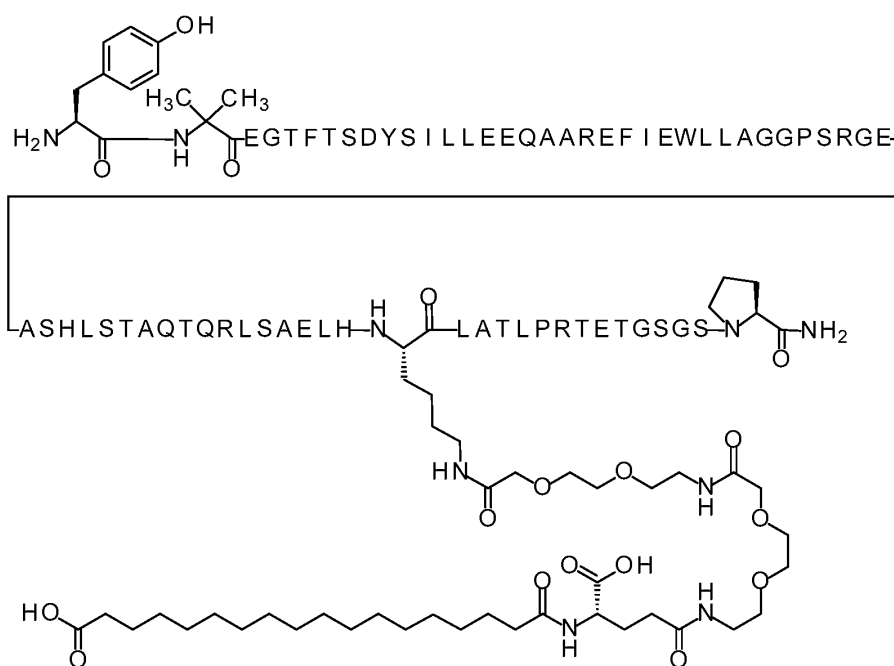
110. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is compound 52



111. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is compound 58



112. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is compound 68



119. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which is capable of activating the human GLP-1 receptor in an assay with whole cells expressing the human GLP-1 receptor.
- 5 120. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which activates the human GLP-1 receptor *in vitro*, preferably with an EC₅₀ of less than 125 pM, even more preferably with an EC₅₀ of less than 100 pM, and most preferably with an EC₅₀ of less than 50 pM, when measured without HSA in an assay as described in Example 4.
- 10 121. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which is capable of activating the human amylin receptor.
122. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
15 embodiments which is capable of activating the human amylin receptor in an assay with whole cells expressing the human amylin receptor.
123. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding
20 embodiments which activates the human amylin receptor *in vitro*, preferably with an EC₅₀ of less than 125 pM, even more preferably with an EC₅₀ of less than 100 pM, and most preferably with an EC₅₀ of less than 50 pM, when measured without HSA in an assay as described in Example 4.
- 25 124. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which is capable of activating the human GIP, GLP-1, and amylin receptors.
- 30 125. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which is capable of activating the human GIP, GLP-1, and amylin receptors in assays with whole cells expressing the human GIP receptor, GLP-1, and amylin receptors.
- 35 126. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments which activates the human GIP, GLP-1 and amylin receptors *in vitro*, preferably with an EC₅₀ of less than 125 pM, even more preferably with an EC₅₀ of

less than 100 pM, and most preferably with an EC₅₀ of less than 50 pM, when measured without HSA in assays as described in Example 4.

- 5 127. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the preceding embodiments, which activates the human GIP, GLP-1 and amylin receptors *in vitro*, when measured without HSA in assays as described in Example 4, and which has a potency ratio of less than 50.
- 10 128. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 127, wherein the potency ratio is less than 20.
129. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 127 or 128, wherein the potency ratio is less than 15, and most preferred less than 11.
- 15 130. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 81 to 114 which has improved pharmacokinetic properties.
131. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 81 to 114 which has an increased half-life.
- 20 132. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 131, which has an increased half-life of 40 hours to 145 hours, when determined in minipigs, preferably of 90 hours to 140 hours, even more preferably 85 hours to 125 hours.
- 25 133. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 81 to 132, which has improved chemical stability.
134. The GLP-1-/GIP-/amylin-receptor tri-agonist according to embodiment 133, which has improved chemical stability and which has a purity loss of no more than 6.0 percent per week, such as determined in Example 7 described herein, preferably a purity loss of less than 3.0 percent per week, such as determined in Example 7 described herein.
- 30 135. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of the embodiments 81 to 134 which has the effect *in vivo* of reducing food intake in normal
- 35

weight rats, as determined in the experimental protocol for efficacy testing on appetite, such as Example 5 described herein; or which has the effect *in vivo* of reducing food intake in DIO rats, as determined in sub-chronic treatment in DIO rats, such as Example 8 described herein.

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136. A pharmaceutically acceptable salt of the GLP-1-/GIP-/amylin-receptor triple agonist according to any one of the preceding embodiments.

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137. A pharmaceutical composition comprising a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of the preceding embodiments, and one or more pharmaceutically acceptable excipients.

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138. The pharmaceutical composition according to embodiment 137, which is for oral or for subcutaneous administration.

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139. The pharmaceutical composition according to the embodiment 137 or embodiment 138, which is a solid pharmaceutical composition.

25

140. The solid pharmaceutical composition according to embodiment 139, which is a tablet.

141. The solid pharmaceutical composition according to embodiment 139 or embodiment 140, comprising a salt of *N*-[8-(2-hydroxybenzoyl)amino] caprylate, preferably sodium *N*-(8-(2-hydroxybenzoyl)amino)caprylate and magnesium stearate.

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142. The solid pharmaceutical composition according to any one of the embodiments 139 to 141, comprising 75-600 mg sodium *N*-(8-(2-hydroxybenzoyl)amino)caprylate and 7-8.5 mg magnesium stearate.

143. The pharmaceutical composition according to any one of embodiments 137 to 142, which is for dosing approximately once daily, such as once every 12-36 hours, such as once every 18-30 hours, such as approximately once every 24 hours; or which is for dosing approximately once weekly, such as once every 6-8 days.

144. An injection device comprising the GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of embodiments 1 to 136, or the pharmaceutical composition according to any one of embodiments 137 to 143.
- 5 145. The GLP-1-/GIP-/amylin-receptor tri-agonist peptide according to any of embodiments 1 to 78 for use as an intermediate in the manufacture of a GLP-1-/GIP-/amylin-receptor tri-agonist peptide derivative according to any of embodiments 81 to 135.
- 10 146. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136 or the pharmaceutical composition according to any one of embodiments 137 to 143 for use as a medicament.
- 15 147. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136 or the pharmaceutical composition according to any one of embodiments 137 to 143 for use in the treatment of type 2 diabetes, obesity, metabolic dysfunction-associated steatohepatitis (MASH), and/or cardiovascular disease.
- 20 148. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136 or the pharmaceutical composition according to any one of embodiments 137 to 143, for use in the treatment of a subject with an initial body mass index (BMI) of 25 or more, 27 or more, or 28 or more, or 30 or more.
- 25 149. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136 or the pharmaceutical composition according to any one of embodiments 137 to 143, for use in the treatment of a subject with an initial body mass index (BMI) of 25 or more, 27 or more, or 28 or more, 30 or more; and in the presence of at least one weight-related co-morbidity.
- 30
- 35 150. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136 or the pharmaceutical composition according to any one of embodiments 137 to 143, for use as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management in an adult subject suffering from obesity, i.e. with an initial body mass index (BMI) of 25 or more, or 27

or more, or 28 or more, 30 or more; or in an adult subject suffering from overweight, i.e. with an initial body mass index (BMI) of 23 or more, or 24 or more, or 25 or more, or 27 or more.

- 5 151. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of
embodiments 1 to 136 or the pharmaceutical composition according to any one of
embodiments 137 to 143, for use as an adjunct to a reduced-calorie diet and
increased physical activity for chronic weight management in an adult subject
suffering from obesity, i.e. with an initial body mass index (BMI) of 25 or more, or 27
10 or more, or 28 or more, 30 or more; or in an adult subject suffering from overweight,
i.e. with an initial body mass index (BMI) of 23 or more, or 24 or more, or 25 or more,
or 27 or more; and in the presence of at least one weight-related co-morbidity.
- 15 152. The use according to embodiment 149 or embodiment 151, wherein the at least one
weight-related co-morbidity is selected from the group consisting of hypertension,
dysglycaemia (prediabetes or type 2 diabetes), dyslipidaemia, high cholesterol,
cardiovascular disease and obstructive sleep apnoea.
- 20 153. Use of a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of
embodiments 1 to 136 in the manufacture of a medicament for the treatment of type 2
diabetes, obesity, metabolic dysfunction-associated steatohepatitis (MASH), and/or
cardiovascular disease.
- 25 154. Use of a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of
embodiments 1 to 136 in the manufacture of a medicament for the treatment of a
subject with an initial body mass index (BMI) of 25 or more, 27 or more, or 28 or
more, 30 or more; and optionally in the presence of at least one weight-related co-
morbidity.
- 30 155. Use of a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of
embodiments 1 to 136 in the manufacture of a medicament for the treatment of an
adult subject suffering from obesity, i.e. with an initial body mass index (BMI) of 25 or
more, or 27 or more, or 28 or more, 30 or more; or an adult subject suffering from
overweight, i.e. with an initial body mass index (BMI) of 23 or more, or 24 or more, or

25 or more, or 27 or more; and optionally in the presence of at least one weight-related co-morbidity.

- 5 156. Use of a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of
embodiments 1 to 136 in the manufacture of a medicament for chronic weight
management in an adult subject suffering from obesity, i.e. with an initial body mass
index (BMI) of 25 or more, or 27 or more, or 28 or more, 30 or more; or in an adult
subject suffering from overweight, i.e. with an initial body mass index (BMI) of 23 or
10 more, or 24 or more, or 25 or more, or 27 or more; and optionally in the presence of
at least one weight-related co-morbidity.
- 15 157. The use according to embodiment 155 or embodiment 155, wherein the
medicament is an adjunct to a reduced-calorie diet and increased physical activity for
chronic weight management in an adult subject suffering from obesity or overweight.
- 20 158. A method for treating type 2 diabetes, obesity, metabolic dysfunction-associated
steatohepatitis (MASH), and/or cardiovascular disease comprising administering a
pharmaceutically relevant amount of a GLP-1-/GIP-/amylin-receptor triple agonist
according to any one of embodiments 1 to 136 to a subject in need thereof.
- 25 159. A method of treating a human subject with an initial body mass index (BMI) of 25 or
more, 27 or more, or 28 or more, or 30 or more, comprising administering to said
human subject a pharmaceutically relevant amount of a GLP-1-/GIP-/amylin-receptor
triple agonist according to any one of embodiments 1 to 136.
- 30 160. A method for reducing excess body weight in a human subject, in combination with
a reduced-calorie diet and increased physical activity, comprising administering to
said human subject a pharmaceutically relevant amount of a GLP-1-/GIP-/amylin-
receptor triple agonist according to any one of embodiments 1 to 136.
161. The method according to embodiment 159 or embodiment 160, wherein said human
subject is an adult subject, suffering from overweight and has an initial body mass
index (BMI) of 23 or more, or 24 or more, or 25 or more, or 27 or more.

162. The method according to embodiment 159 or embodiment 160, wherein said human subject is an adult subject, suffering from obesity and has an initial body mass index (BMI) of 25 or more, or 27 or more, or 28 or more, 30 or more.
- 5 163. The method according to any one of embodiments 159 to 162, wherein said human subject has at least one weight-related comorbidity selected from the group consisting of hypertension, dysglycaemia (prediabetes or type 2 diabetes), dyslipidaemia, high cholesterol, cardiovascular disease and obstructive sleep apnoea.
- 10 164. A method for preparing the GLP-1-/GIP-/amylin-receptor triple agonist according to any one of embodiments 1 to 136.
- 15 165. The method according to embodiment 164, comprises a step of solid phase peptide synthesis.

Examples

Materials and Methods

List of Abbreviations

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

	Ado:	8-amino-3,6-dioxaoctanoic acid
	Aib:	2-aminoisobutyric acid
	amu:	atomic mass unit
	BHK	Baby Hamster Kidney
10	Boc:	t-butyloxycarbonyl
	CAD:	Charged Aerosol Detector
	cAMP:	cyclic adenosine monophosphate
	CRE:	cAMP response element
	DCM:	dichloromethane
15	DIC:	<i>N,N'</i> -diisopropylcarbodiimide
	DIO:	Diet Induced Obese
	DMB:	2,4-dimethoxybenzyl
	DMEM:	Dulbecco's Modified Eagle's Medium
	DMF:	<i>N,N</i> -dimethyl formamide
20	DTT:	1,4-dithiothreitol
	EC50:	half maximal effective concentration
	EDTA:	ethylenediaminetetraacetic acid
	ES:	Electrospray
	FBS:	Foetal Bovine Serum
25	Fmoc:	9-fluorenylmethyloxycarbonyl
	FWHM:	Full Width at Half Maximum
	GIP:	Glucose-dependent Insulinotropic Polypeptide
	GLP-1:	Glucagon-Like Peptide-1
	hAMYR3:	human Amylin receptor 3
30	hGIPR:	human Glucose-dependent Insulinotropic Polypeptide Receptor

	hGLP-1R:	human Glucagon-Like Peptide-1 Receptor
	HEPES:	<i>N</i> -(2-Hydroxyethyl)piperazine- <i>N'</i> -(2-ethanesulfonic acid)
	HFIP:	1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroisopropanol
	HPLC:	High Performance Liquid Chromatography
5	HSA:	Human Serum Albumin
	i.v.:	intravenously
	LCMS or LC-MS:	Liquid Chromatography Mass Spectrometry
	LLOQ:	Lower limit of Quantitation
	Luc:	luciferase
10	MeCN:	acetonitrile
	MRI:	Magnetic resonance imaging
	MS:	Mass Spectroscopy
	Mtt:	4-methyltrityl
	NCA:	non-compartmental pharmacokinetic method
15	nd:	not determined
	OtBu:	tert-butoxy
	Oxyma Pure®:	cyano-hydroxyimino-acetic acid ethyl ester
	Pbf:	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
	PBS:	phosphate-buffered saline
20	PK:	pharmacokinetic
	QD:	quaque die (once a day)
	QTof:	Quadrupole Time of Flight
	RAMP3:	receptor modifying protein 3
	RT:	Room Temperature
25	s.c.:	subcutaneously
	SD:	Sprague Dawley
	SEM:	Standard Error of Mean
	SPPS:	Solid Phase Peptide Synthesis
	tBu:	tert-butyl
30	TFA:	trifluoroacetic acid
	TIPS:	triisopropylsilane

TQ: Triple Quadrupole
 Trt: triphenylmethyl or trityl
 UPLC: Ultra Performance Liquid Chromatography
 UV: Ultraviolet

5 Fatty diacid and special amino acid building blocks

For synthesis of octadecanedioic acid mono-tert-butyl ester (C₁₈ diacid mono-tert-butyl ester): see patent application WO 2010/102886 (pages 27–28). The corresponding mono-tert-butyl esters of C₁₂–C₂₀ diacid, in particular C₁₆ diacid and C₂₀ diacid, can be prepared accordingly.

- 10 Fmoc-Leu-Ser($\psi^{\text{Me, Me}}\text{pro}$)-OH, Fmoc-Tyr(*t*Bu)-Ser($\psi^{\text{Me, Me}}\text{pro}$)-OH, and Fmoc-Gly-(DMB)Gly-OH were commercially available from TechnoComm Ltd.

General method for peptide synthesis

- The preparation of the peptides (for reference compounds and compounds of the invention) was carried out with SPPS using Fmoc based chemistry on a Symphony X from Protein Technologies, a PurePep Chorus from Protein Technologies, a MultiPep 2 from CEM, a Vapourtec RS-500 from Vapourtec or a CS136XT from CSBioon. The Fmoc-protected amino acids used in the methods were the standard recommended: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(*O**t*Bu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(*O**t*Bu)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Val-OH, Fmoc-Lys(Mtt)-OH, Boc-His(Trt)-OH, Fmoc-Aib-OH, Fmoc-Glu-*O**t*Bu, and Fmoc-Ado-OH supplied from e.g. Gyros Protein Technologies, Bachem, Iris Biotech, or NovabioChem. The building blocks Fmoc-Leu-Ser($\psi^{\text{Me, Me}}\text{pro}$)-OH, Fmoc-Tyr(*t*Bu)-Ser($\psi^{\text{Me, Me}}\text{pro}$)-OH, and Fmoc-Gly-(DMB)Gly-OH, e.g. commercially available from TechnoComm Ltd, were introduced where applicable.

Fmoc-PAL AM resin or Rink-Amide AM resin were used, which were commercially available from NovabioChem. The subsequent amino acids were introduced in a stepwise procedure by the Symphony X peptide synthesizer following the SPPS principles.

- Fmoc-deprotection was achieved with 20% piperidine in DMF with 0.1 M Oxyma Pure for 2 x 10 min. Introduction of the substituent (i.e. protraction moiety comprising a “protractor” P and an optional “linker L_P”) at the alpha-position of the N-terminal amino acid was accomplished using a standard Fmoc-protected amino acid. The peptide couplings were performed with DIC and collidine. Amino acid/Oxyma Pure solutions (0.3 M/0.3 M in DMF at

a molar excess of 5-10-fold) was added to the resin first. Then, the same molar equivalent of DIC was added (1.5 M in DMF), followed by collidine (1.5 M in DMF). Most commonly, it was mixed for 2 hours. In some cases, the coupling time was increased, additional DIC was added, or the coupling step was repeated. Afterwards, a capping step was performed with 1 M acetic anhydride in DMF and collidine. Introduction of the protraction moiety at the epsilon-nitrogen of a lysine (Lys, K) within the sequence was achieved using Fmoc-Lys(Mtt)-OH. Following the synthesis of the peptide backbone sequence the Mtt group was removed by treatment with HFIP/DCM/TIPS (75:23:2) (5 min), followed by a wash with DCM. The resin was then resuspended in HFIP/DCM/TIPS (75:23:2) (2x25 min), and subsequently washed with DCM and DMF. The protraction moieties were introduced in a stepwise procedure as described above, using suitably protected building blocks for the linker L_P, such as the standard Fmoc-protected amino acids such as Fmoc-8-amino-3,6-dioxaoctanoic acid or Fmoc-Glu-OtBu. Introduction of the protractor, fatty acid group, was achieved using the suitable building block, such as but not limited to, octadecanedioic acid mono-tert-butyl-ester.

15 General cleavage method

The peptides were cleaved from the resin with TFA/TIPS/H₂O/DTT (90:4:3:3) for 2-3 hours. Hereafter, the peptide was drained into cold diethyl ether, and centrifuged. The ether was decanted off, and the peptide precipitate was washed with ether two additional times.

General method for purification and quantification of the derivative

20 The crude peptide was dissolved in acetic acid/MeCN/Milli-Q water (45:10:45 or 40:20:40) and orthogonally purified by reversed-phase preparative HPLC (Waters Delta Prep 4000) on a column comprising C18-silica gel. The first elution was performed with an increasing gradient from 20-50% of MeCN in Milli-Q water comprising 1% ammonium bicarbonate. Relevant fractions were analysed with UPLC. Fractions containing the target peptide were pooled and diluted with Milli-Q water (1:1) prior to a second reversed-phase preparative HPLC. The second elution was performed with an increasing gradient from 20-50% of MeCN in Milli-Q water comprising 0.1% TFA. Relevant fractions were analysed with UPLC. Fractions containing the pure target peptide were pooled. The resulting solution was analysed (UPLC, LCMS) and the peptide derivative was quantified using a CAD specific HPLC detector (Thermo-Fischer Vanquish HPLC-CAD). The product was dispensed into glass vials. The vials were capped with Millipore glass fibre prefilters. Freeze-drying afforded the trifluoroacetate salt of the derivative as a white solid.

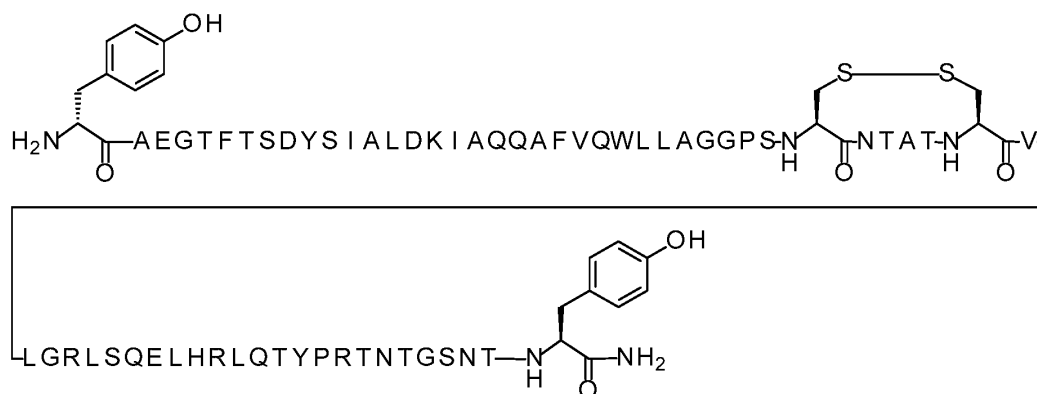
Synthesized compounds

The compounds were prepared using the methods described above.

Example 1: Reference Compounds

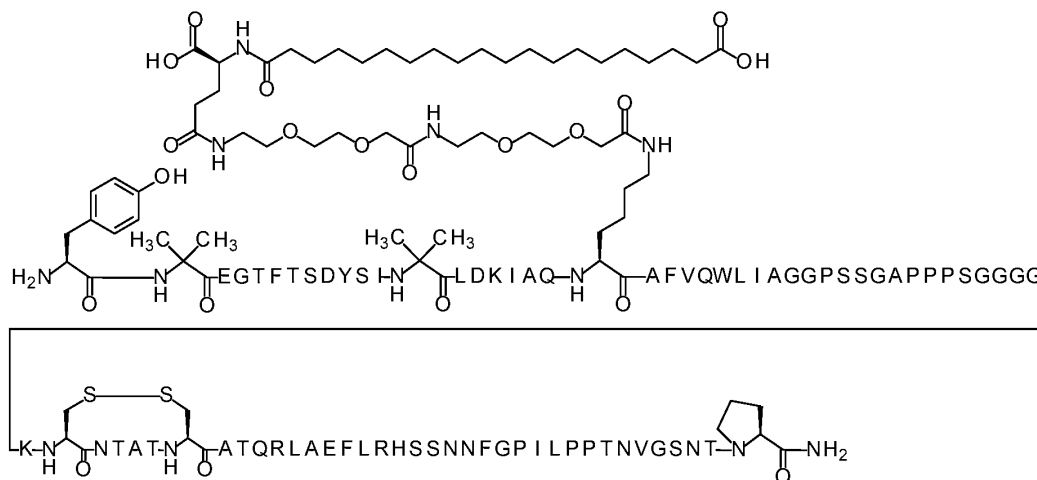
5 Reference compound 1

(GLP-1-/GIP-/amylin-receptor tri-agonist disclosed in WO 2023/288313, example 1, peptide / compound no. 16)



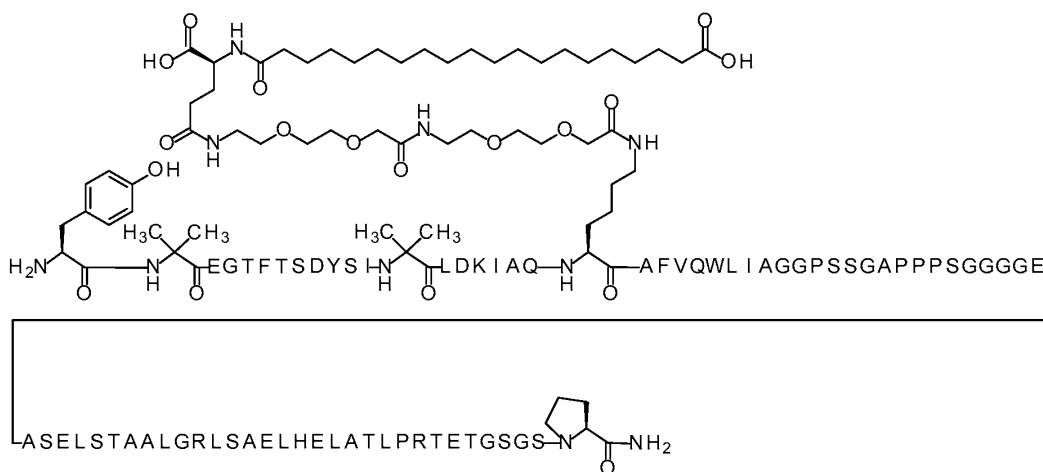
10 Reference compound 2

(Tri-agonist based on conjugation of tirzepatide and cagrilintide)



Reference compound 3

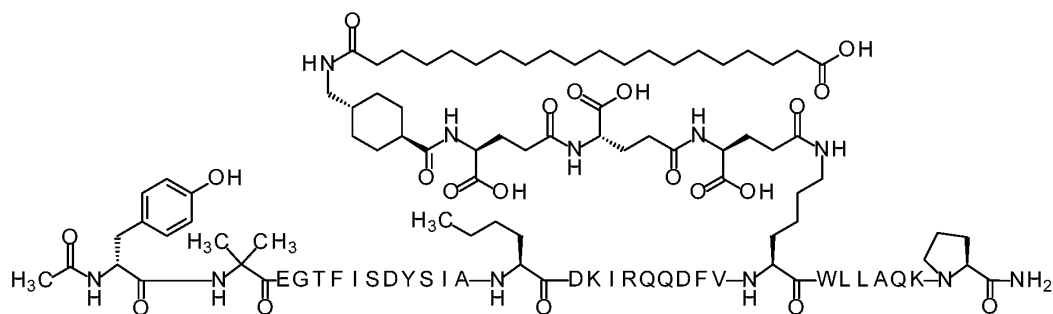
(Tri-agonist based on conjugation of tirzepatide and amylin receptor agonist disclosed in example 21 in WO 2016/034604)



5

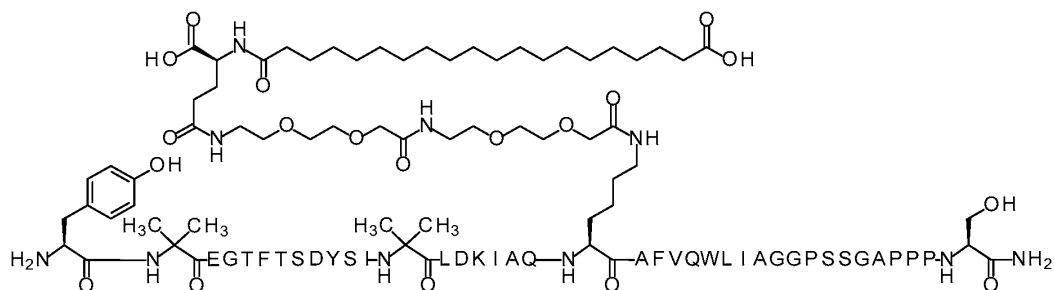
Reference compound 4

(GIP receptor agonist disclosed in WO 2019/211451, example 1, compound 31)



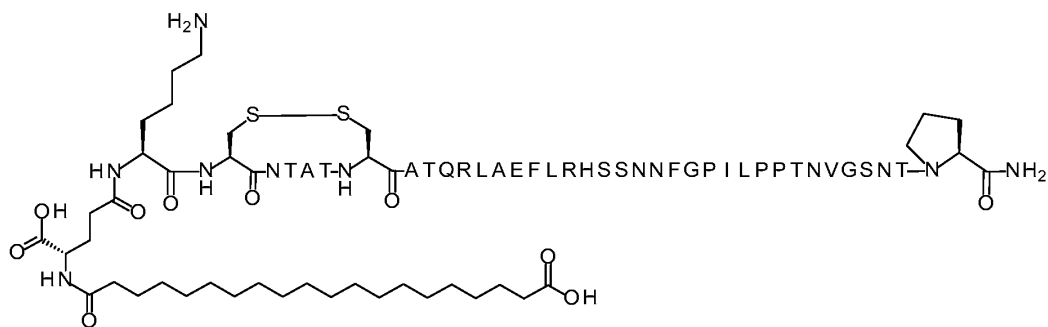
10

(GLP-1/GIP co-agonist tirzepatide)



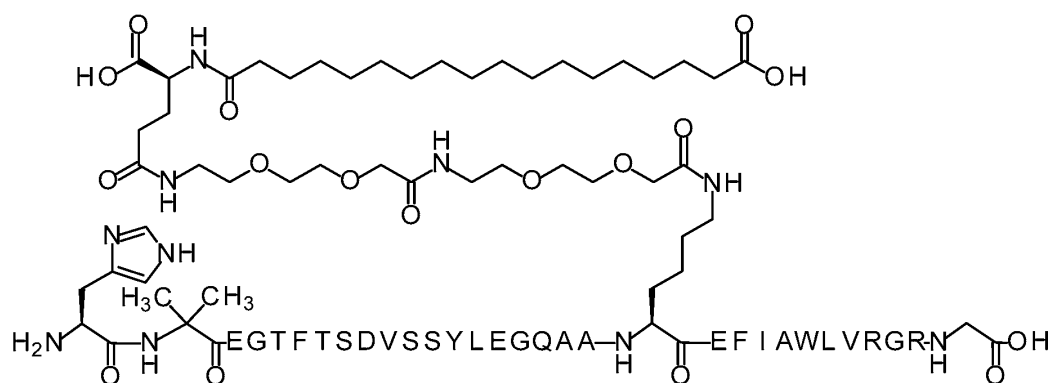
5 Reference compound 6

(Amylin receptor agonist cagrilintide, WO 2012/168432, example 53)



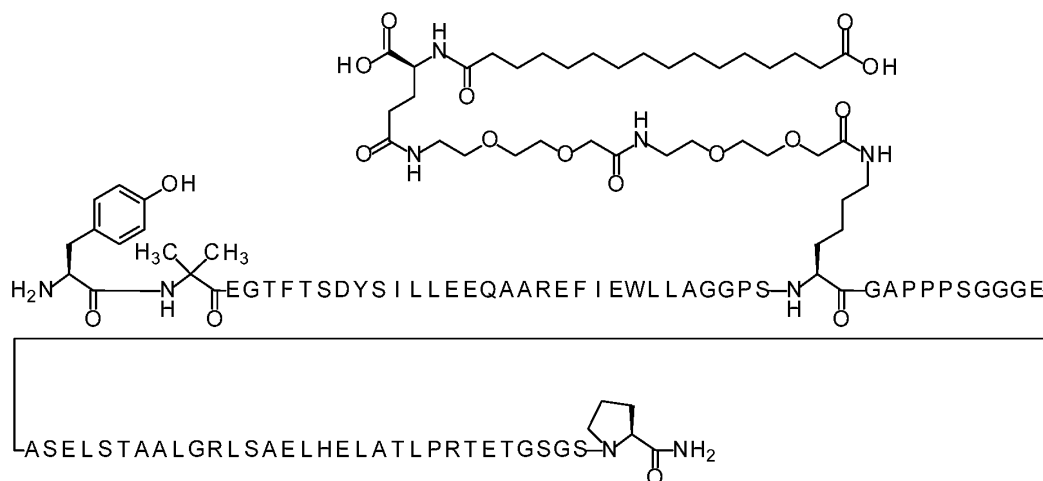
Reference compound 7

10 (GLP-1 receptor agonist semaglutide, WO 2006/097537, example 4)



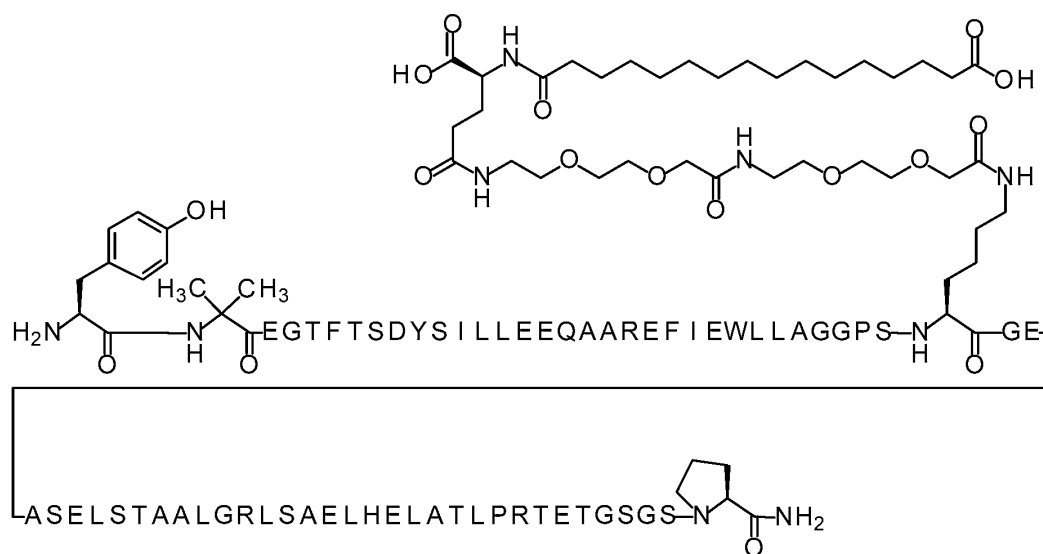
Example 2: GLP-1-/GIP-/amylin-receptor tri-agonists according to the invention

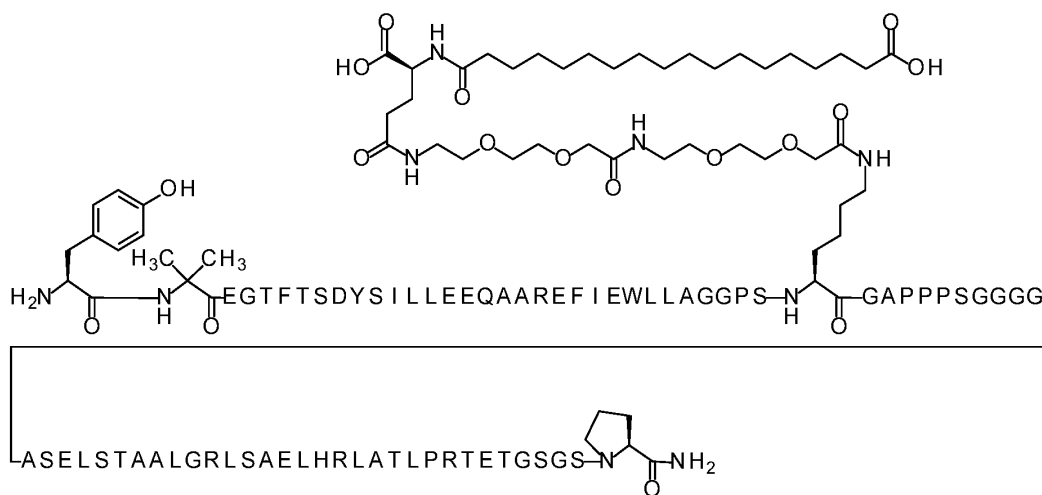
Compound 10



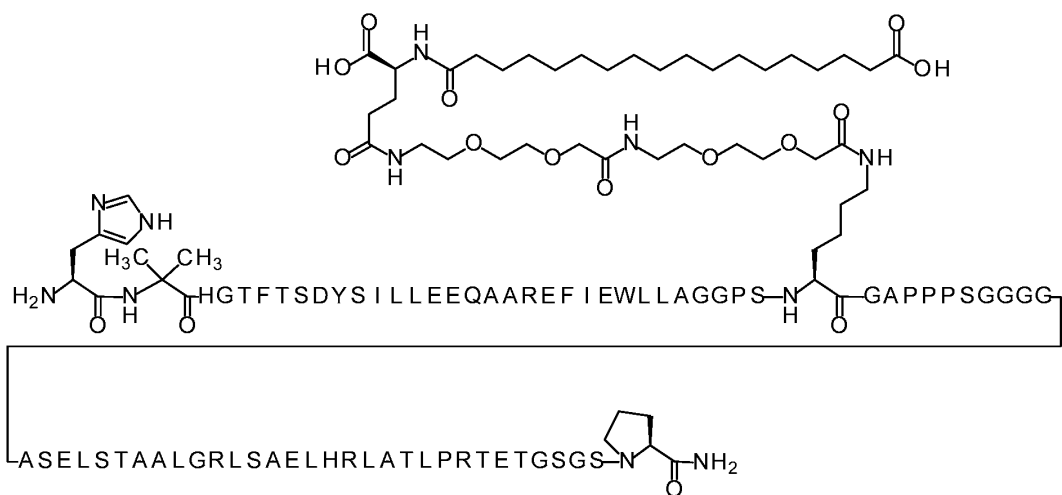
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Compound 11

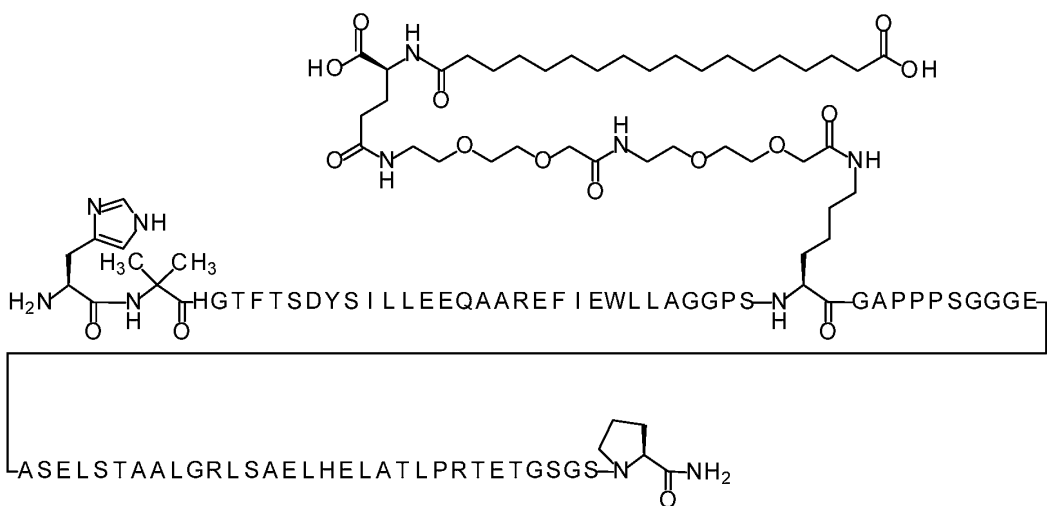


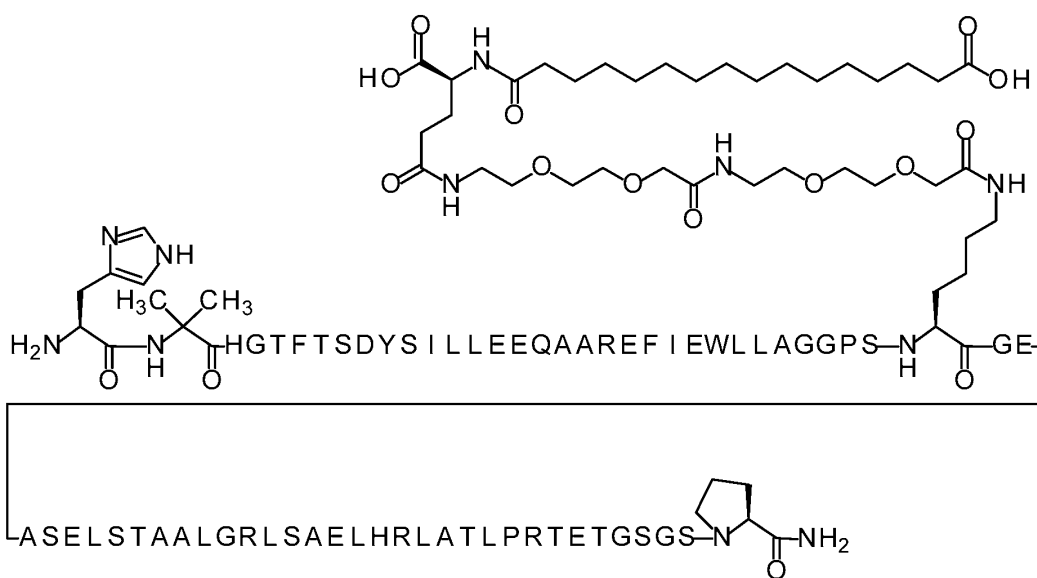


Compound 14

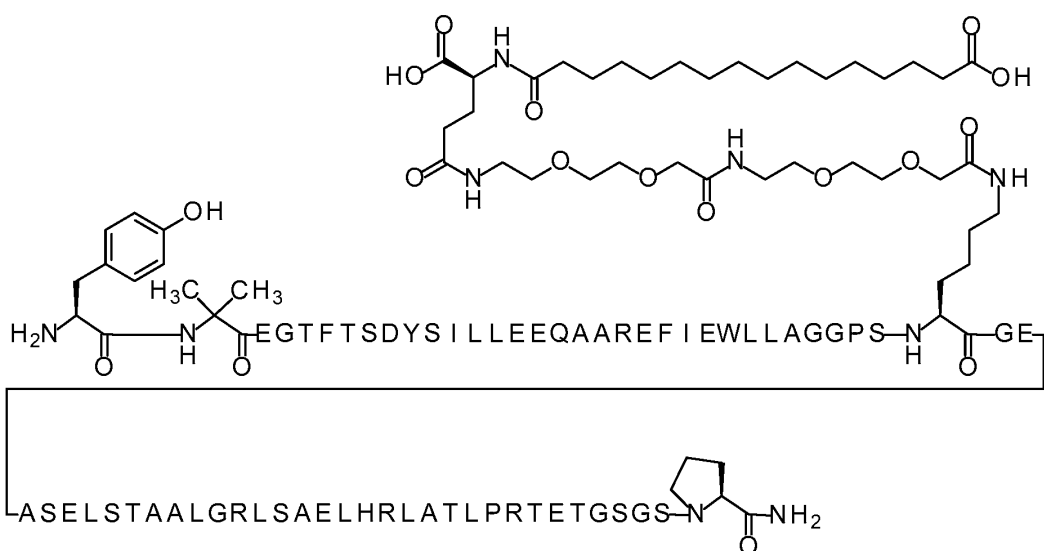


Compound 15

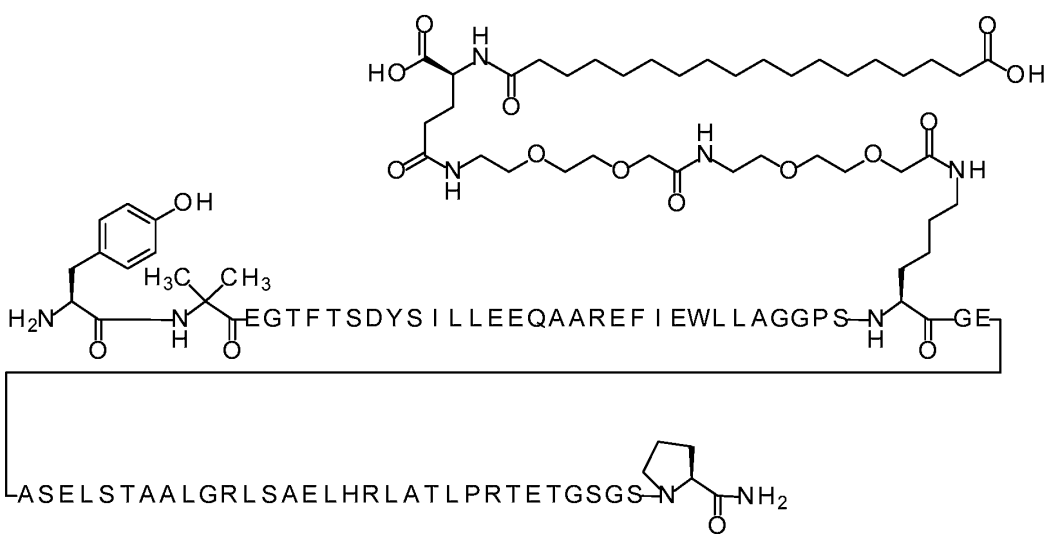




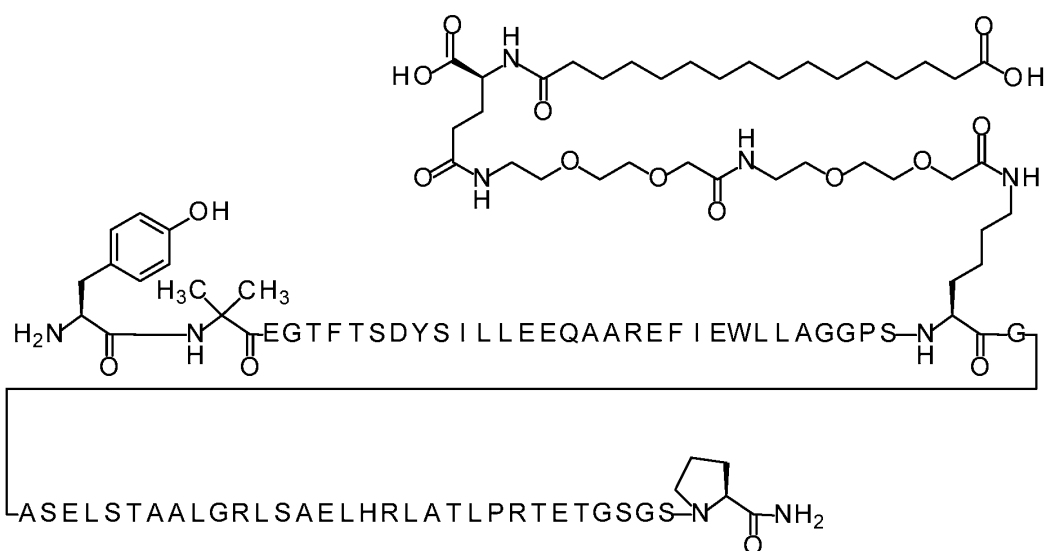
Compound 18



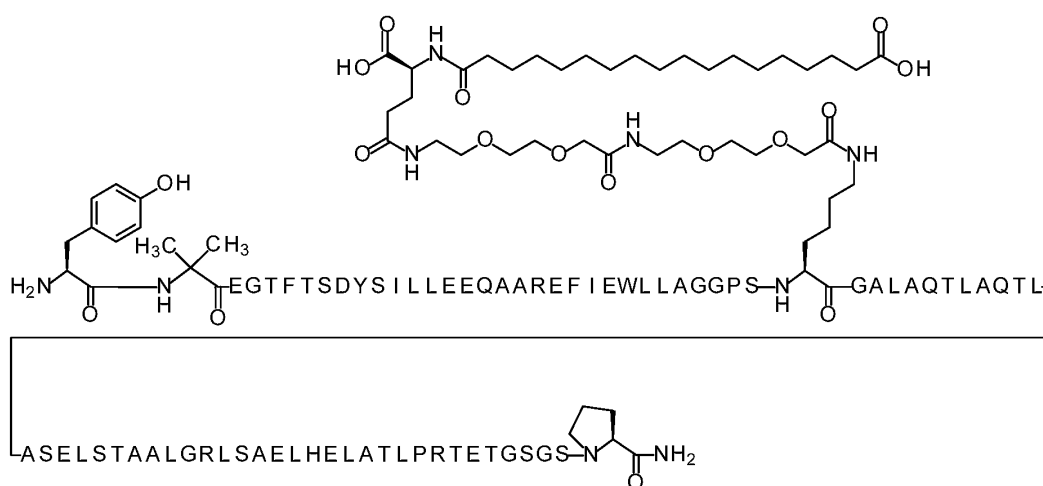
Compound 19



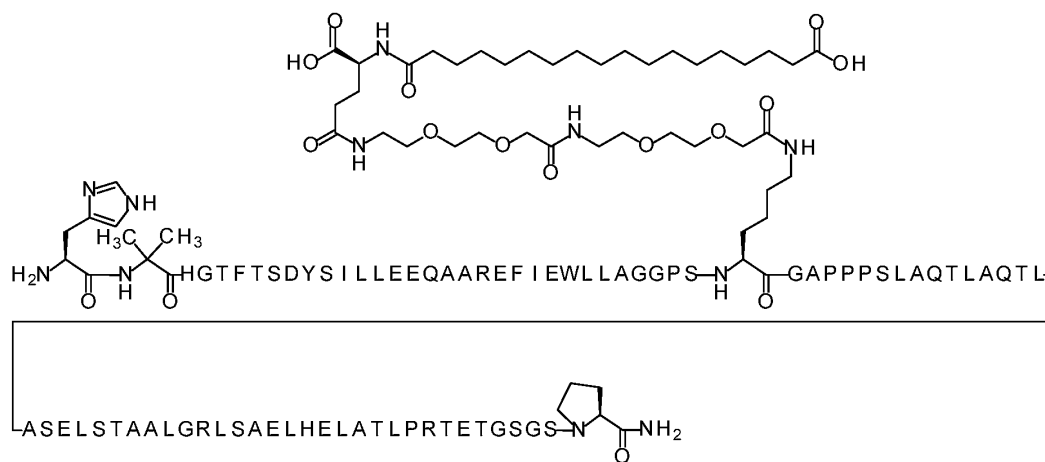
Compound 20



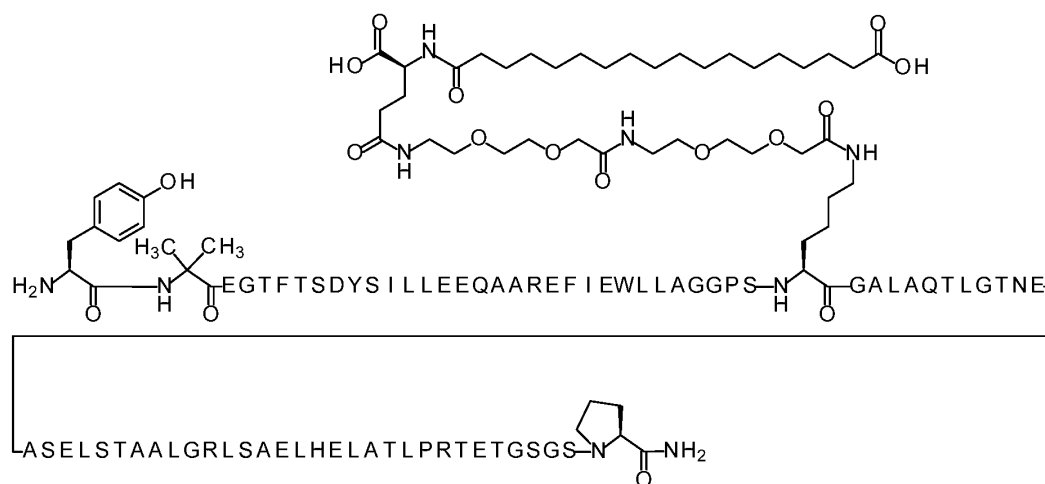
Compound 21

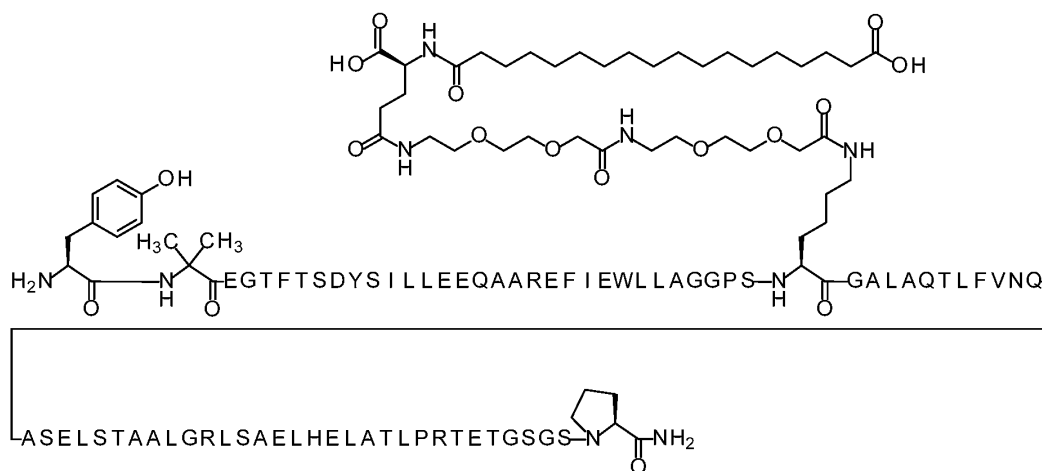


Compound 22

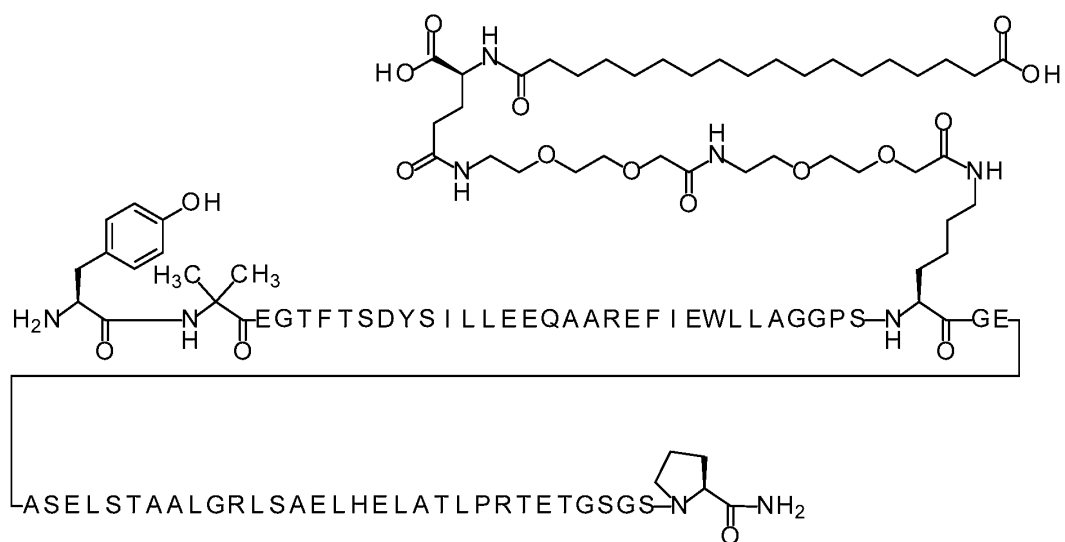


Compound 23

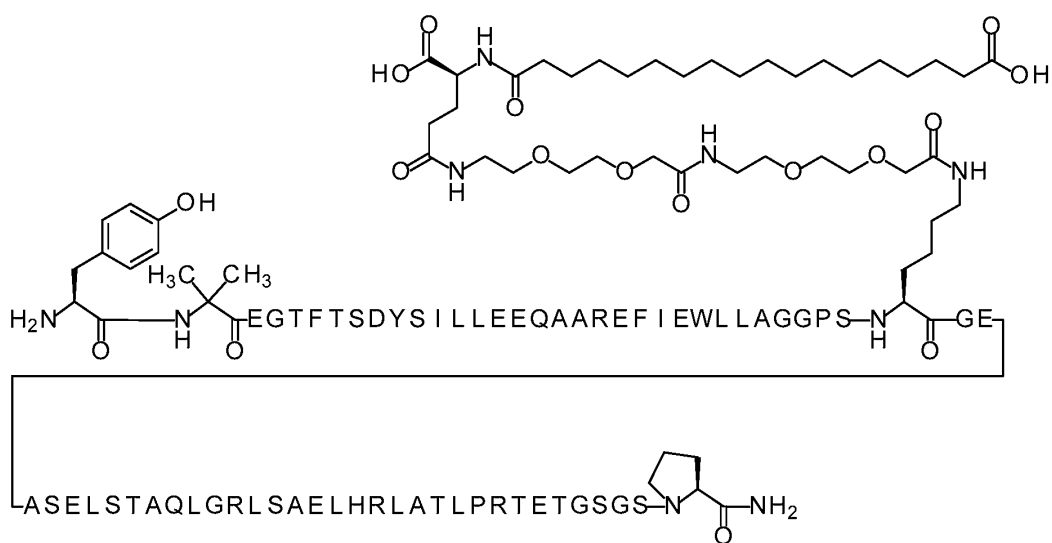


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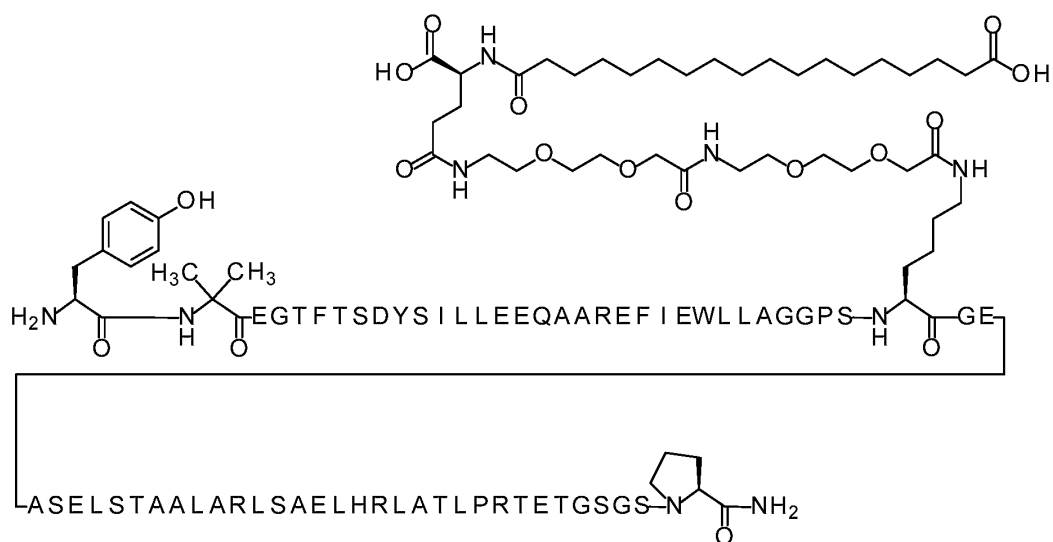
Compound 26



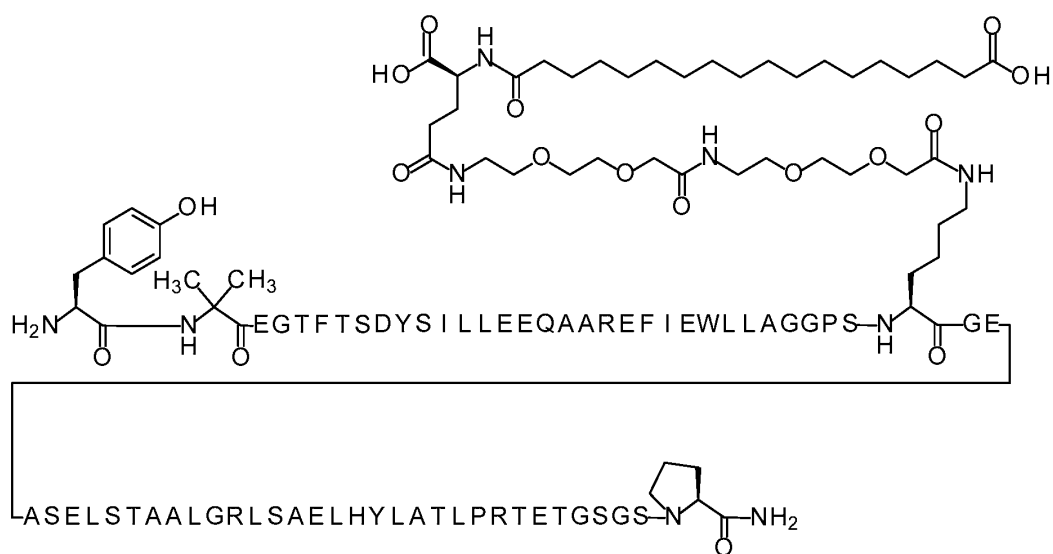
Compound 27

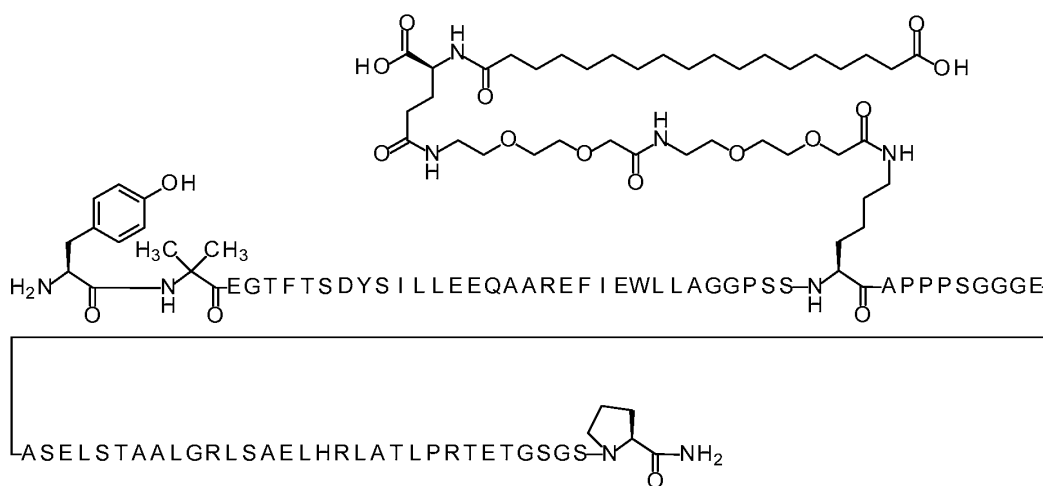


Compound 28

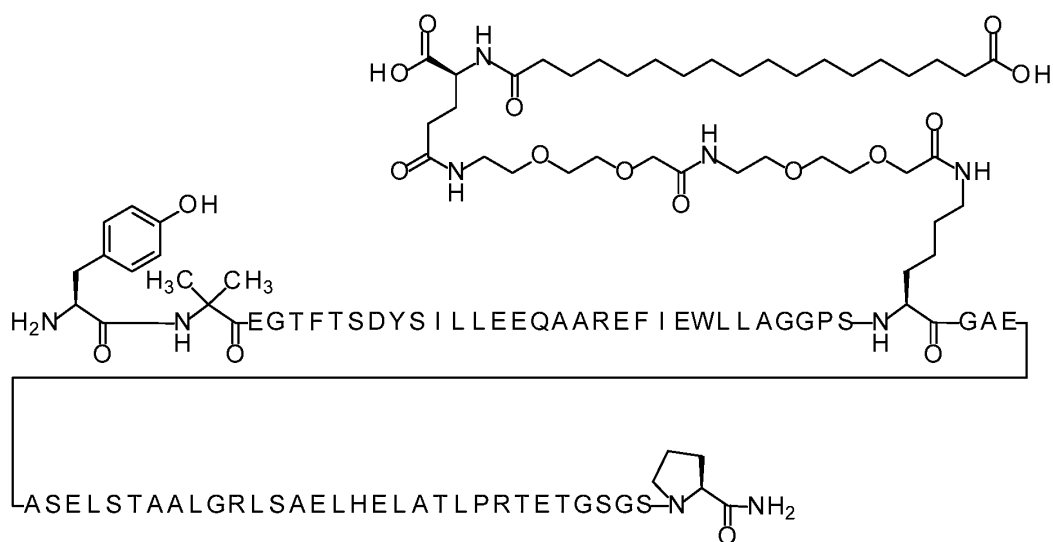


Compound 29

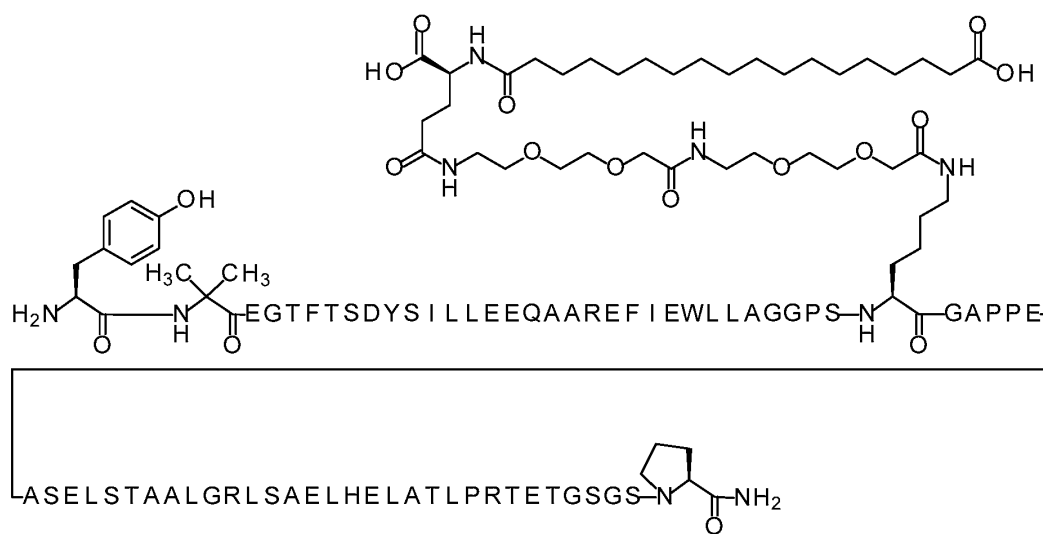




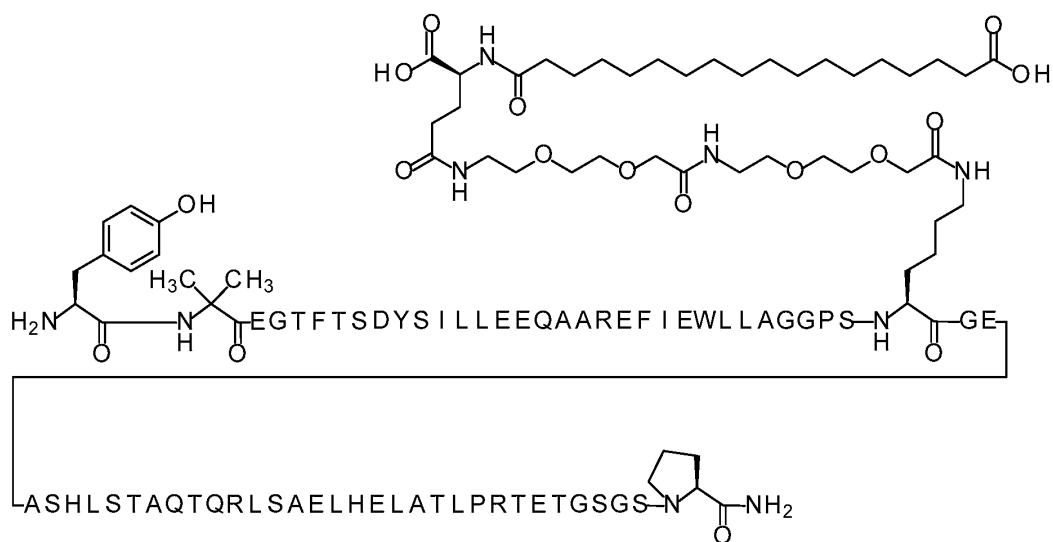
Compound 32



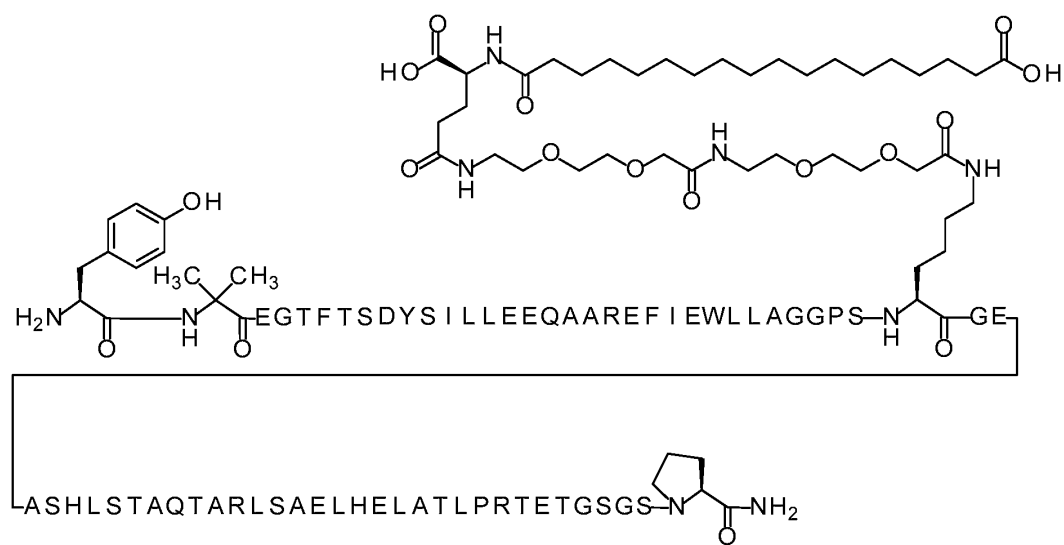
Compound 33

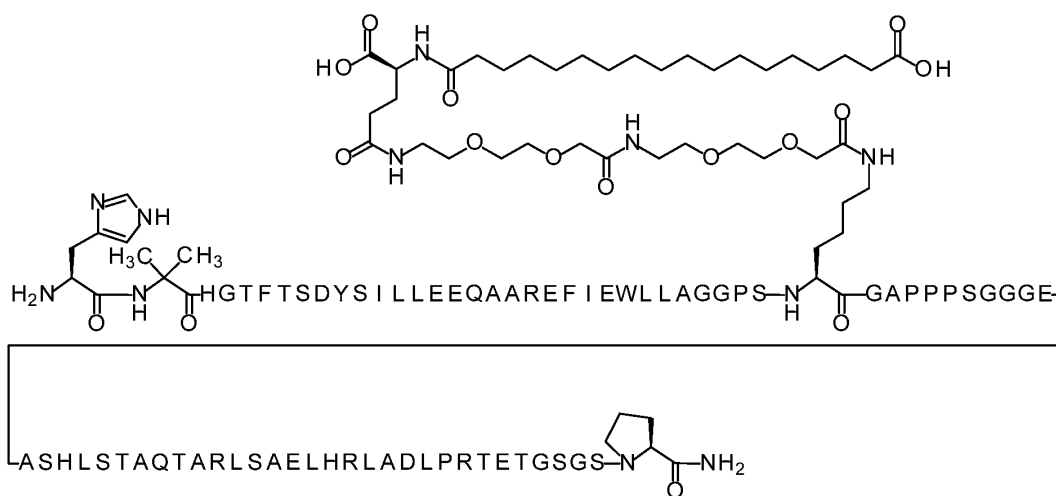


Compound 34

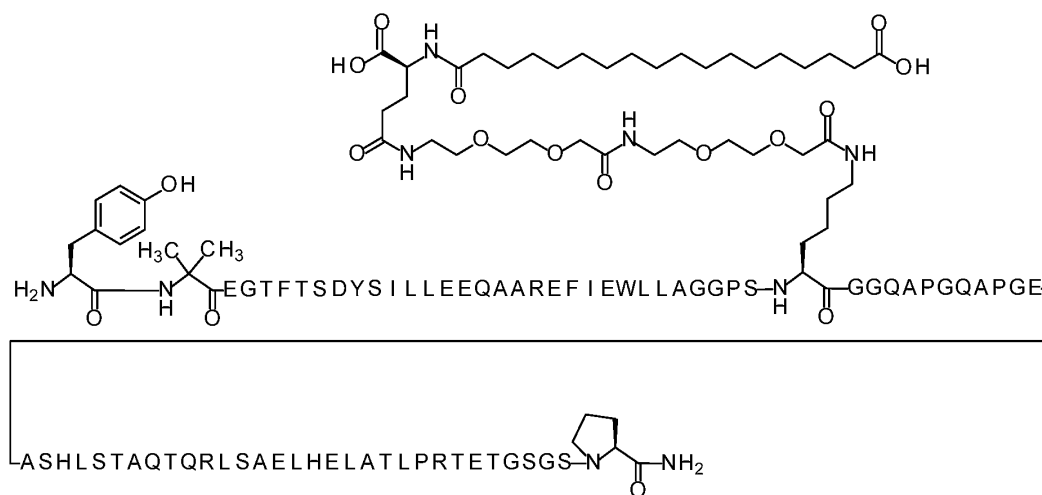


Compound 35

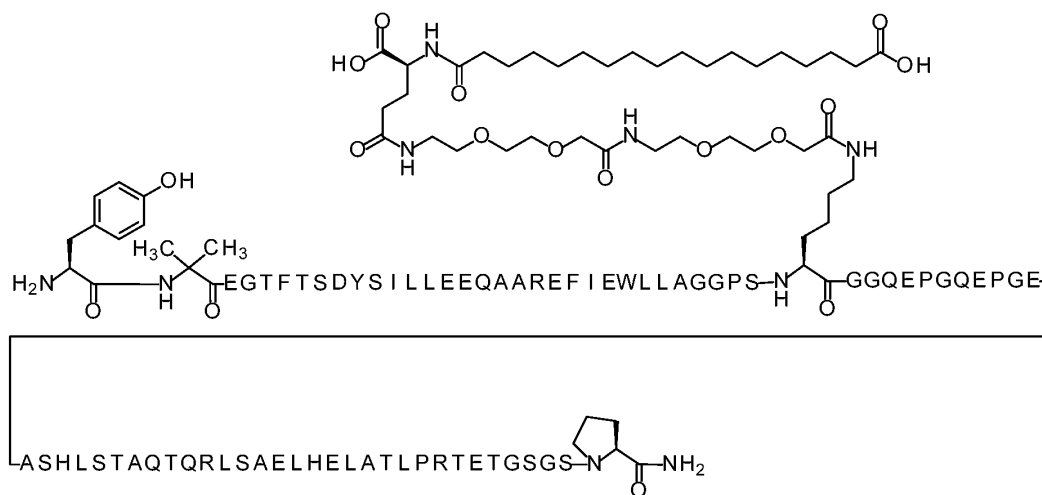




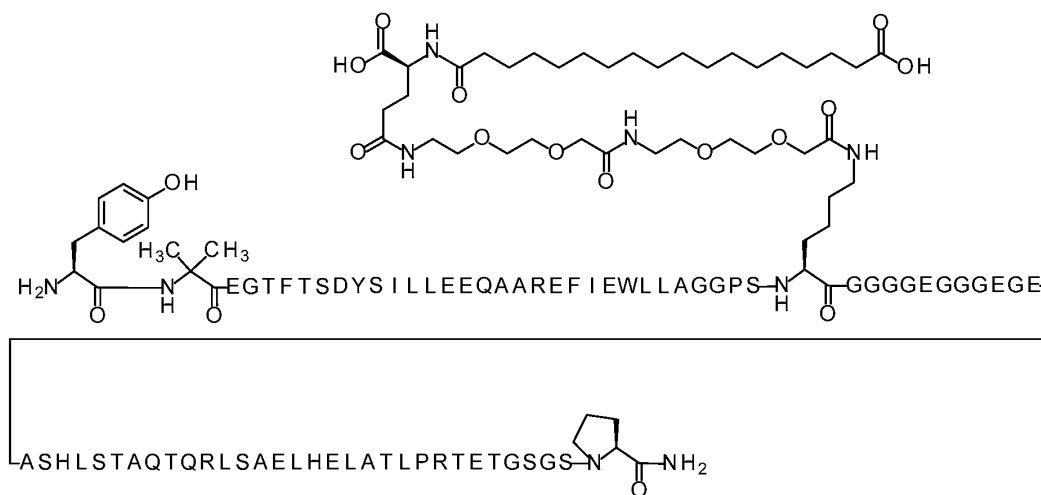
Compound 38



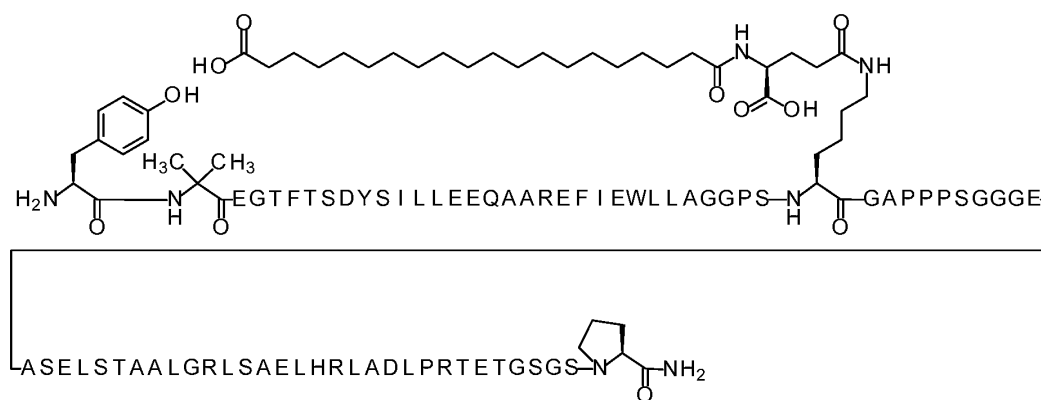
Compound 39



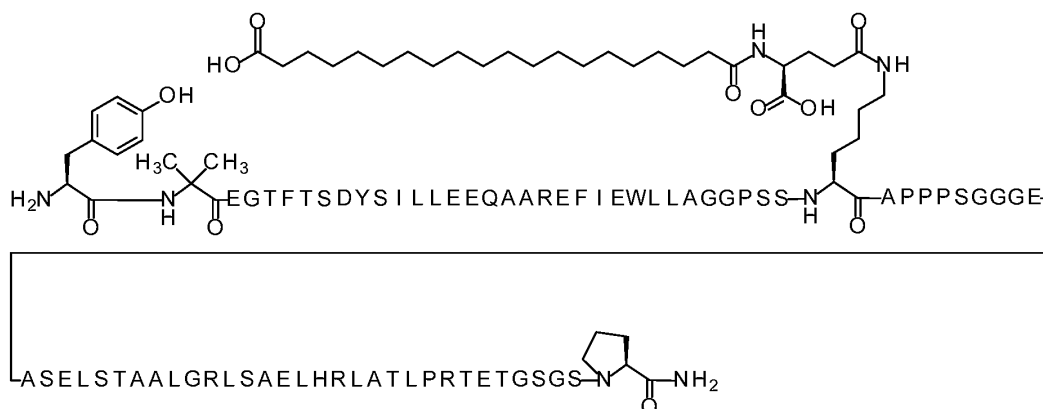
Compound 40



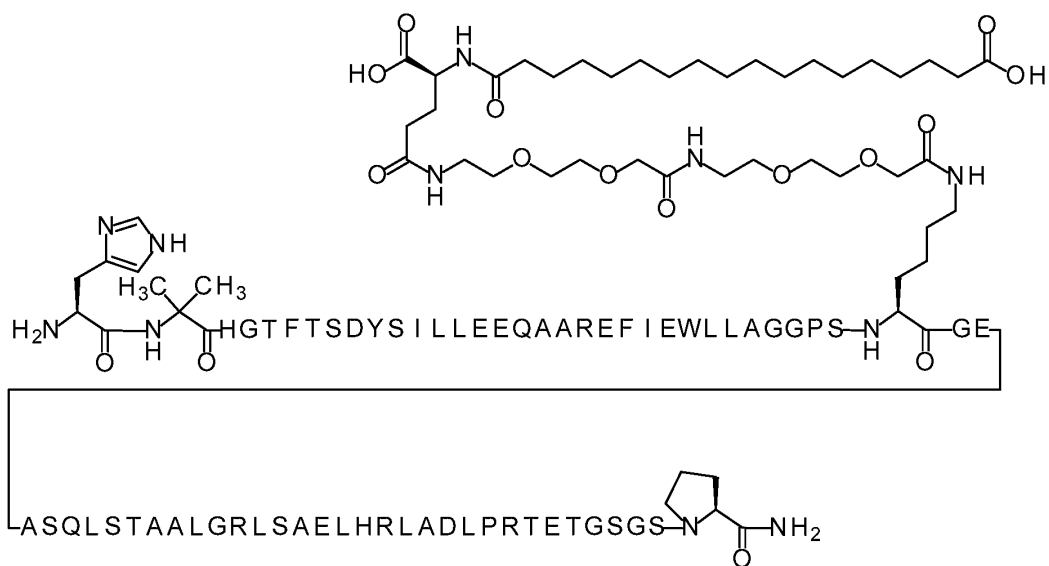
Compound 41



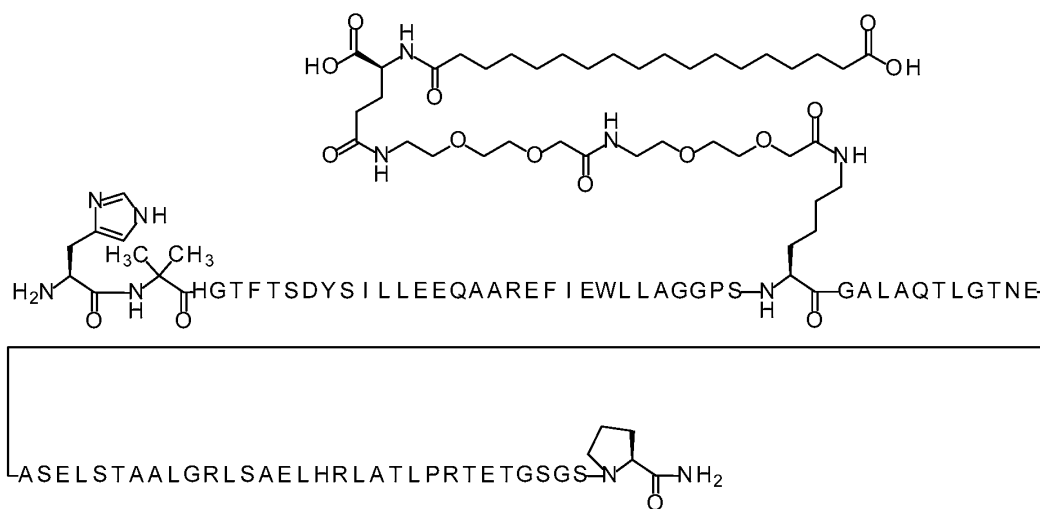
Compound 42



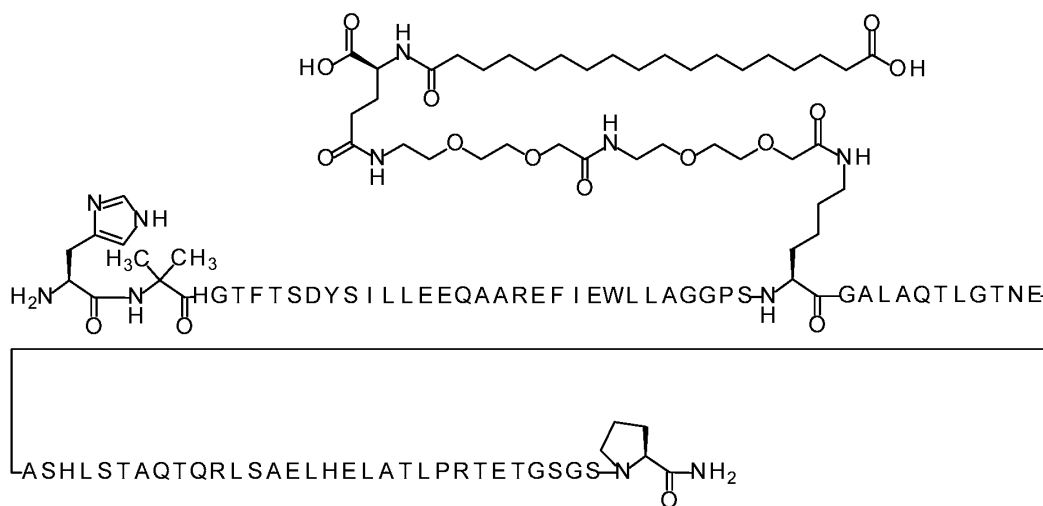
Compound 43



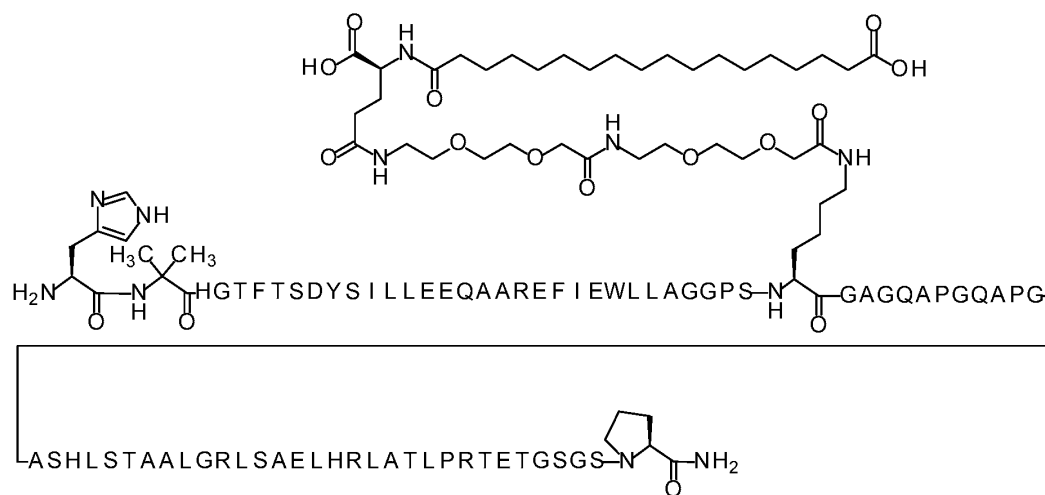
Compound 44



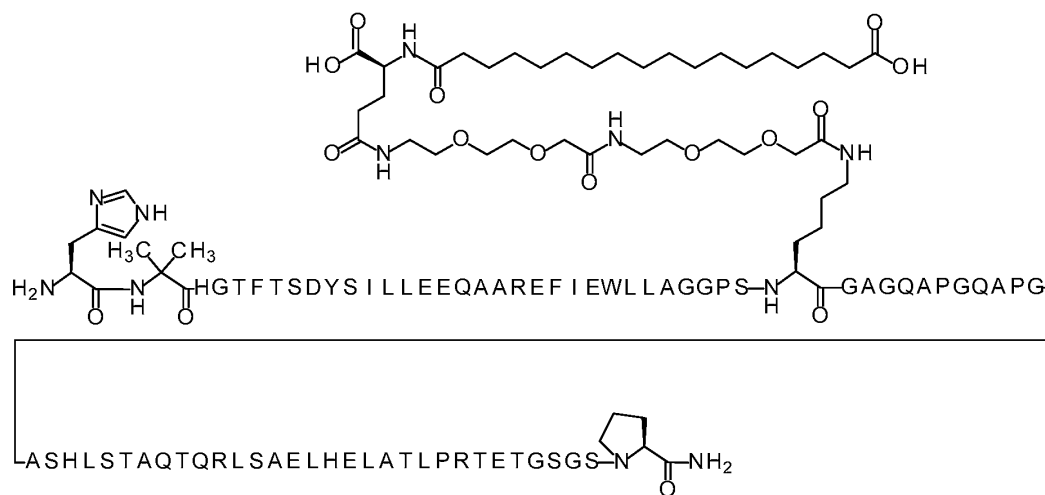
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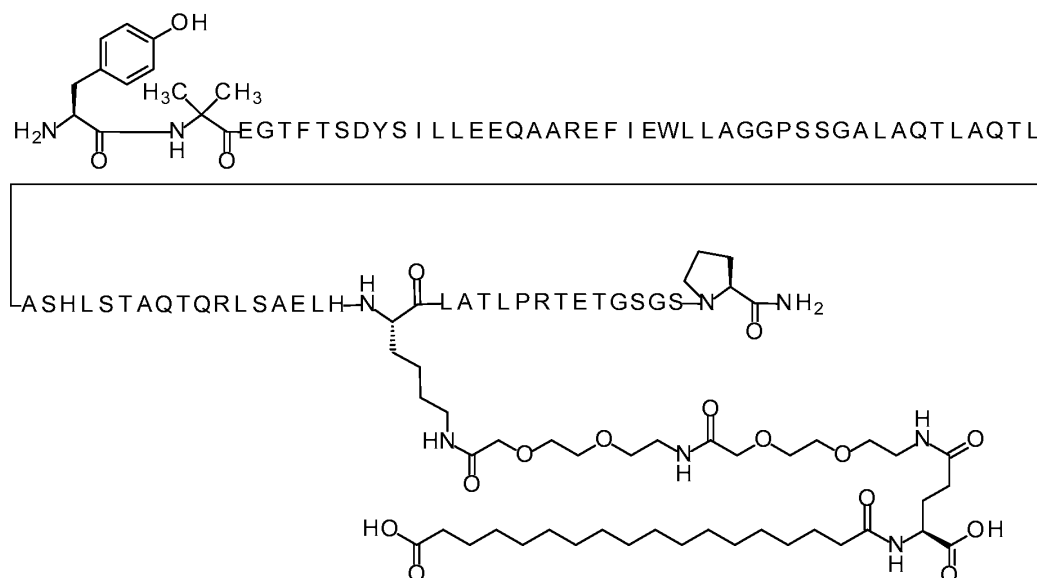


Compound 46

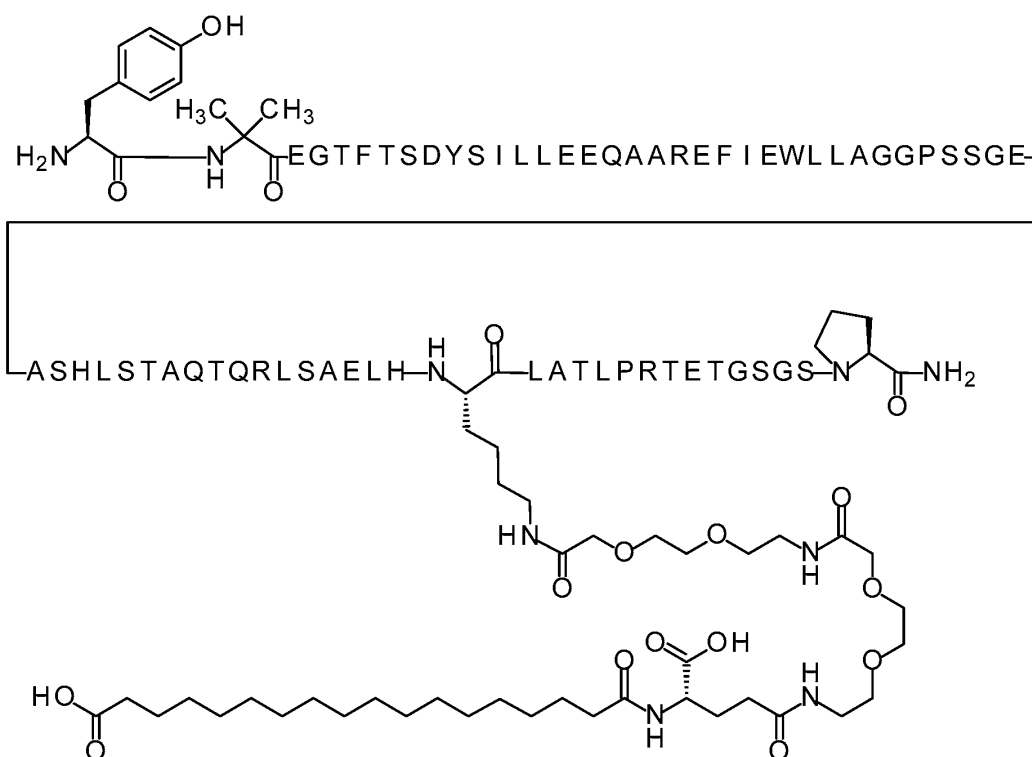


Compound 47

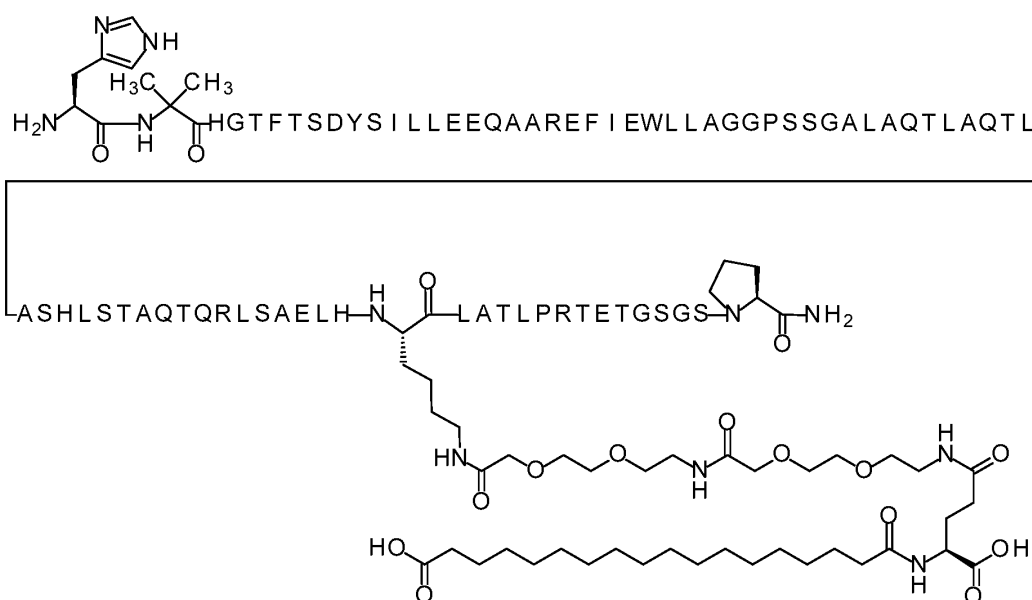


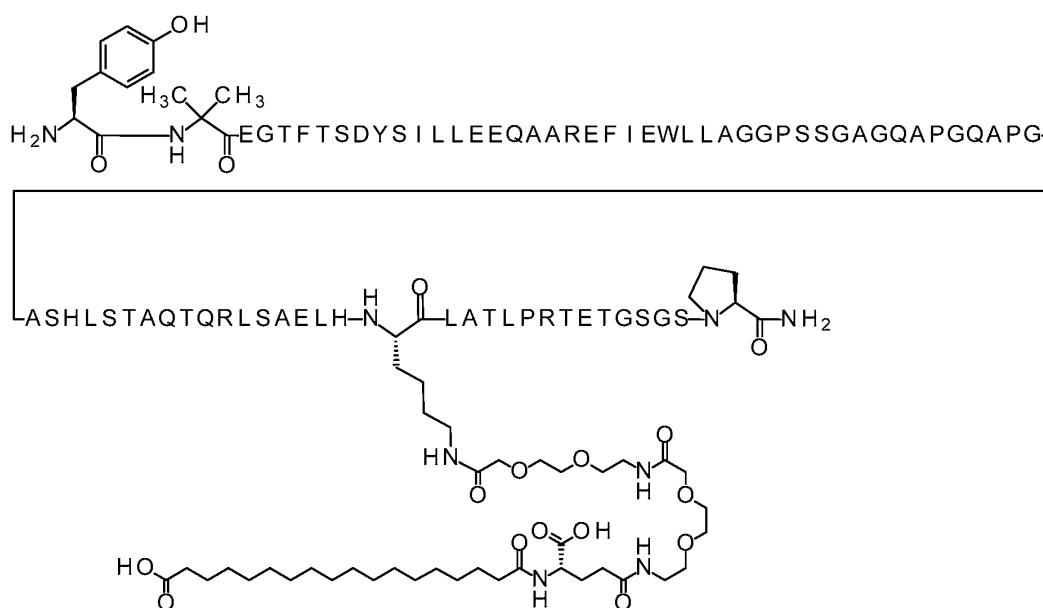


Compound 50

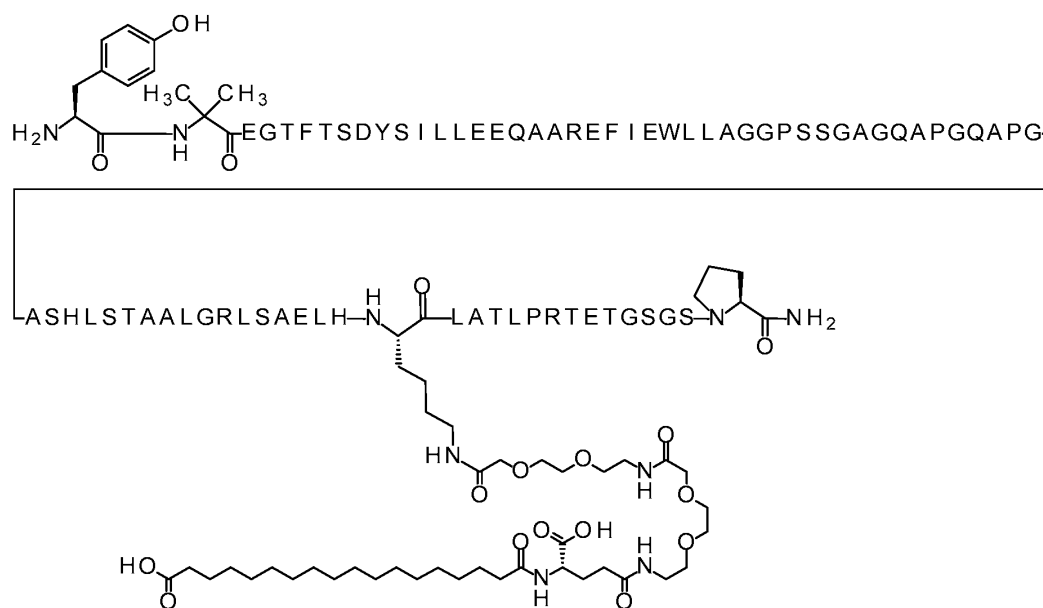


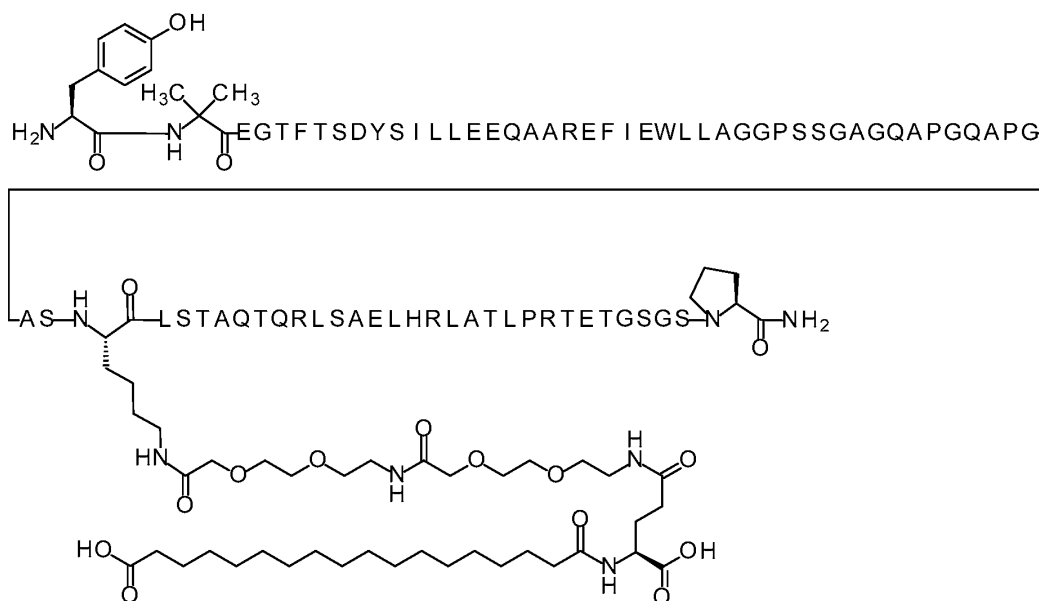
Compound 51



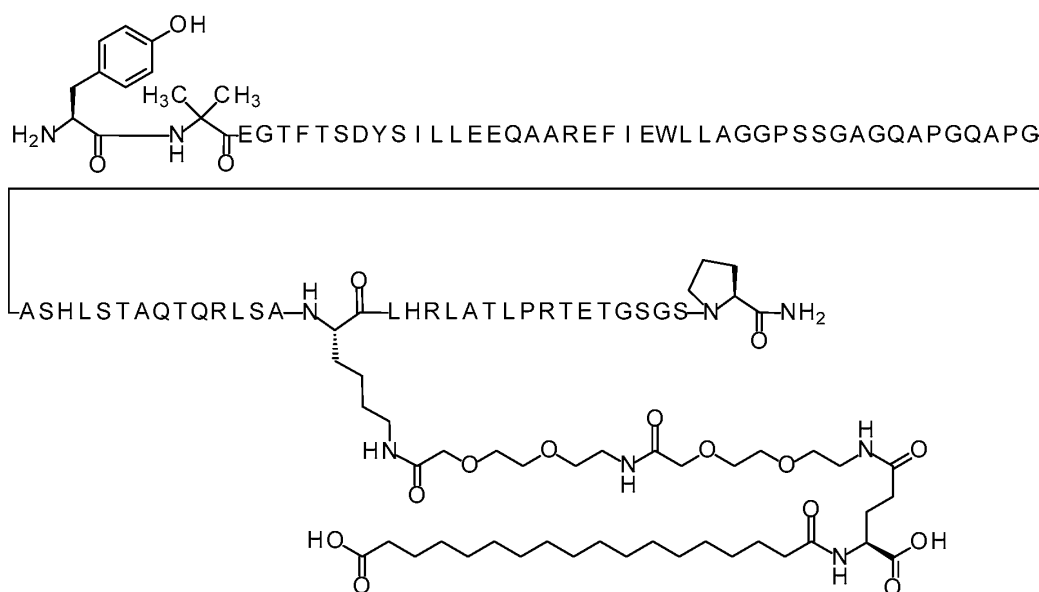


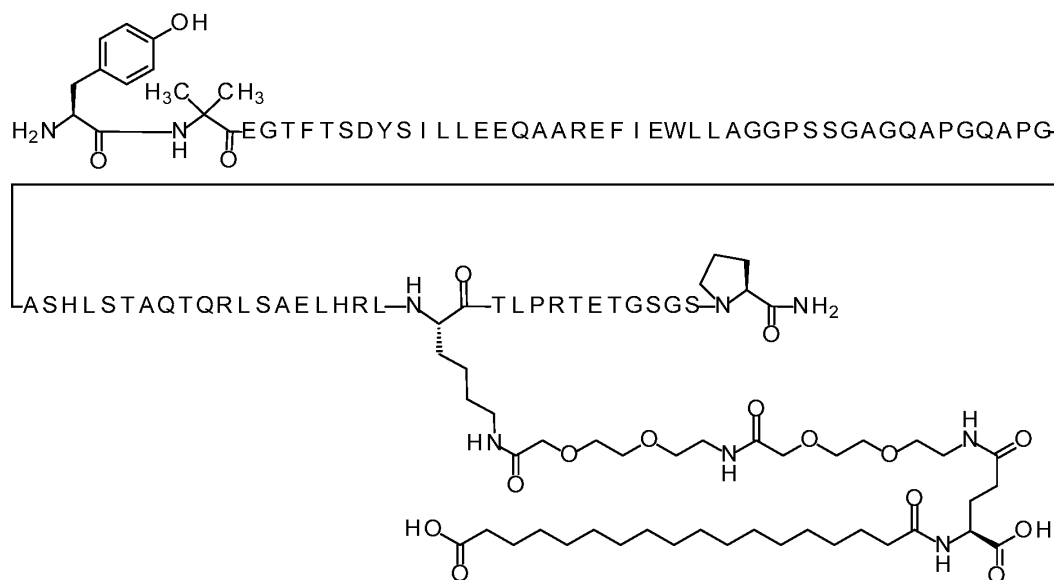
Compound 53



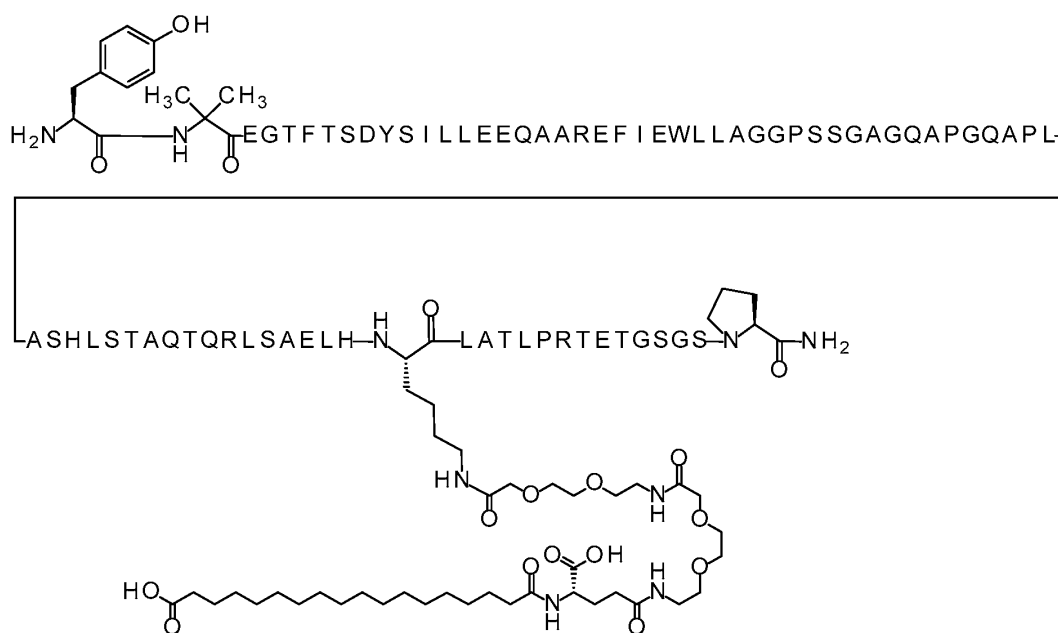


Compound 55

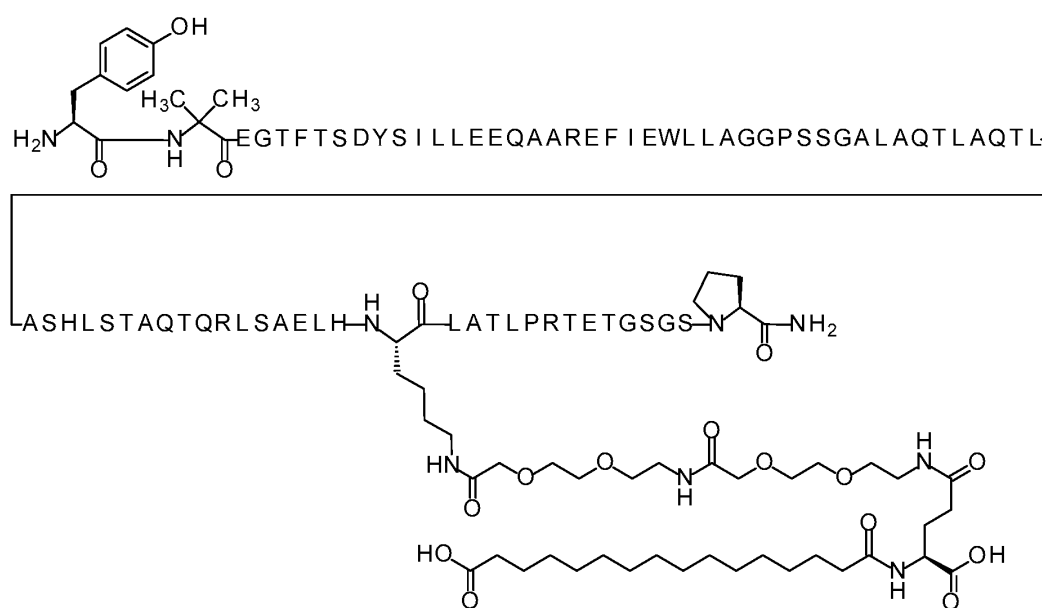


[illegible]

Compound 58

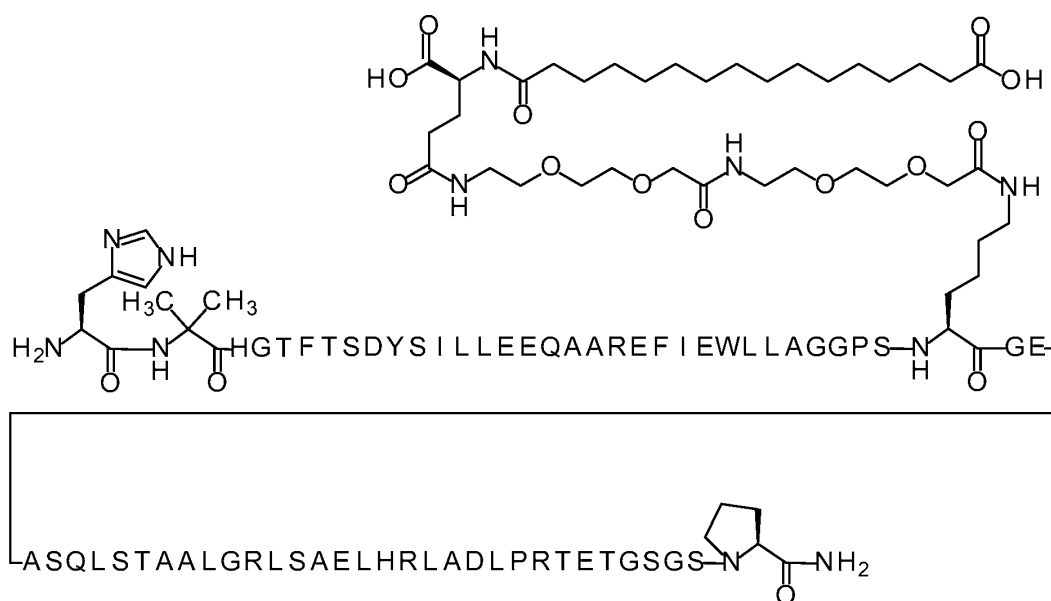


Compound 59

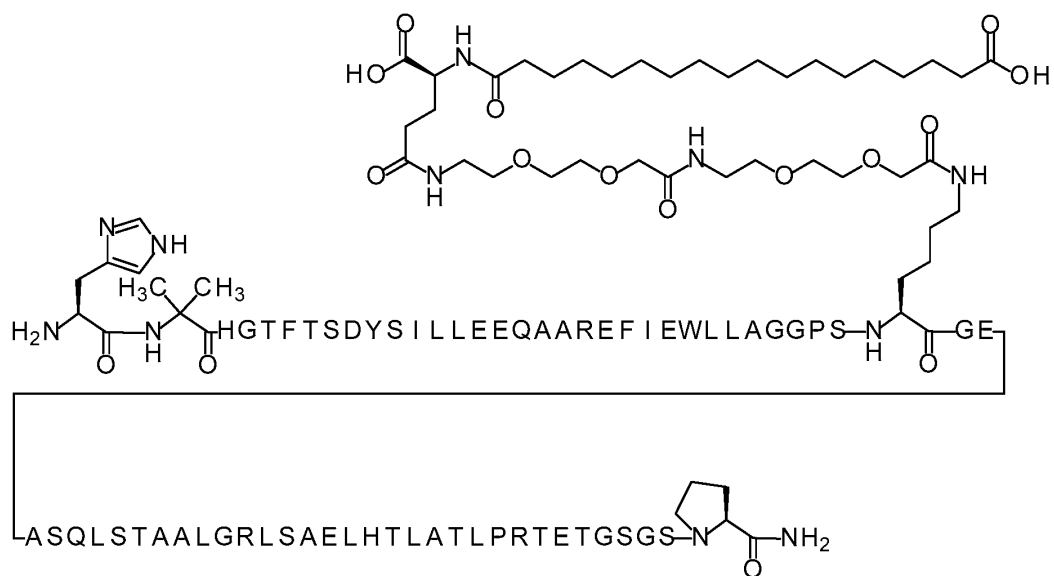


[illegible][illegible]

Compound 62

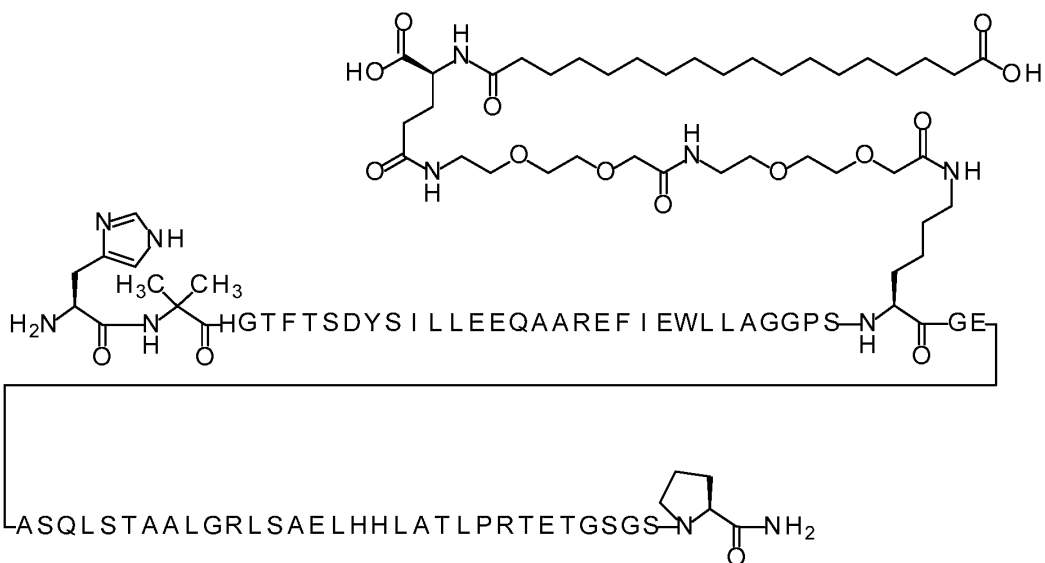


Compound 63

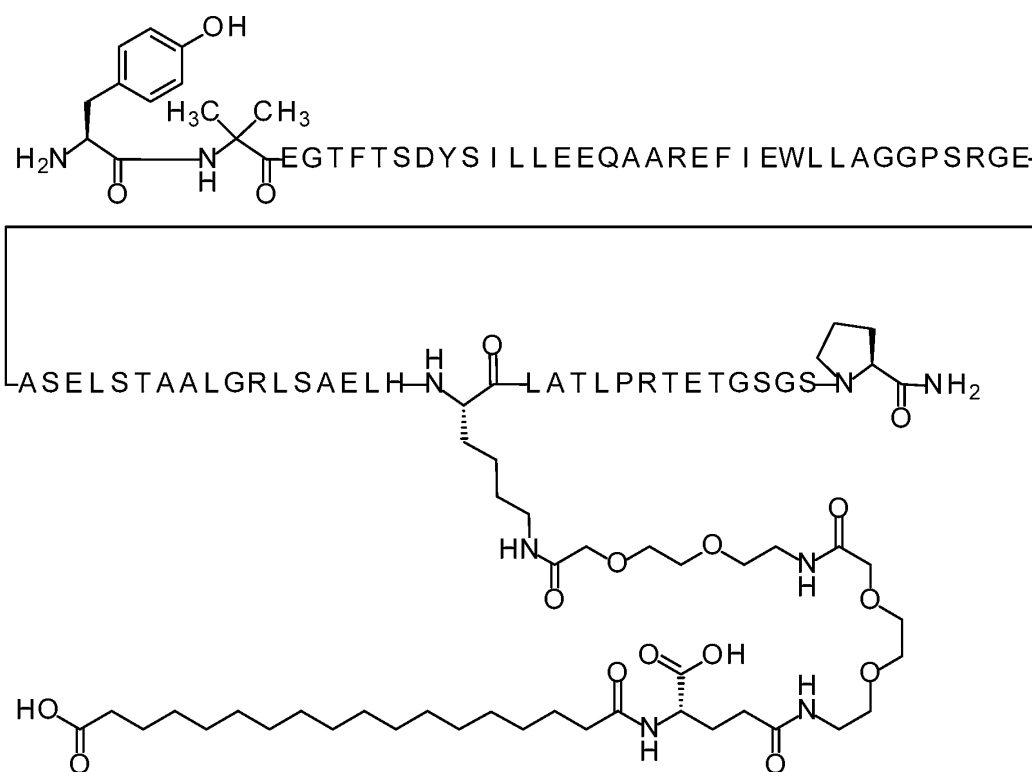




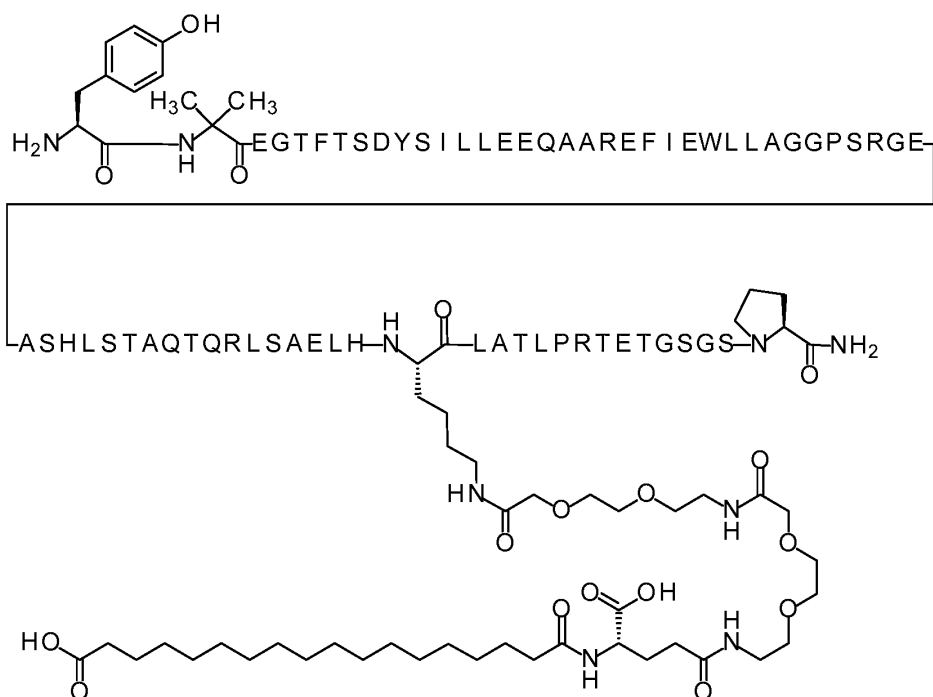
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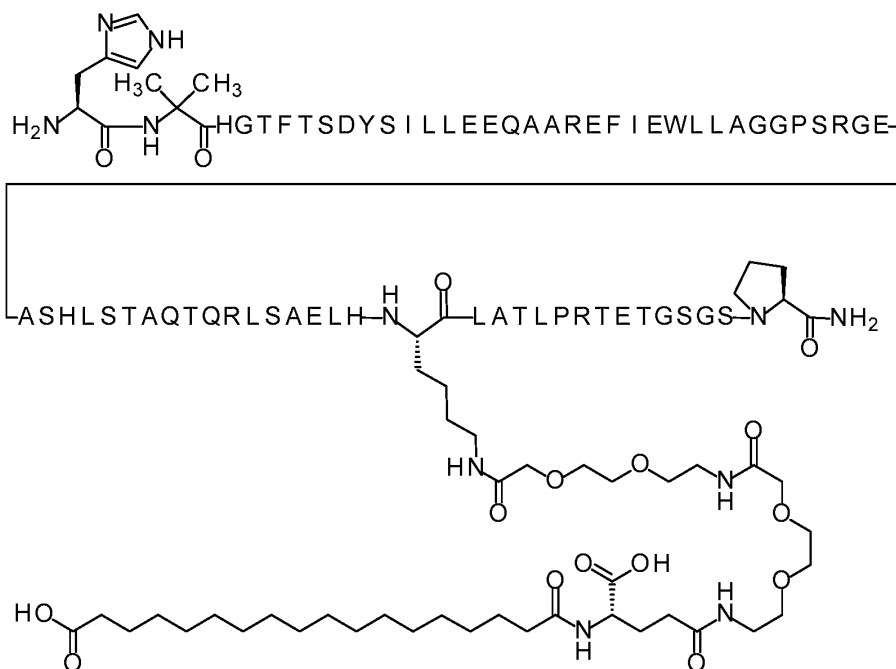
Compound 67



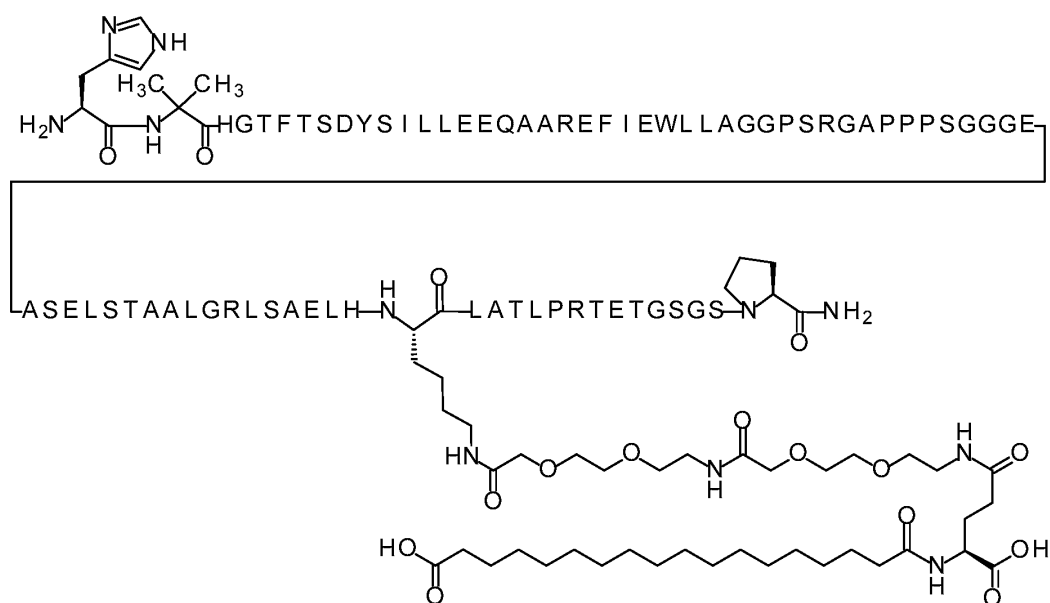
Compound 68



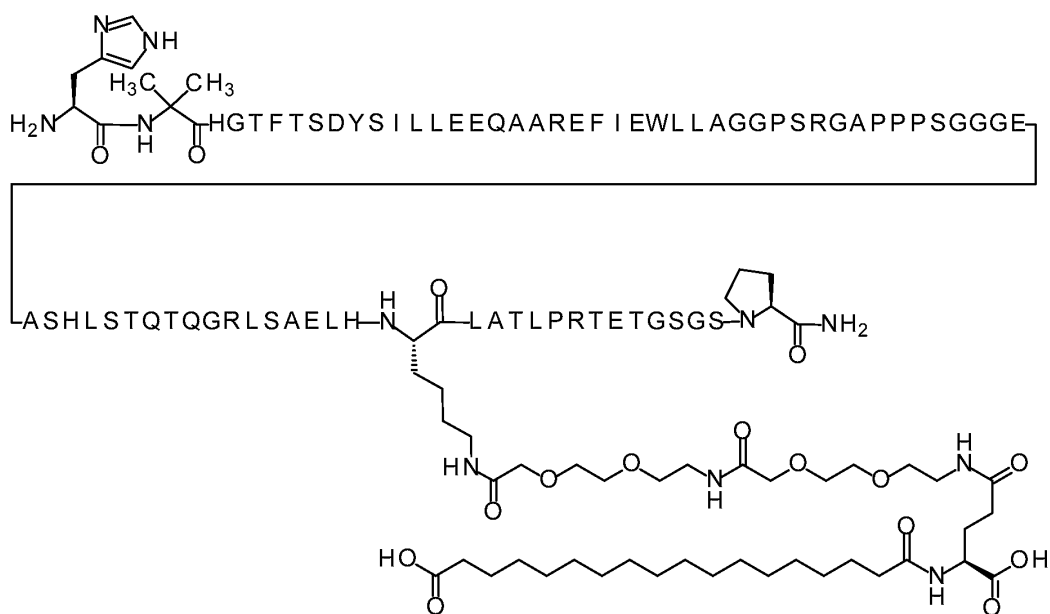
Compound 69



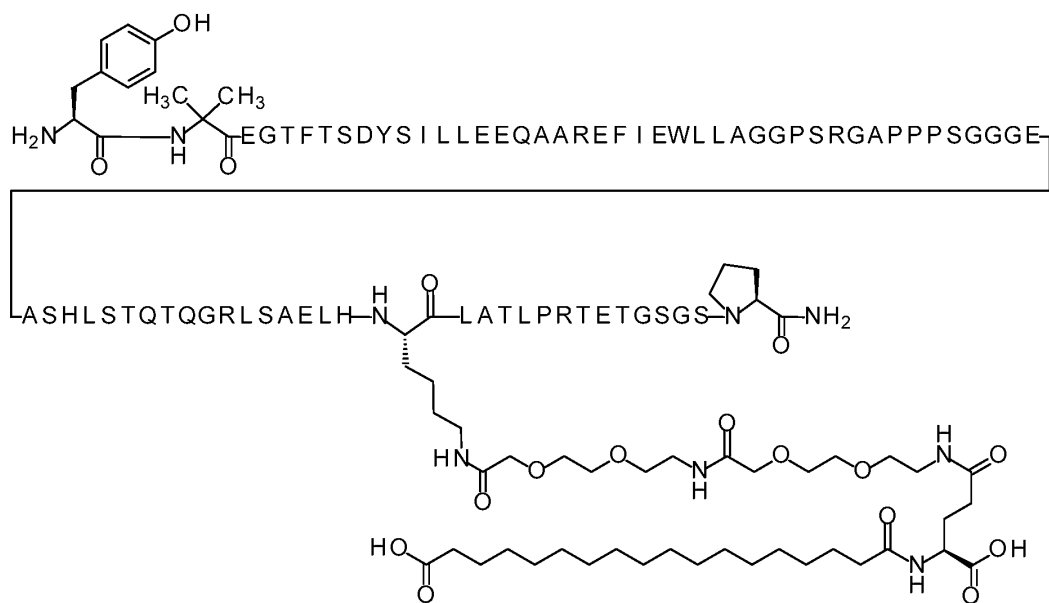
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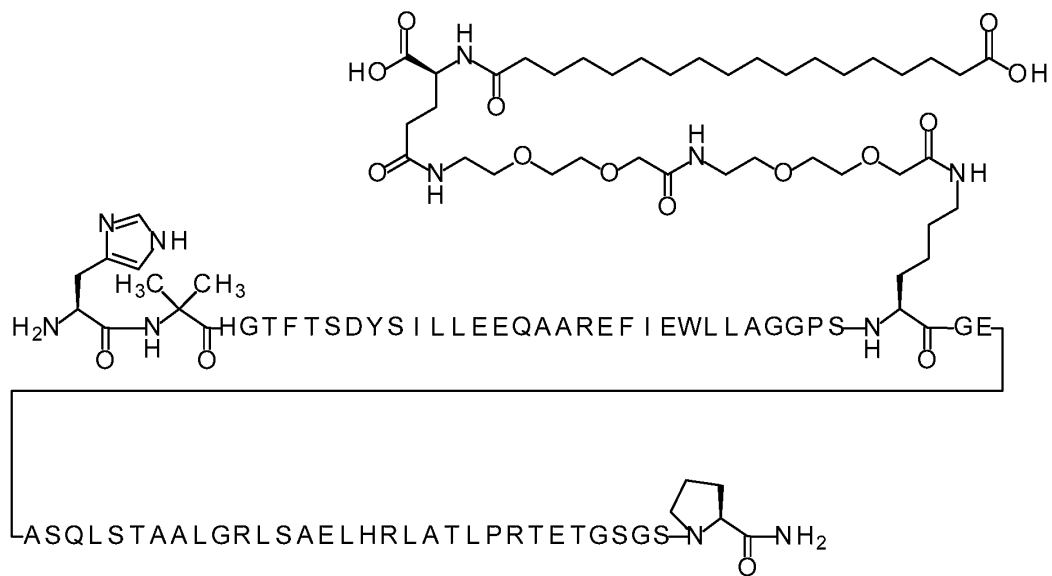
Compound 71



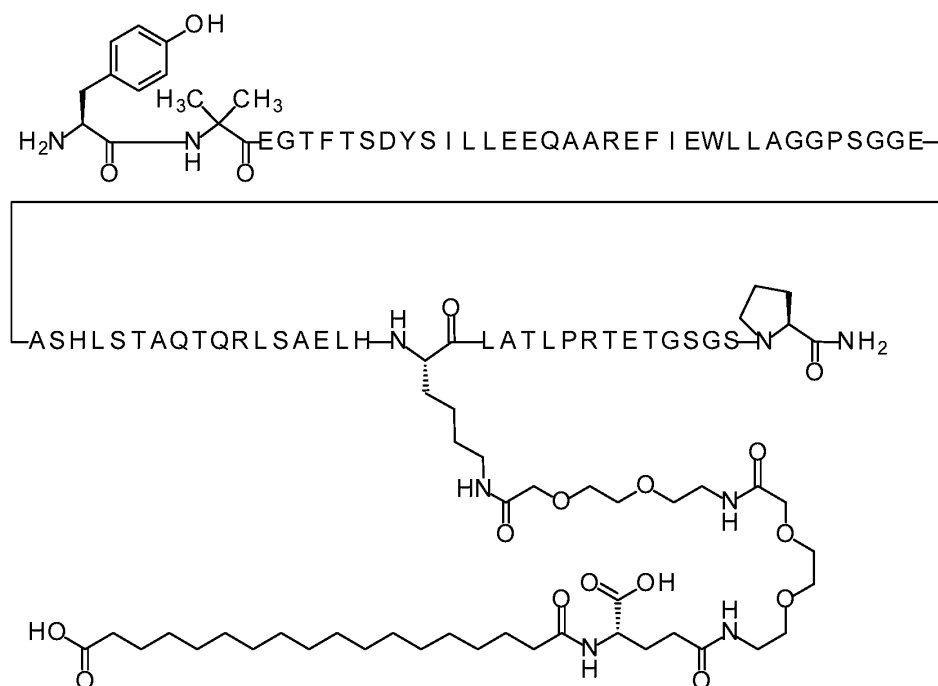
Compound 72



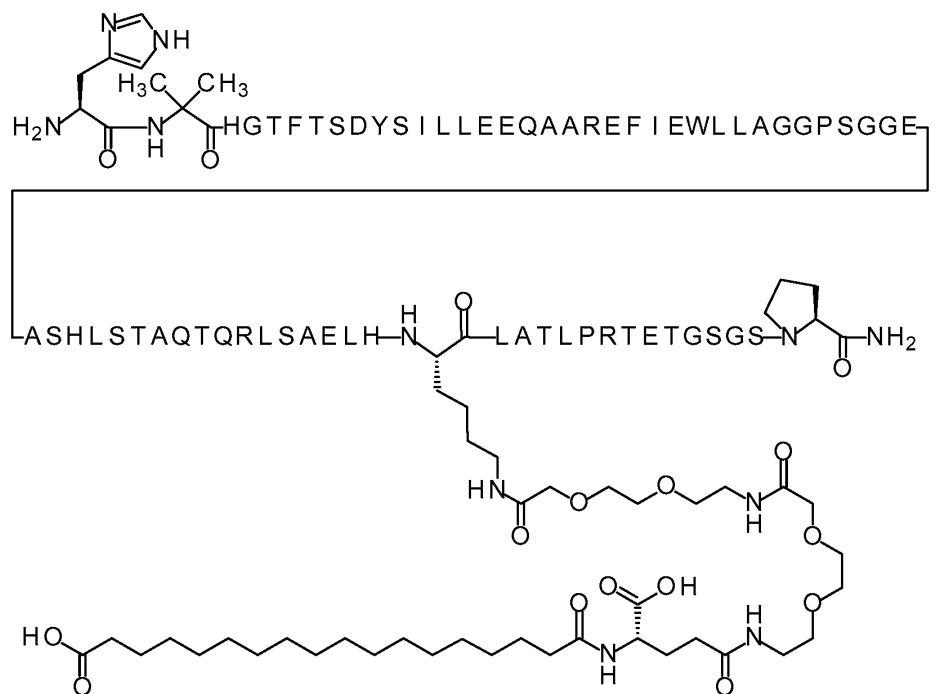
Compound 73



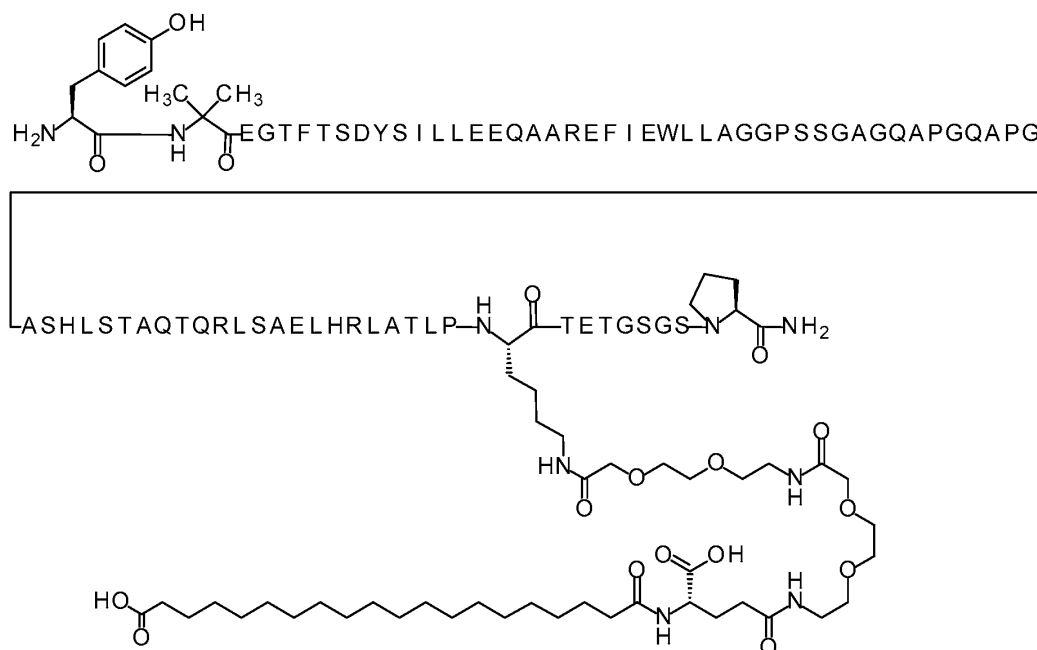
Compound 74



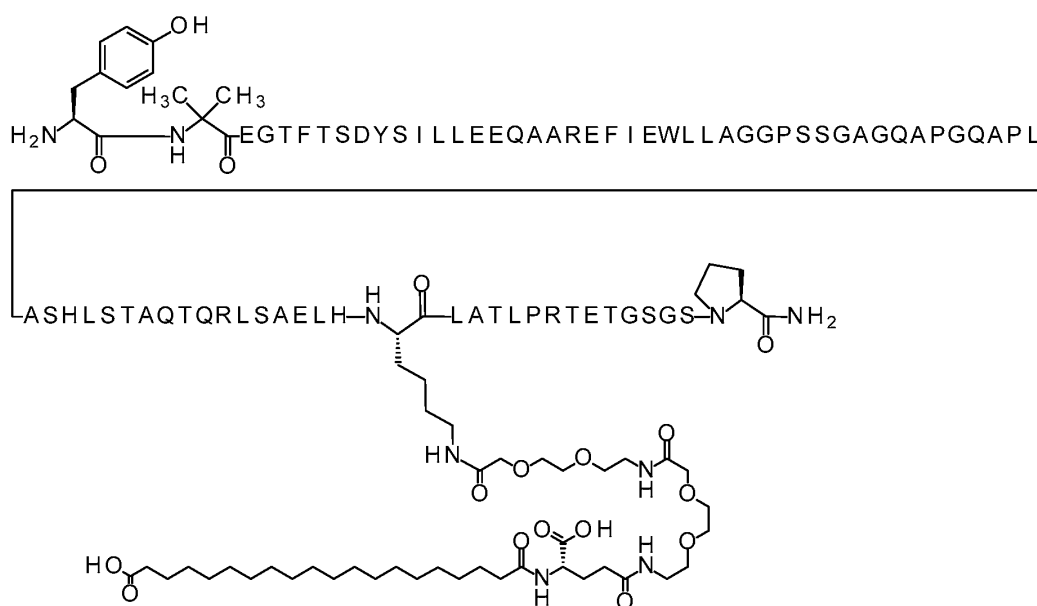
Compound 75



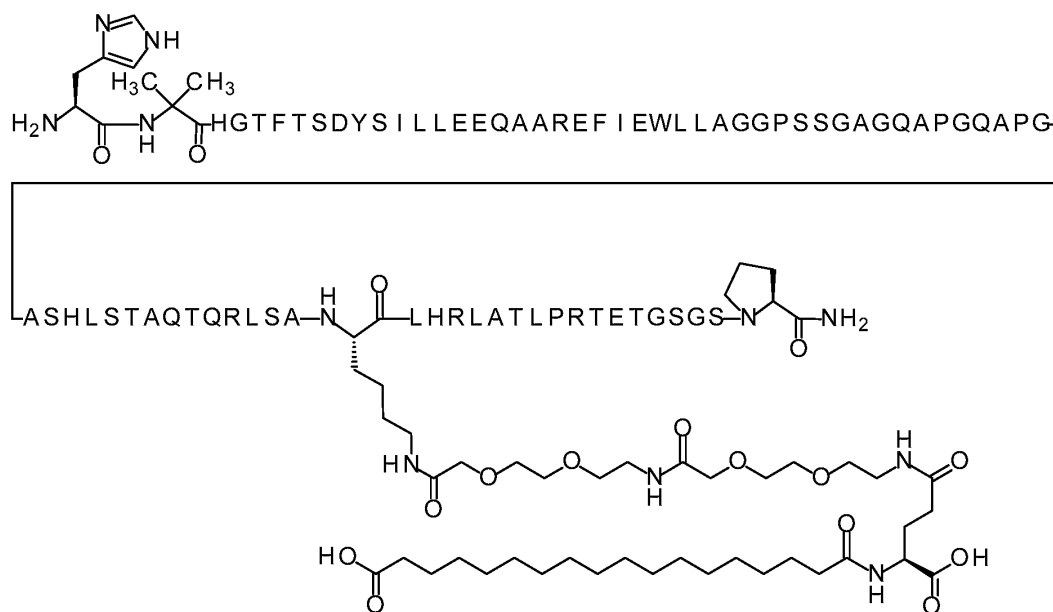
Compound 76



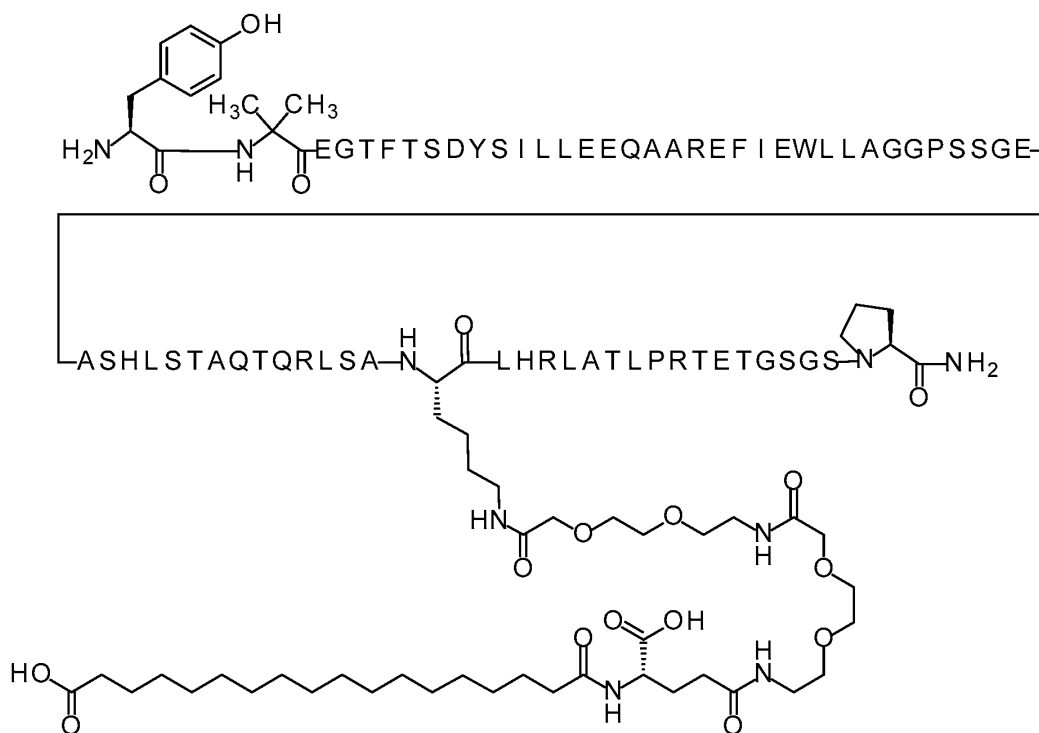
Compound 77

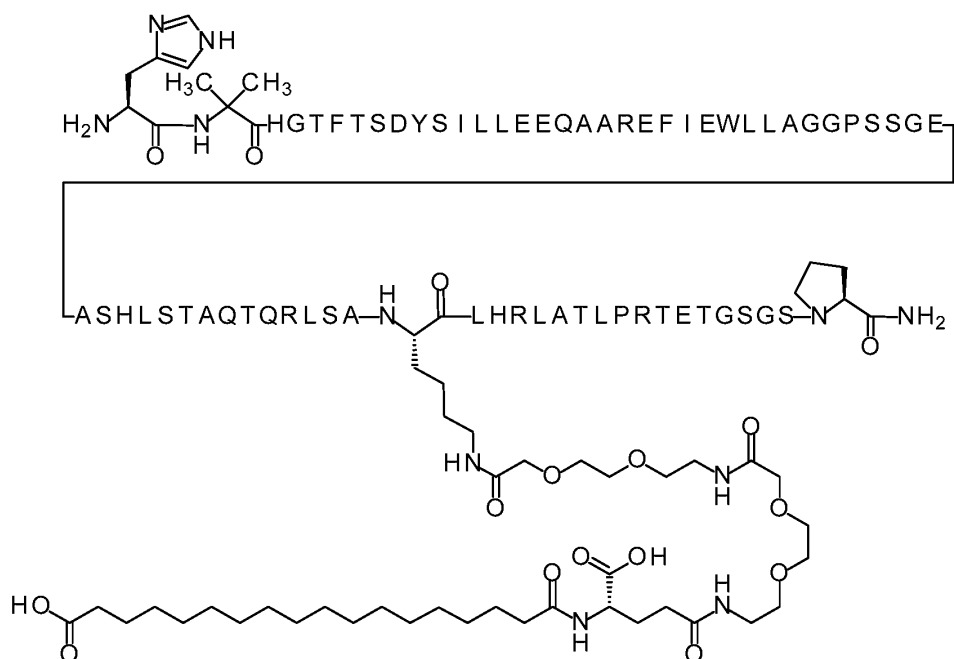


Compound 78

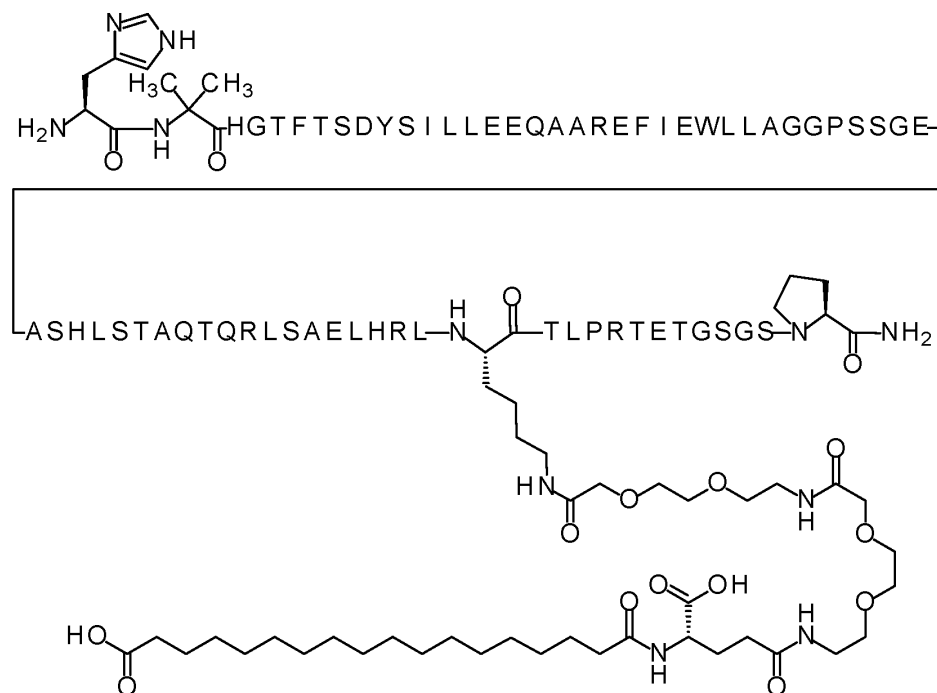


Compound 79

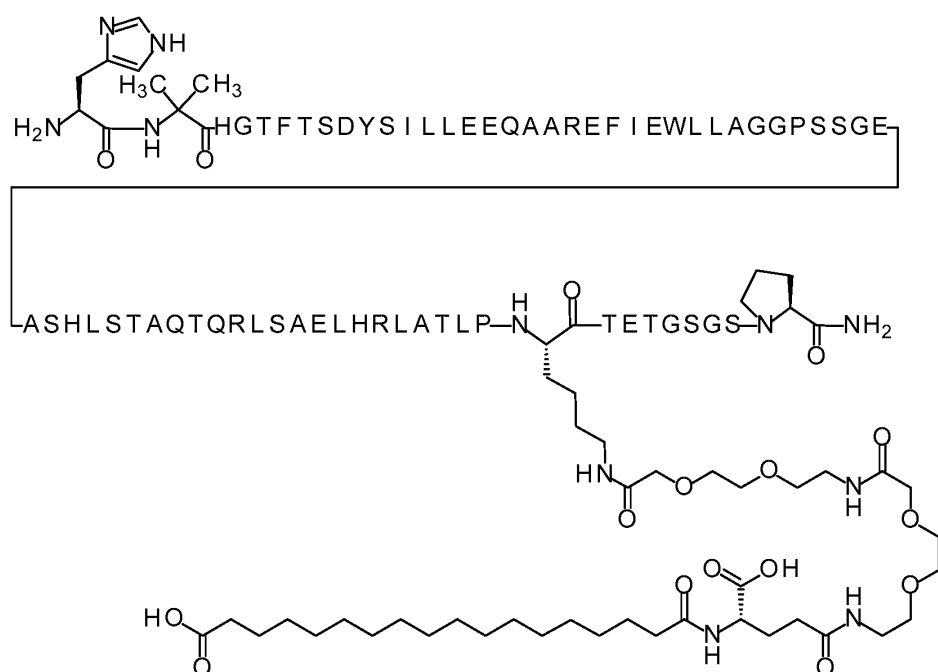




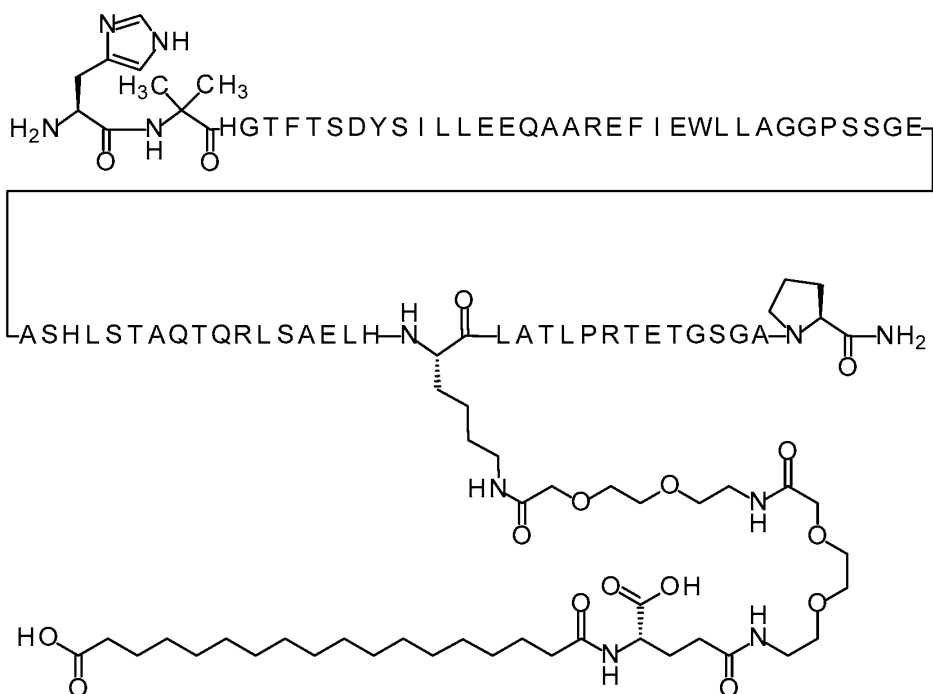
Compound 83



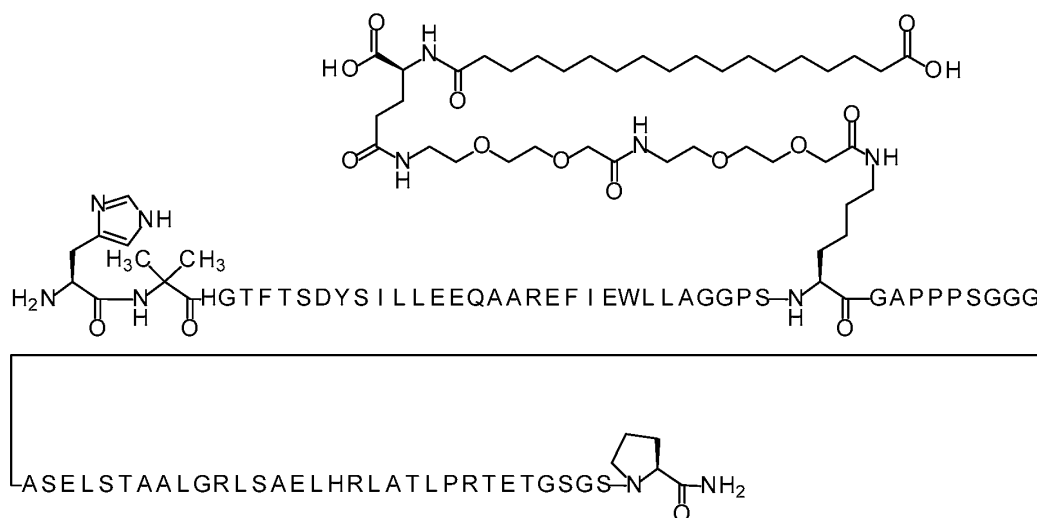
Compound 84



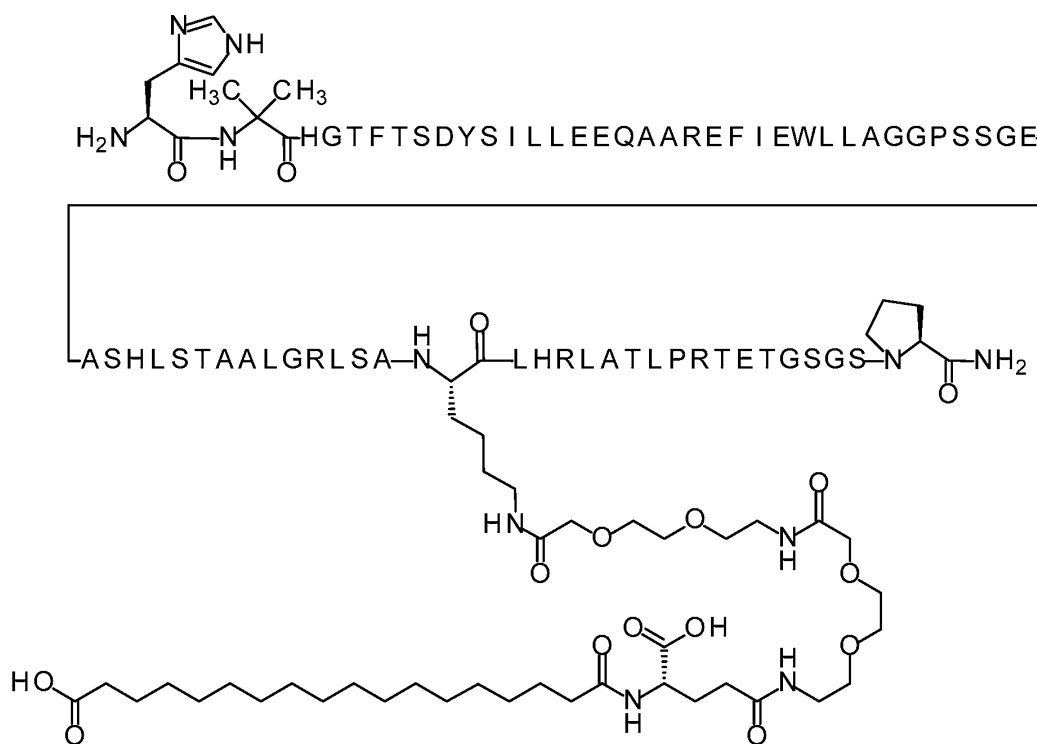
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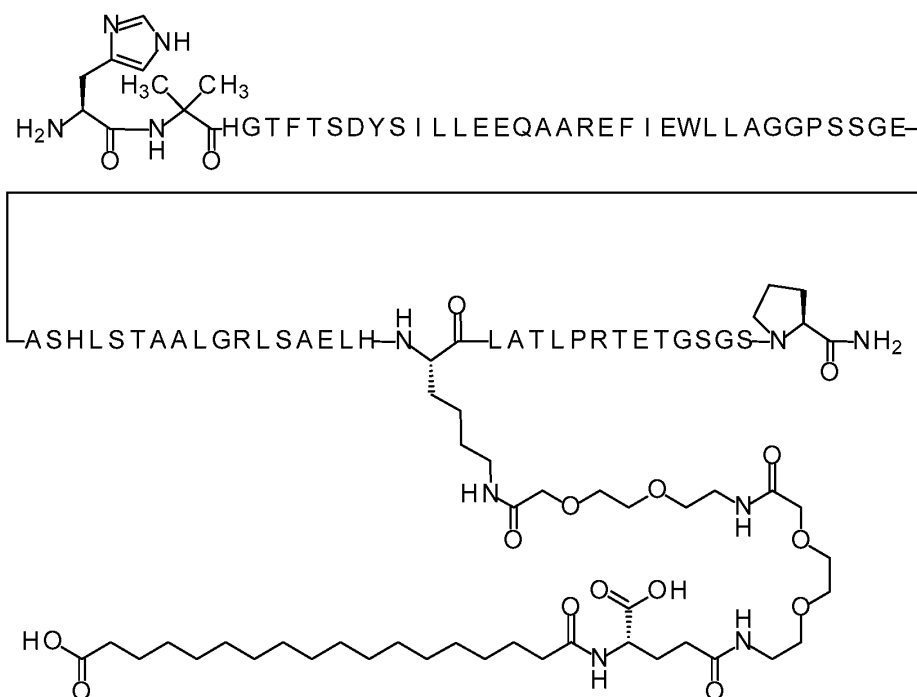
Compound 86



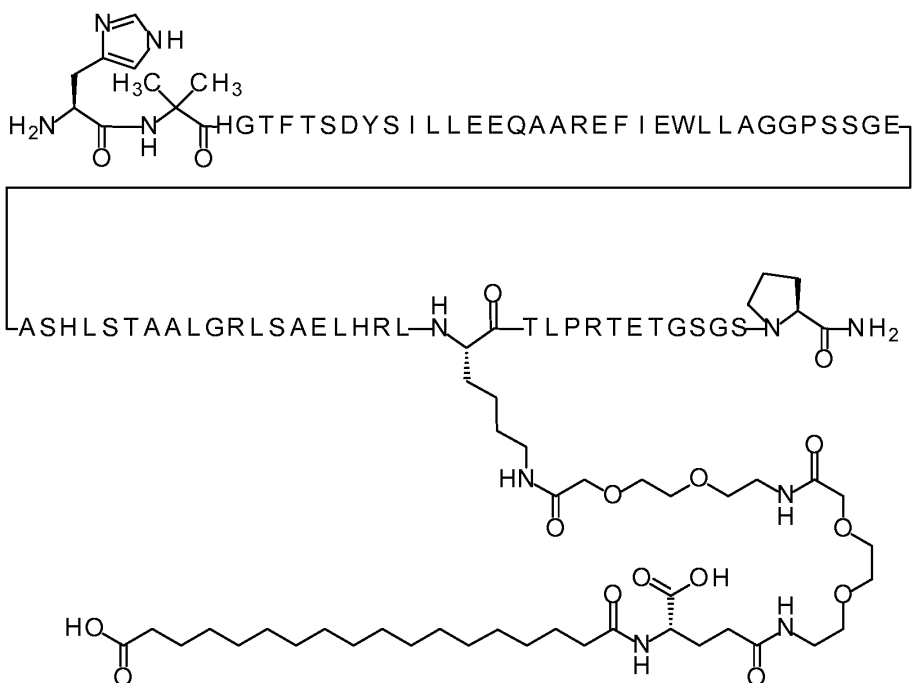
Compound 87



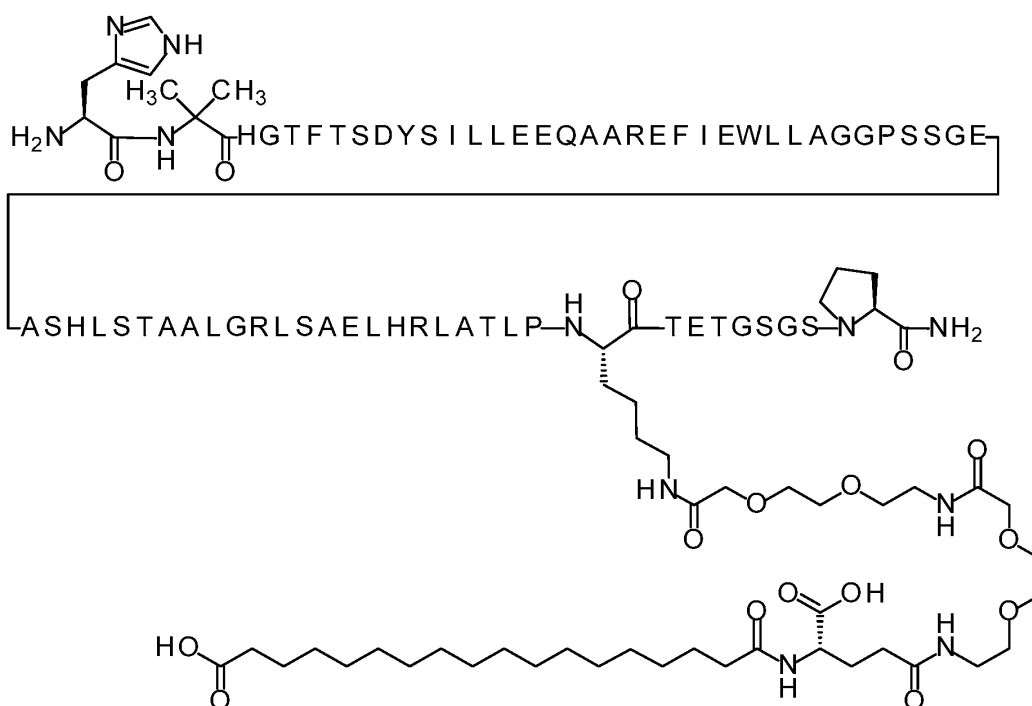
Compound 88



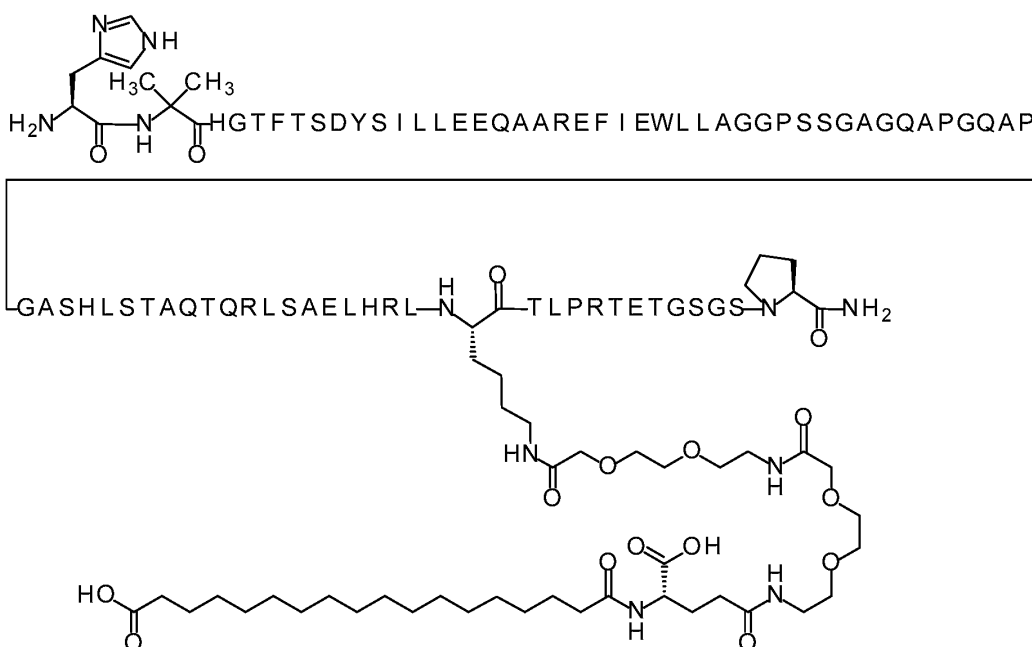
Compound 89



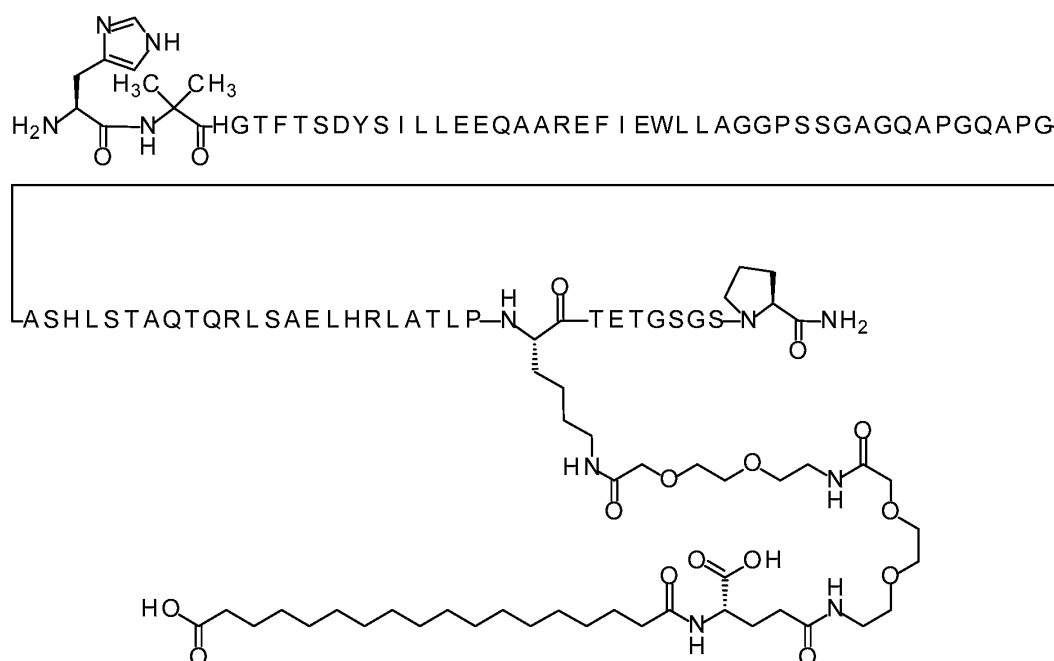
Compound 90



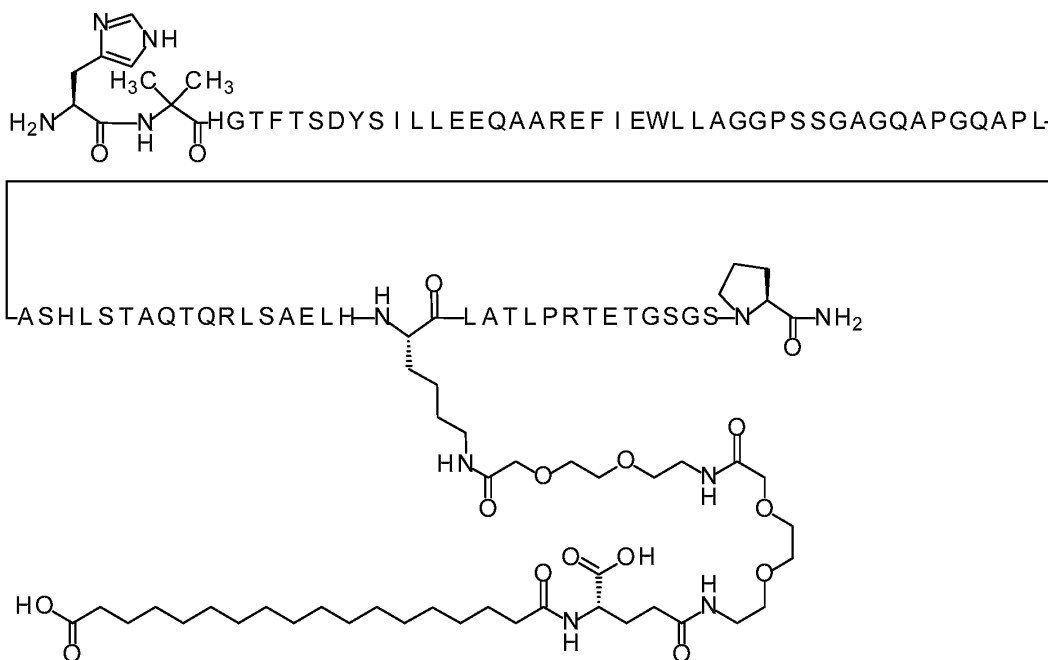
Compound 91



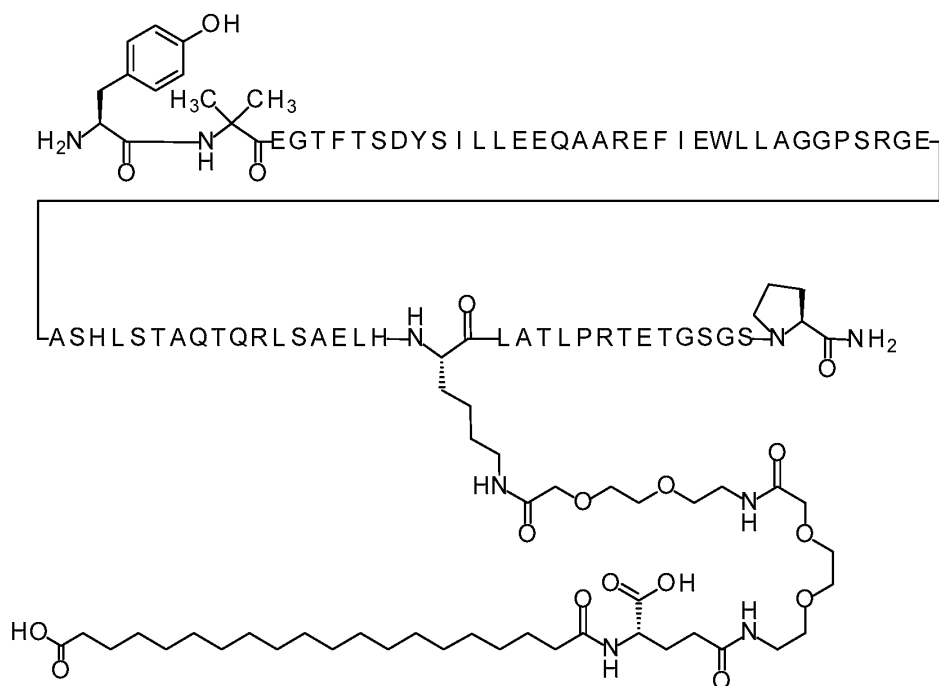
Compound 92



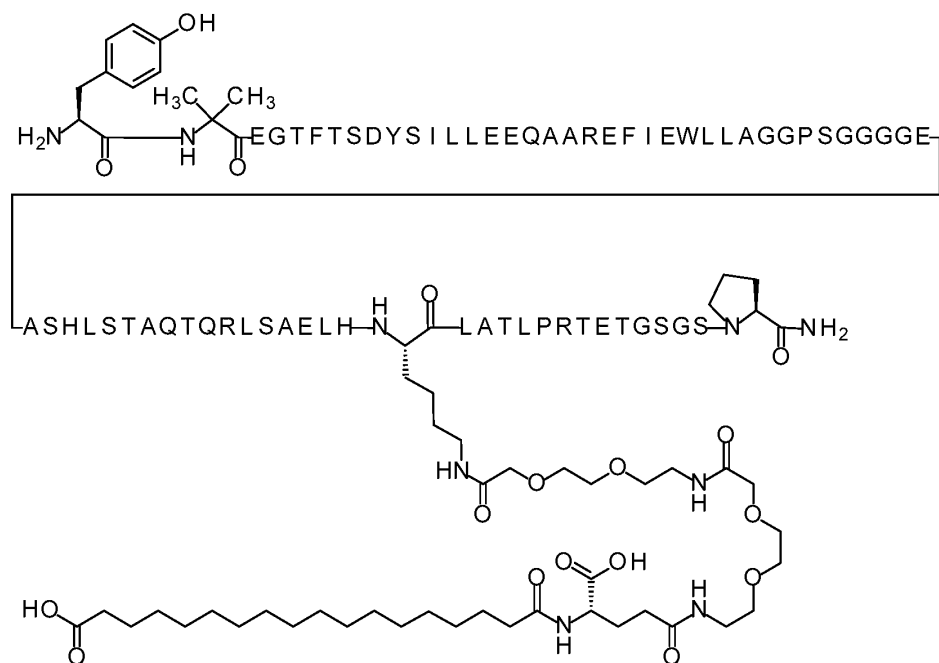
Compound 93



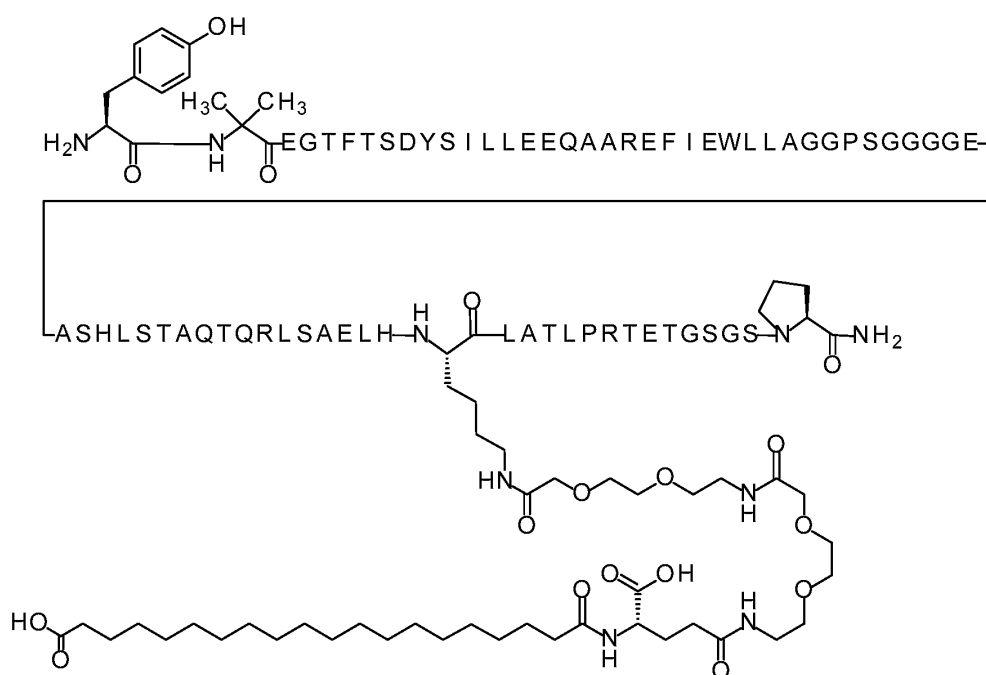
Compound 94



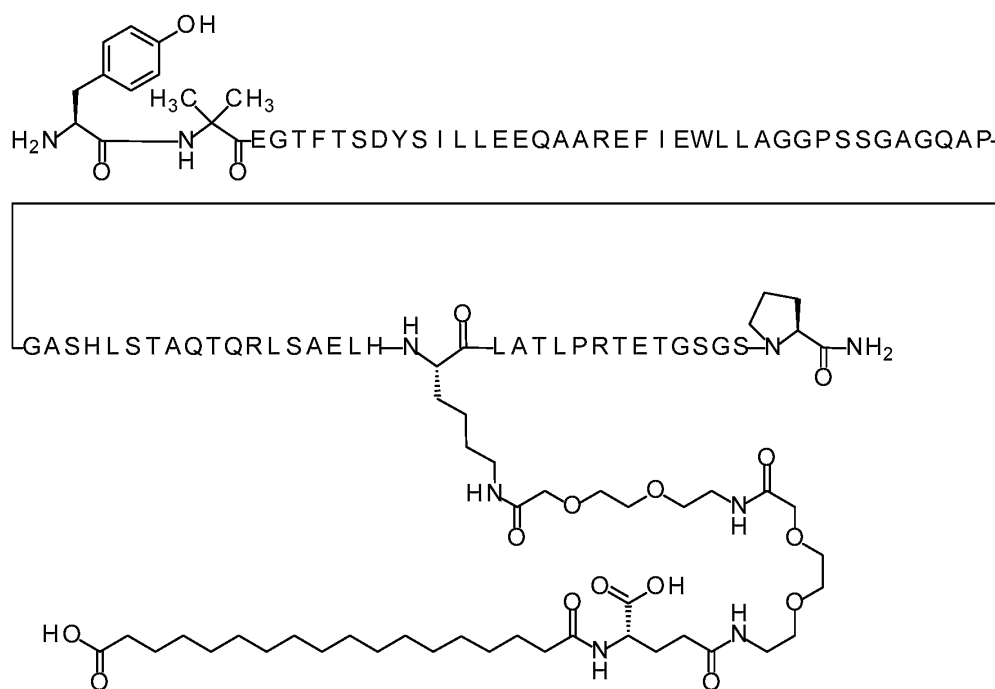
Compound 95



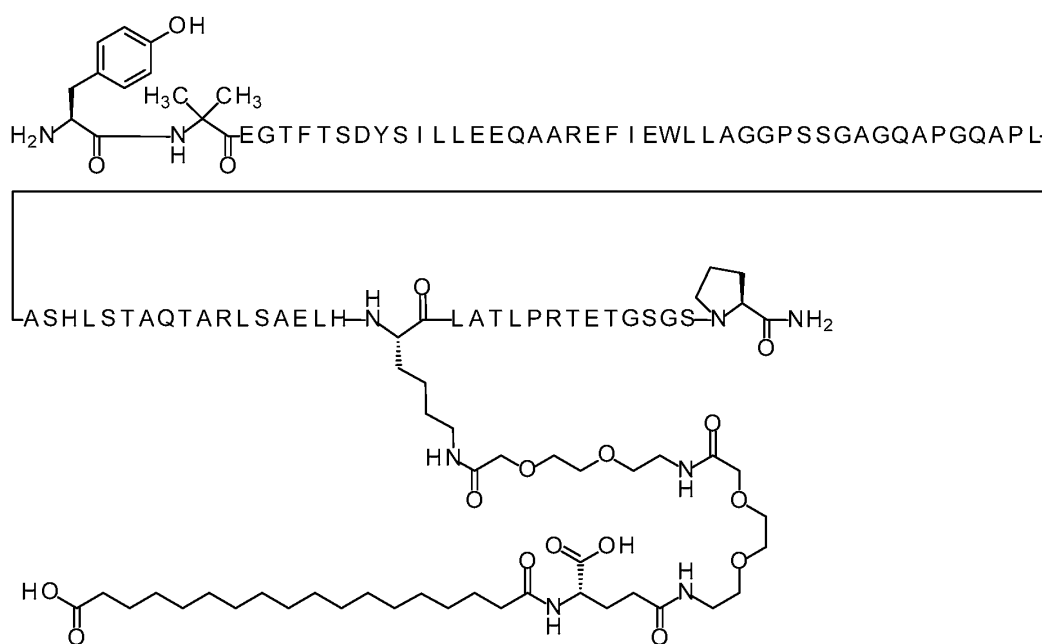
Compound 96



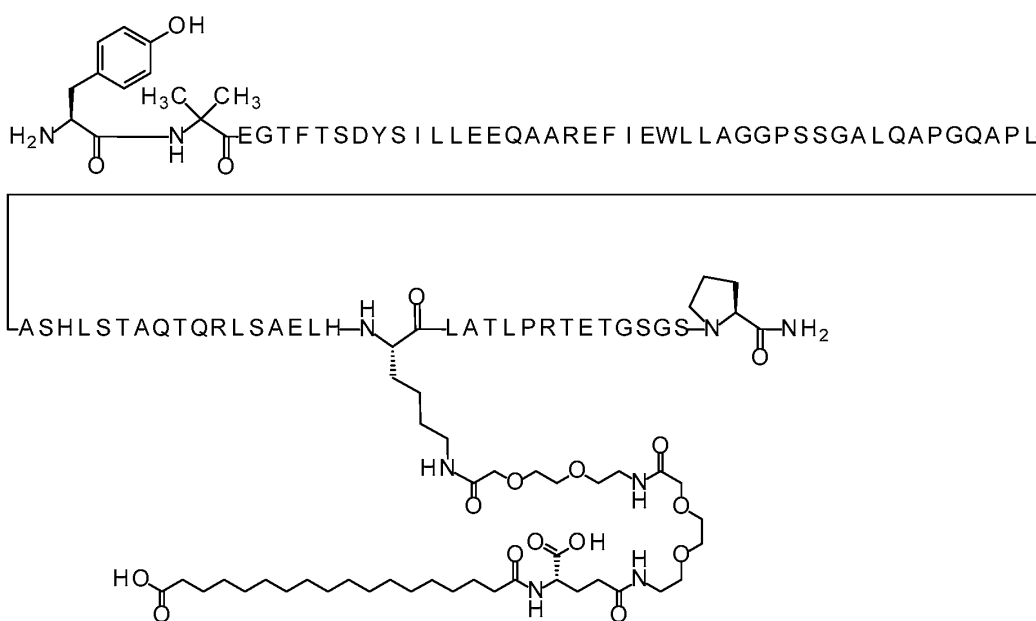
Compound 97

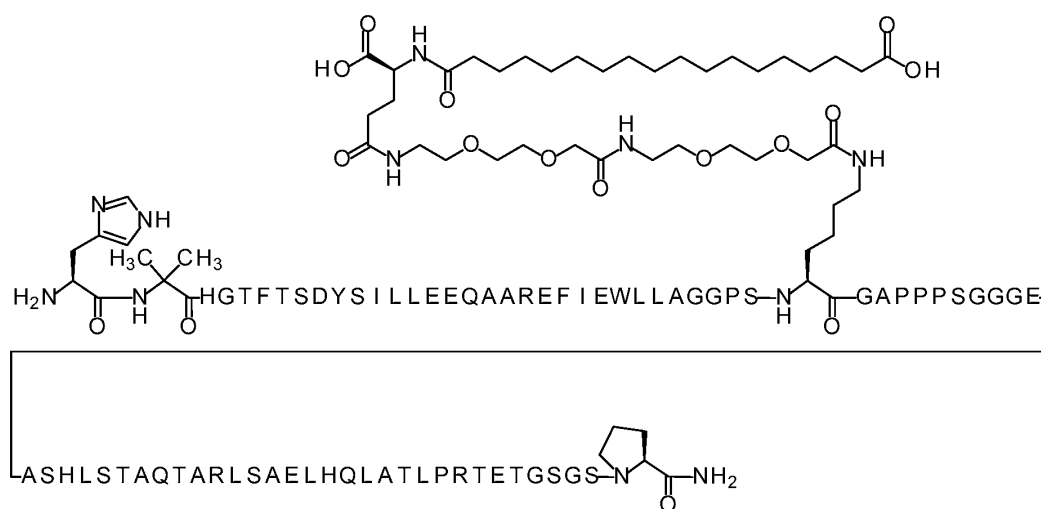


Compound 98

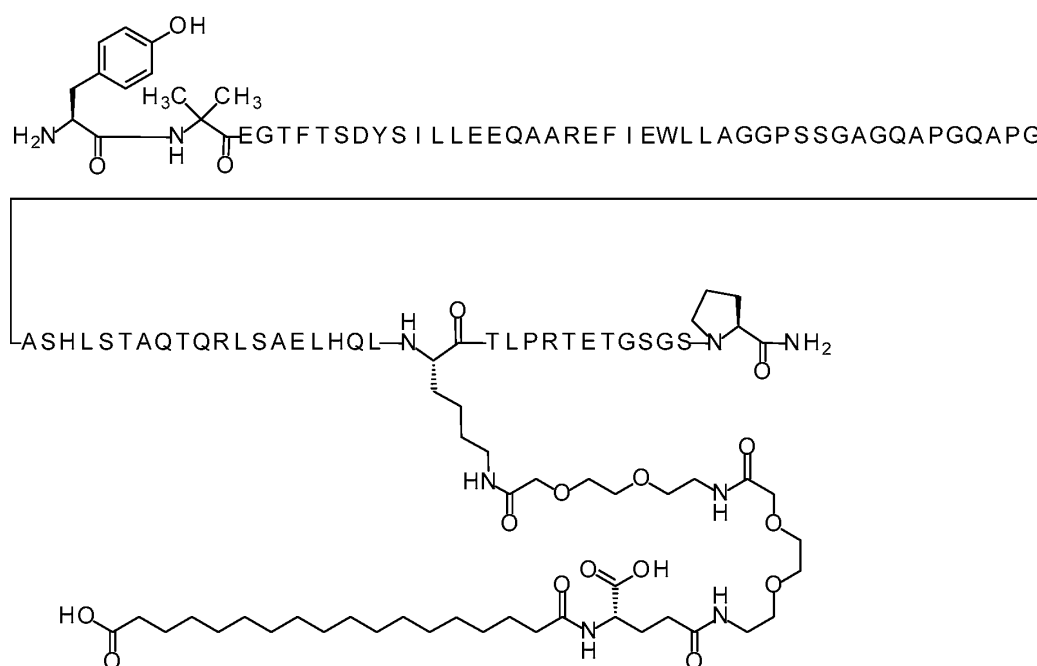


Compound 99

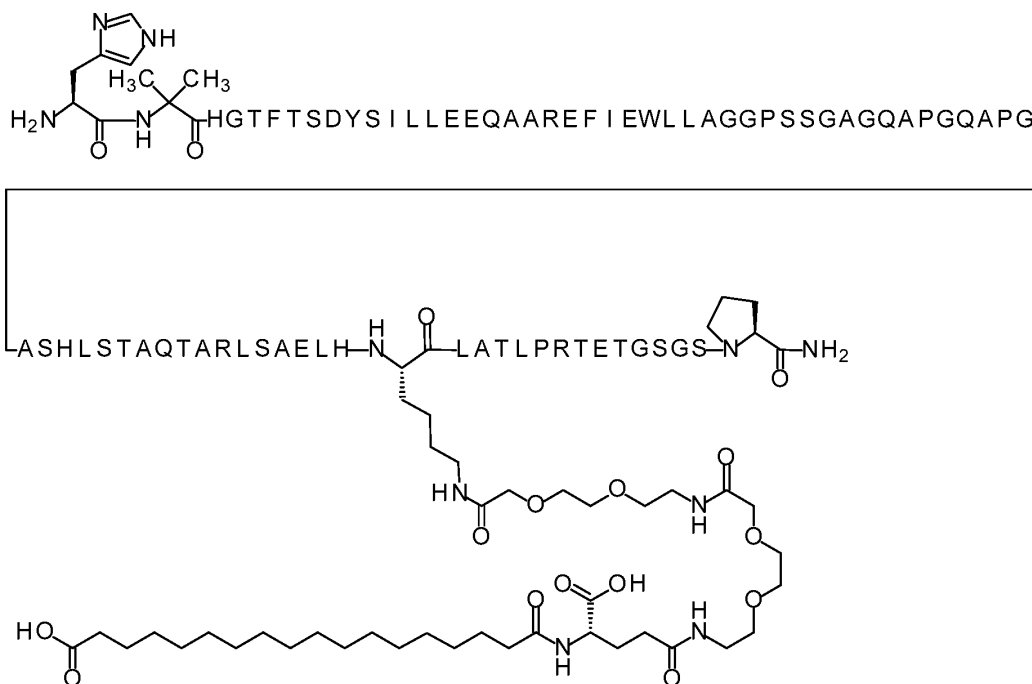




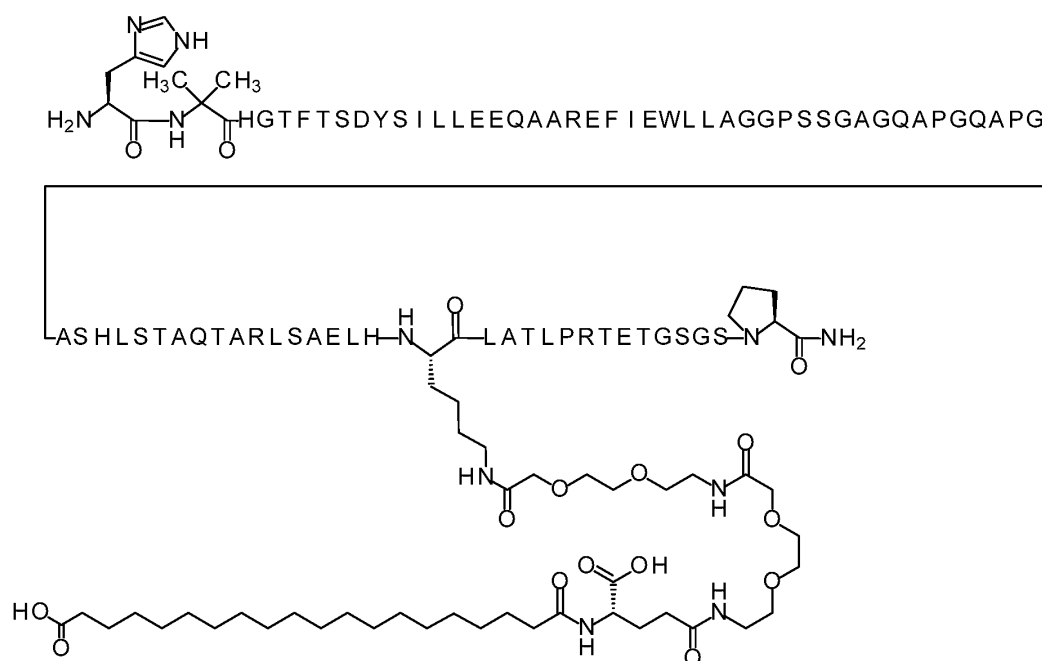
Compound 102



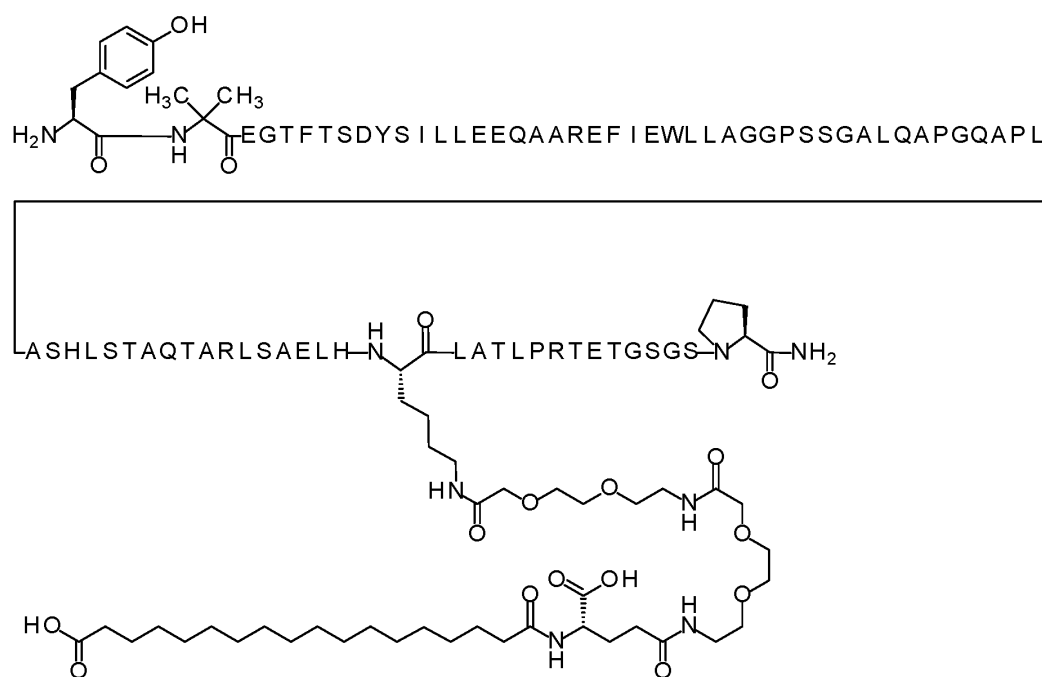
Compound 103



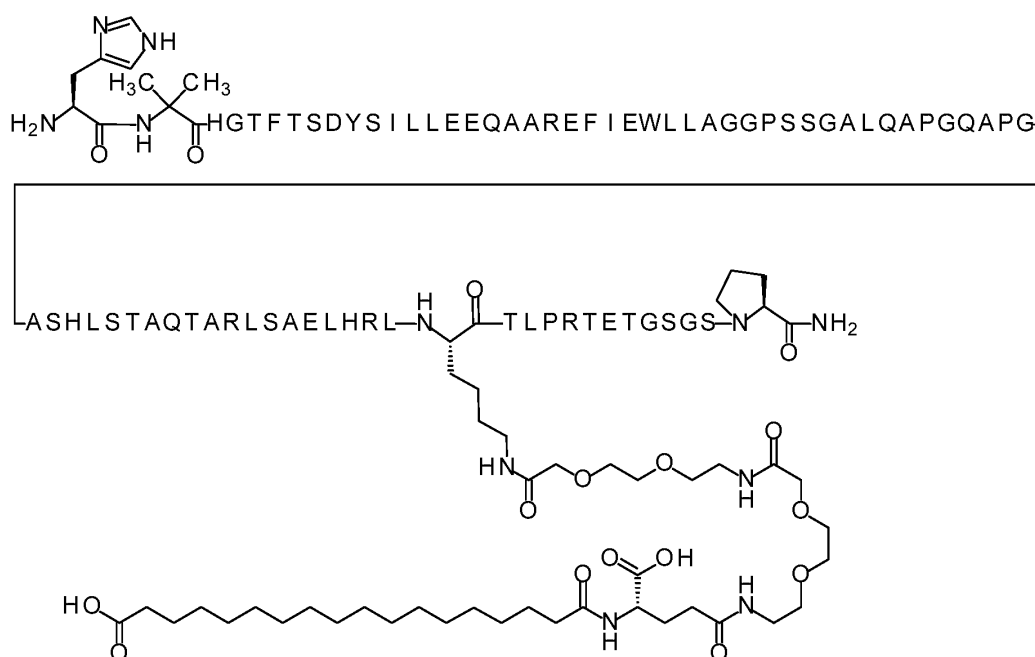
Compound 104



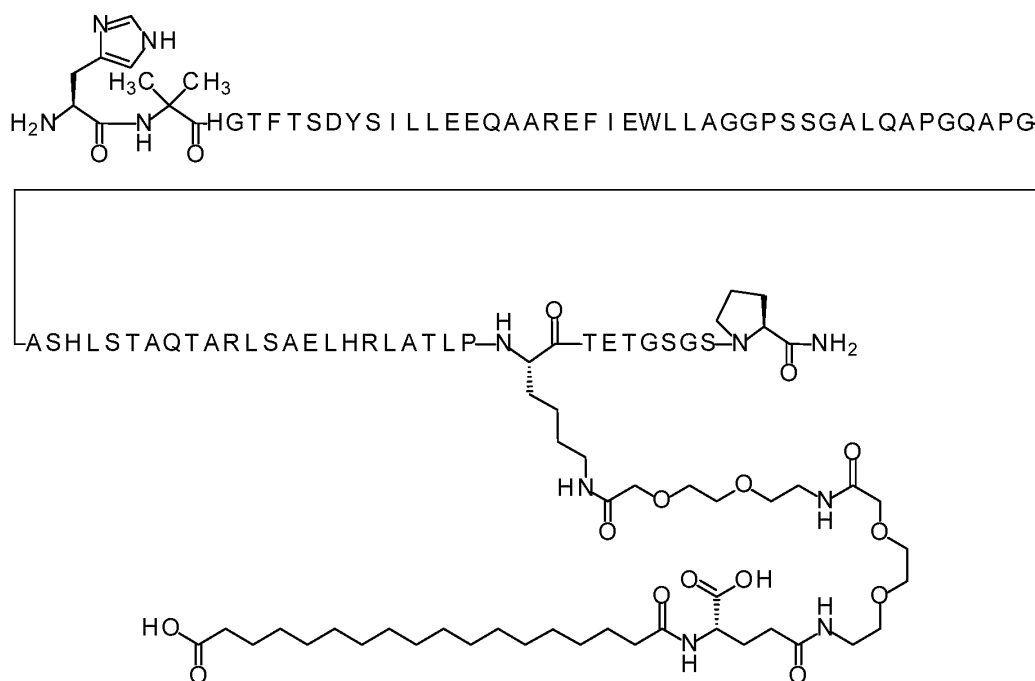
Compound 105



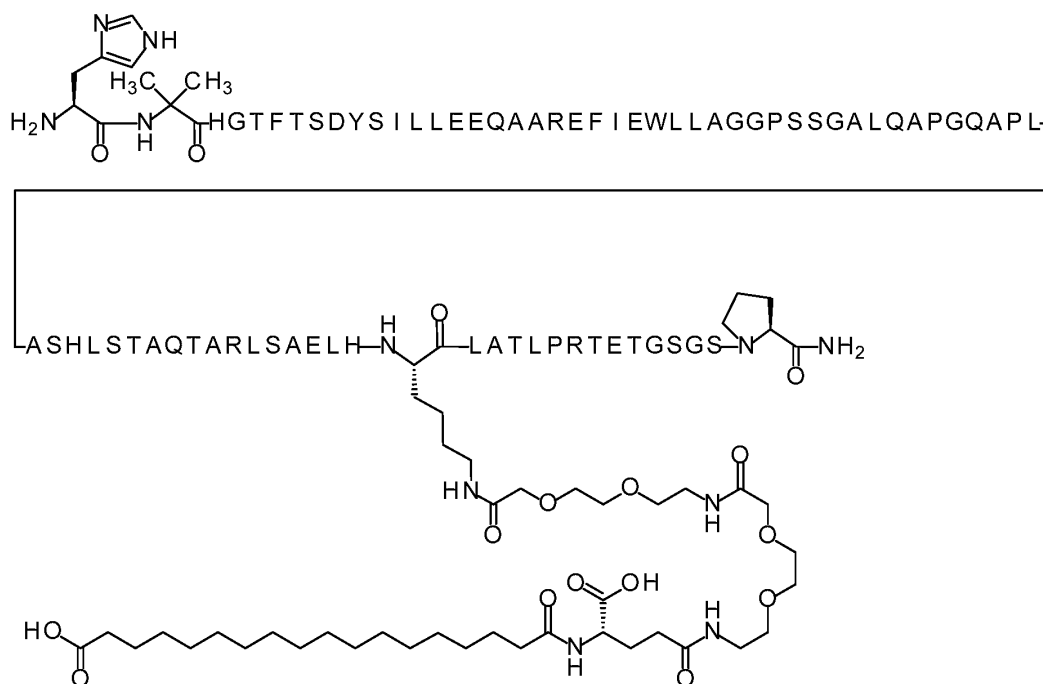
Compound 106



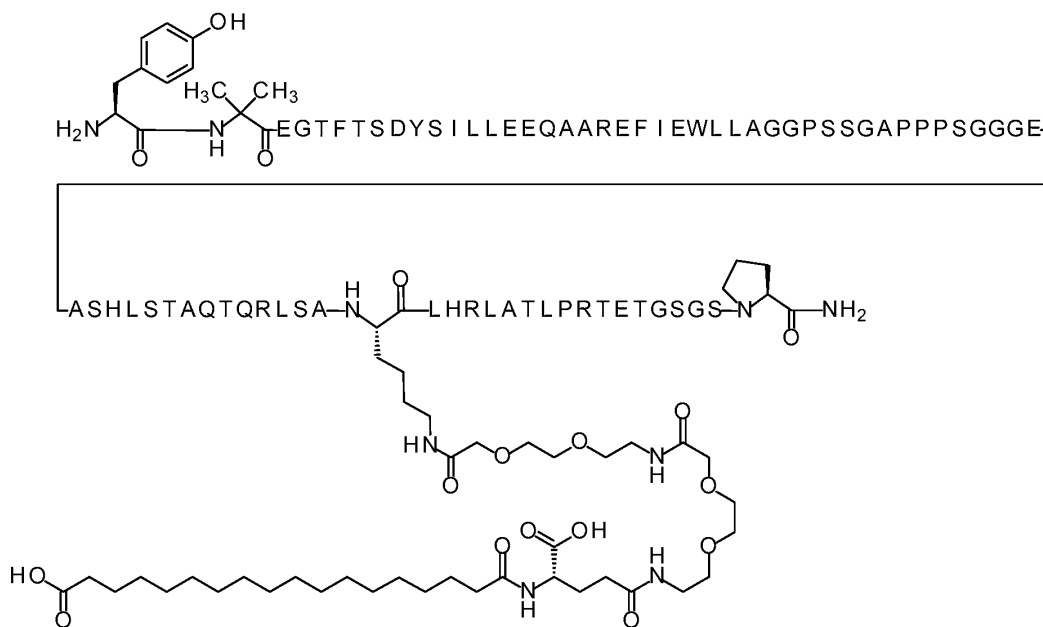
Compound 107

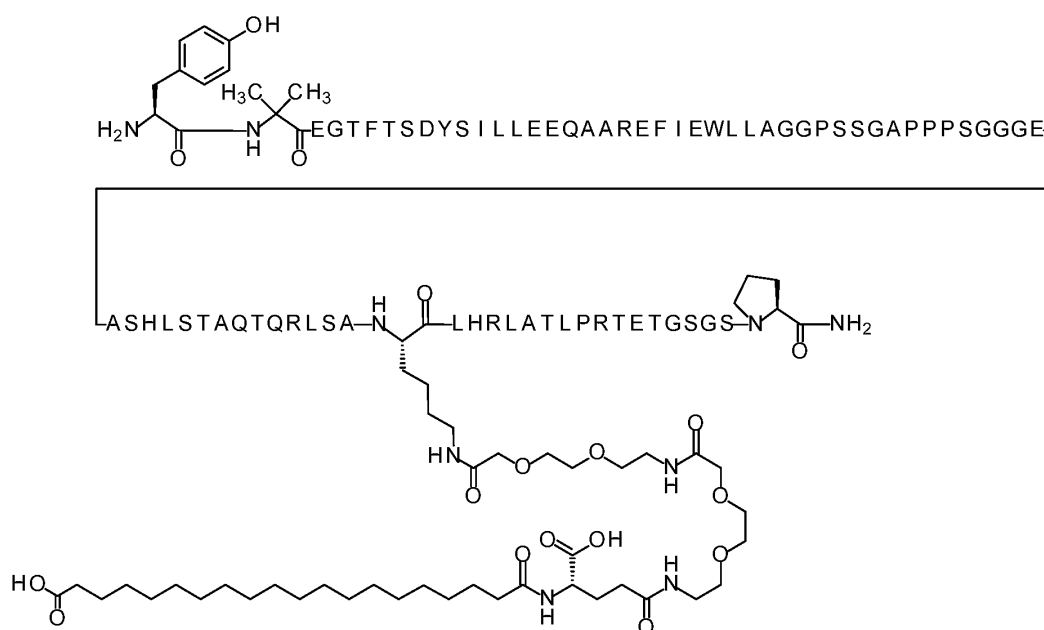


Compound 108

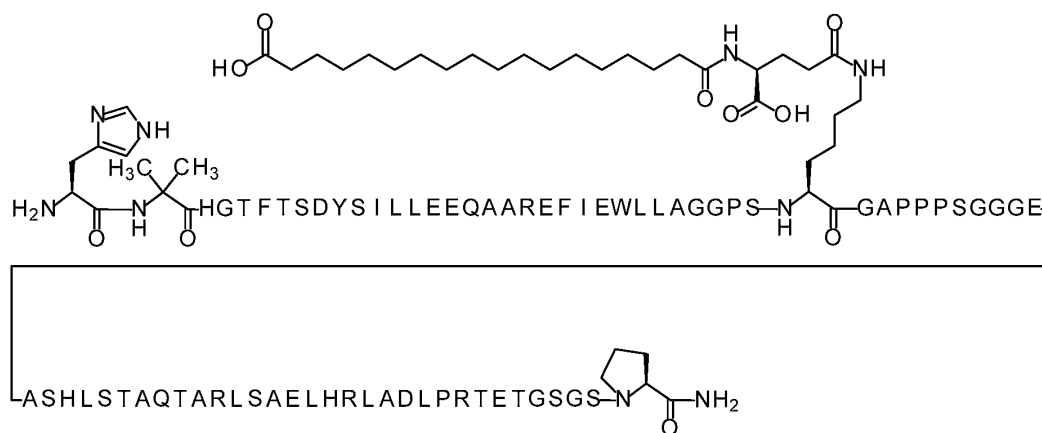


Compound 109

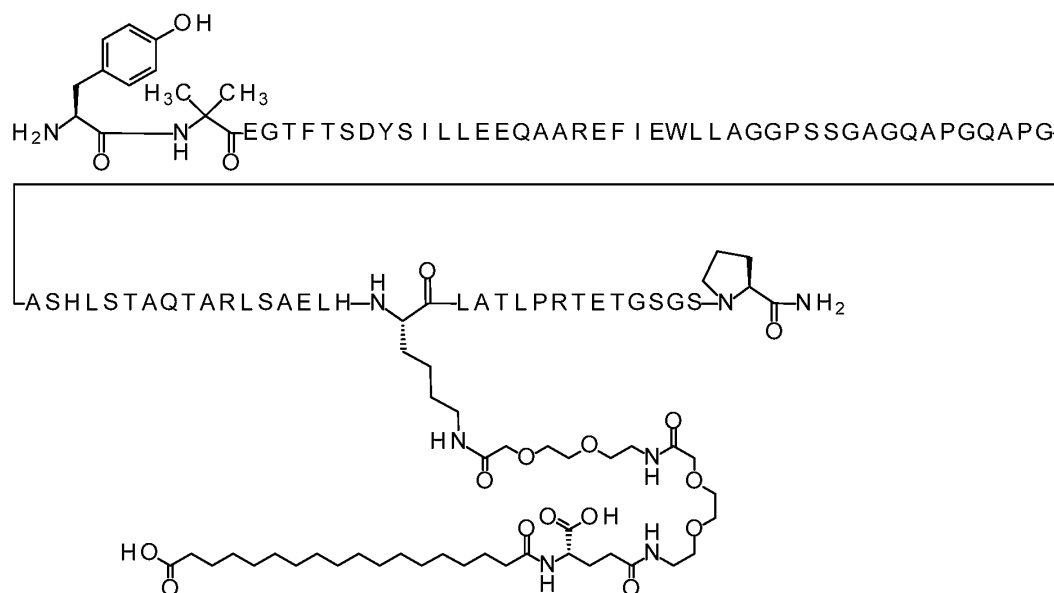


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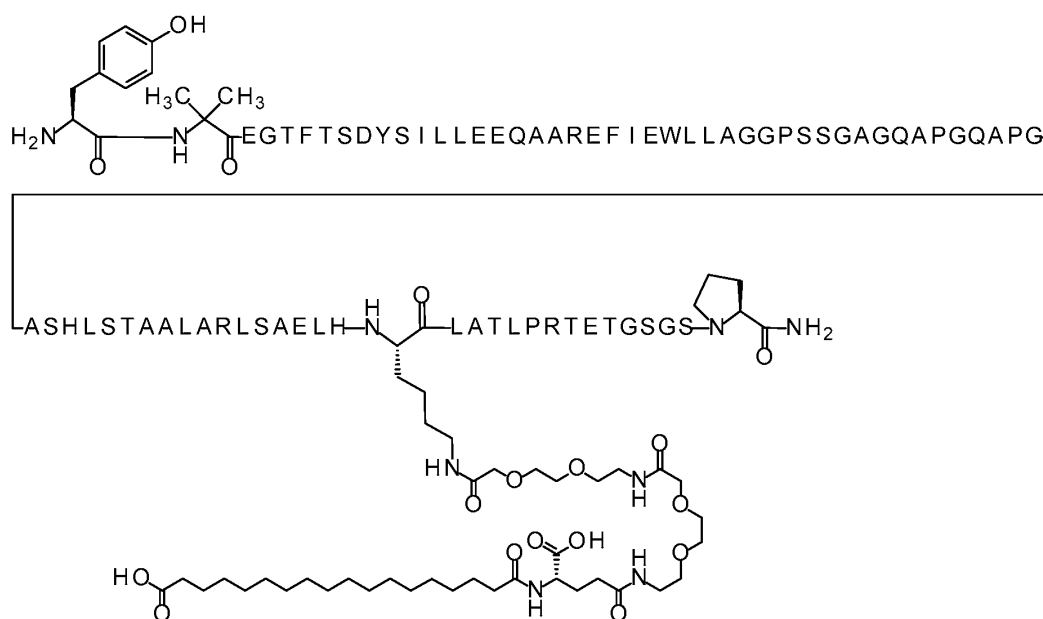
Compound 112



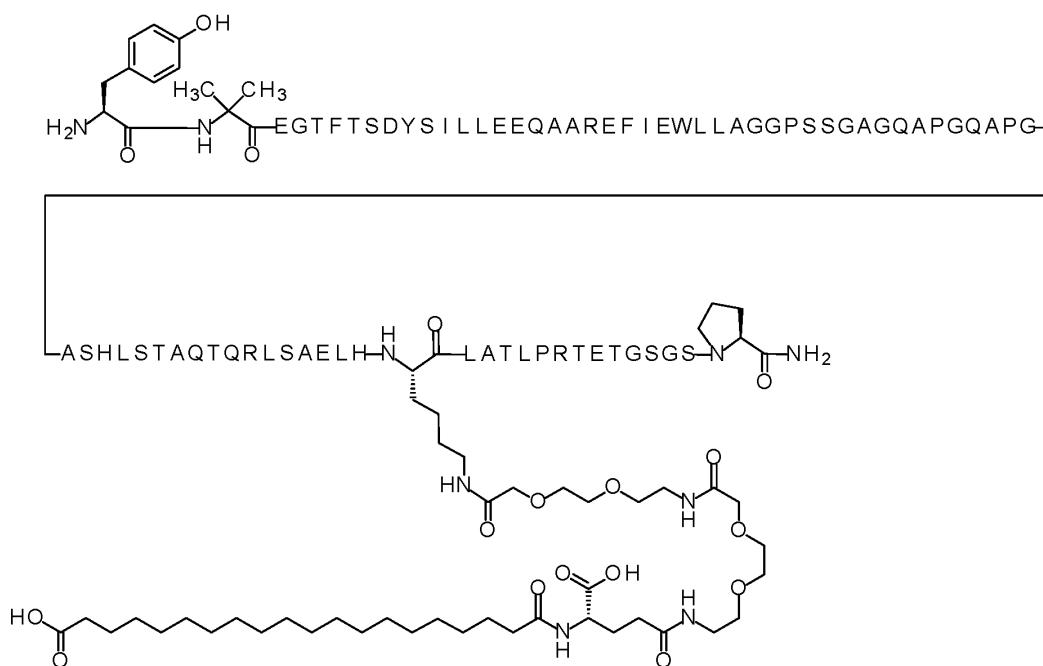
Compound 113

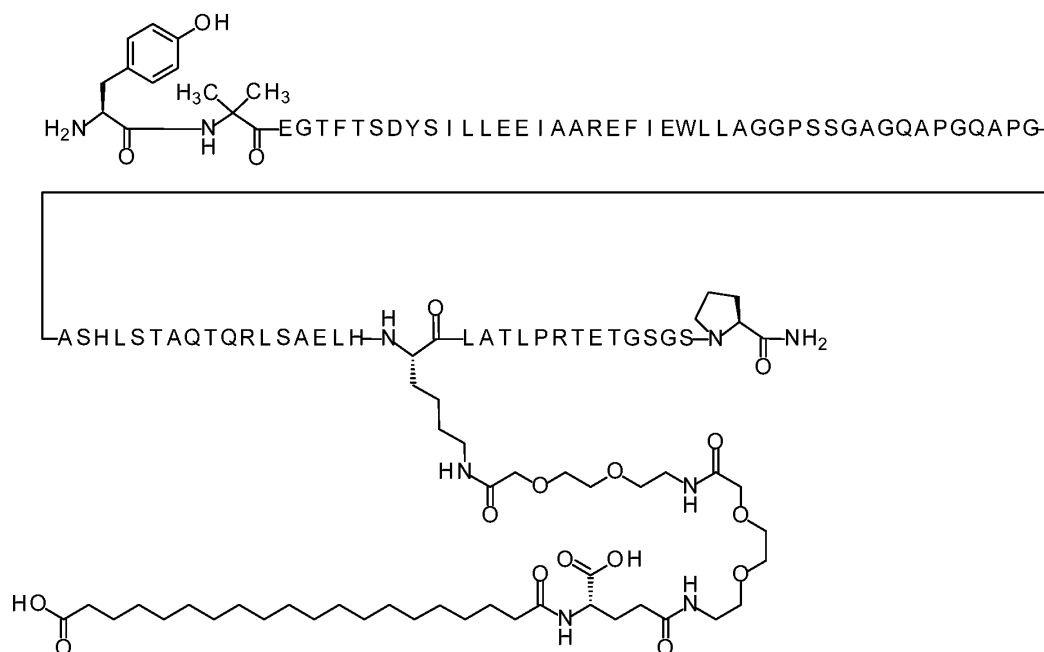


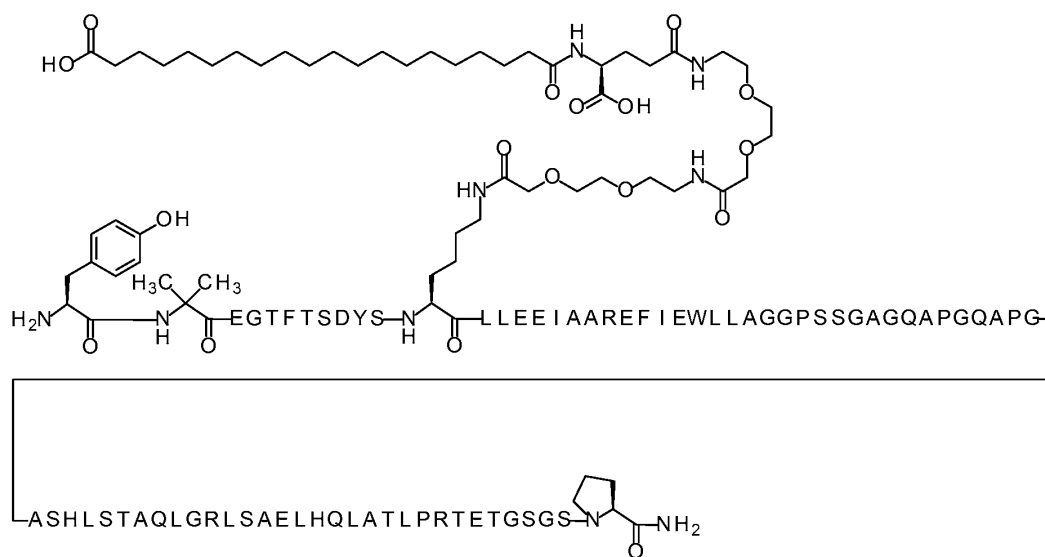
Compound 114



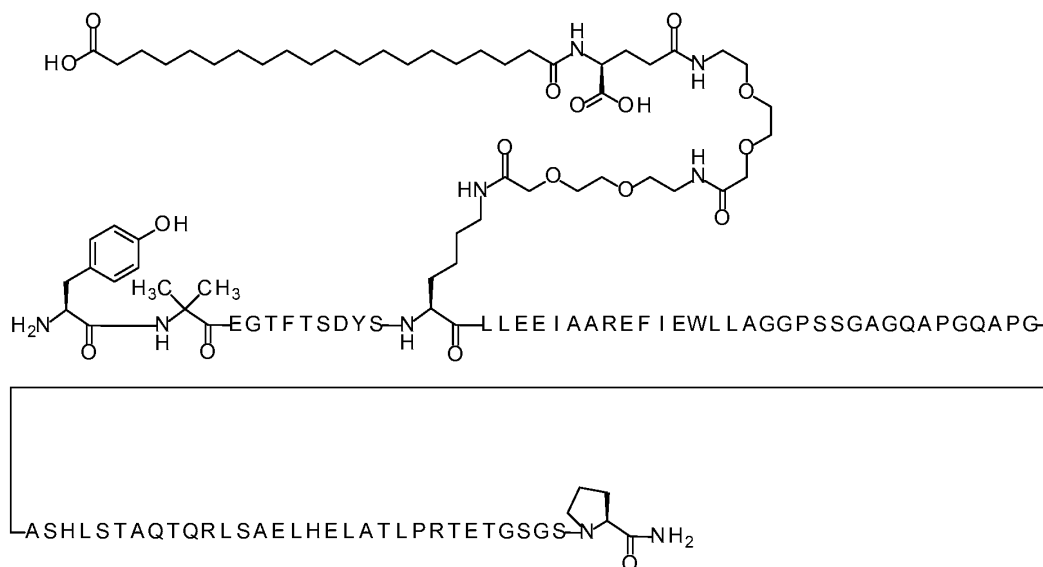
Compound 115



[illegible]

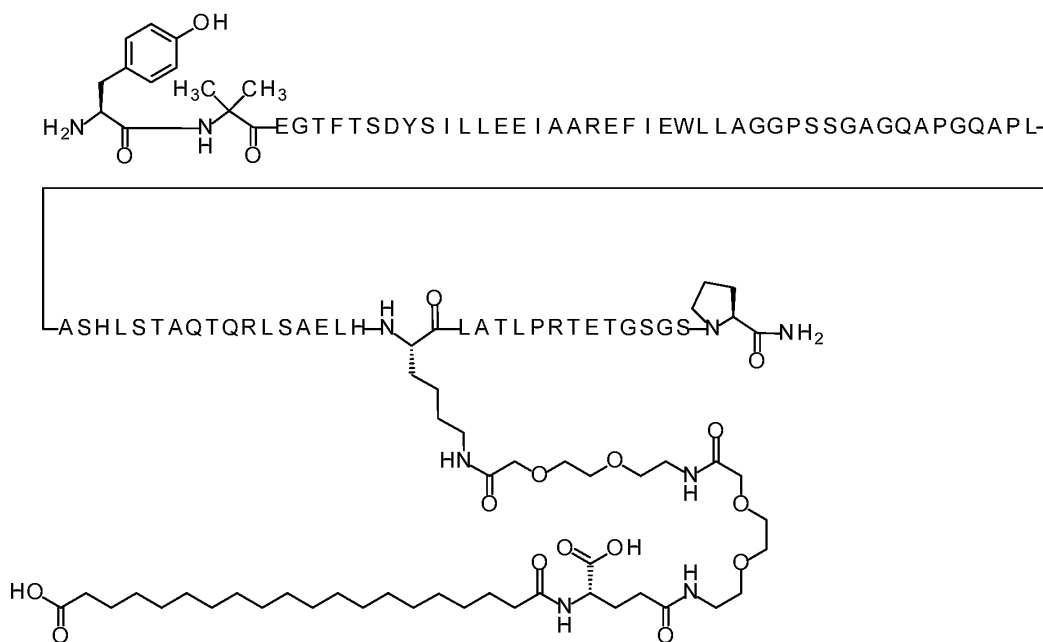


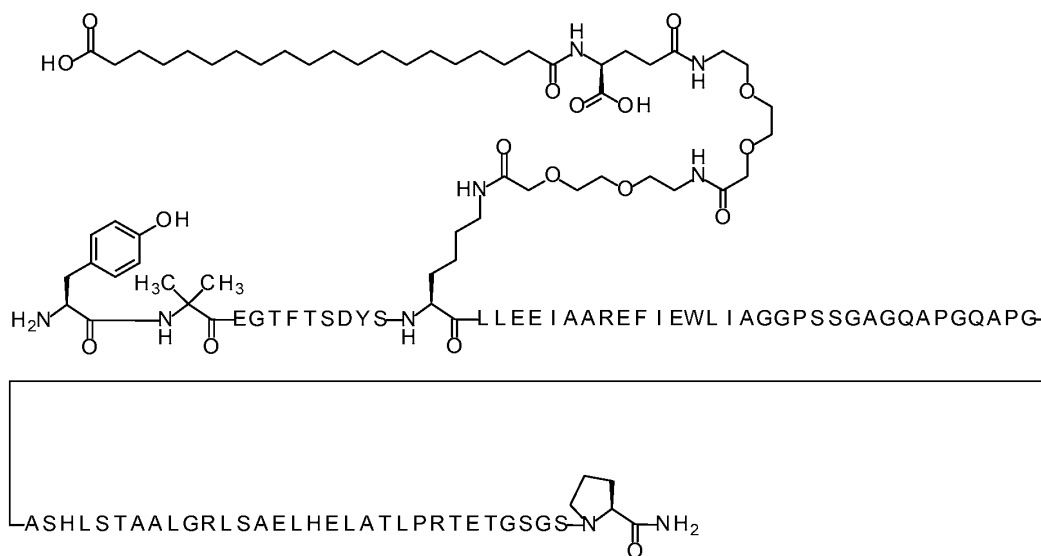
Compound 124



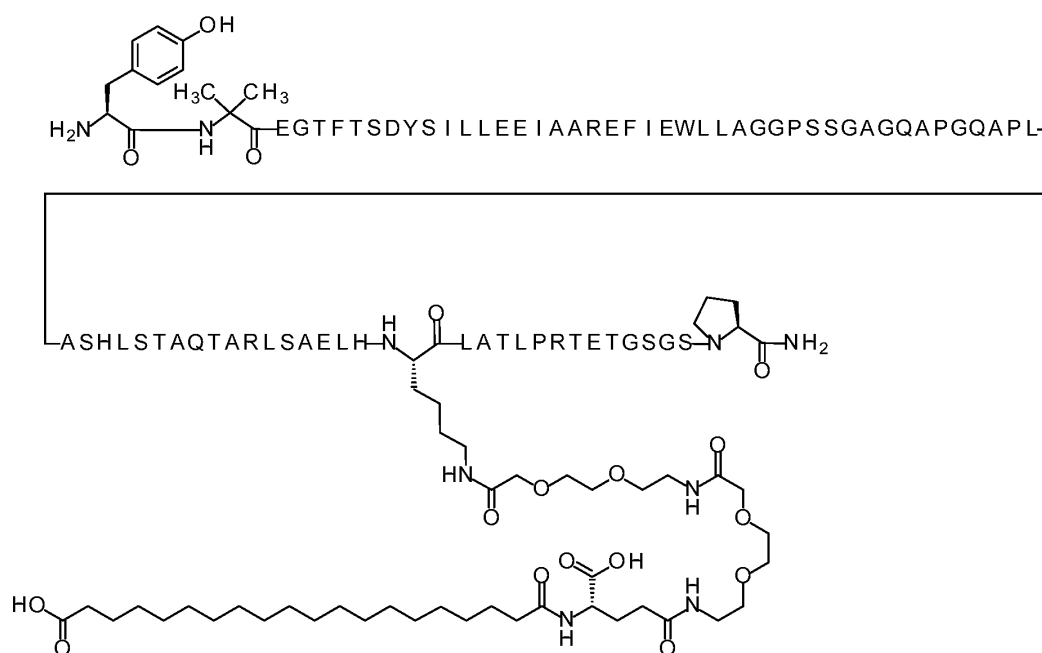
Compound 125

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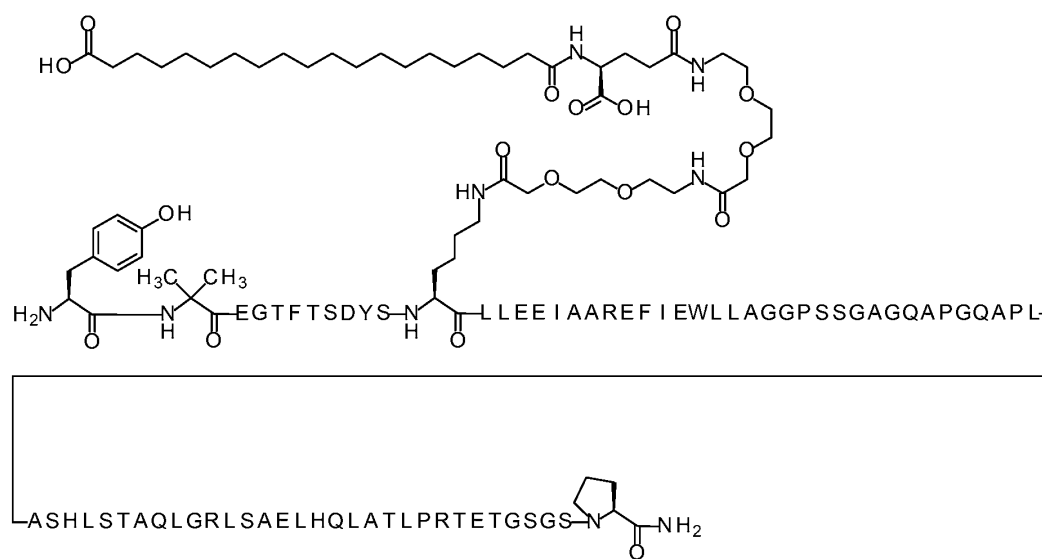




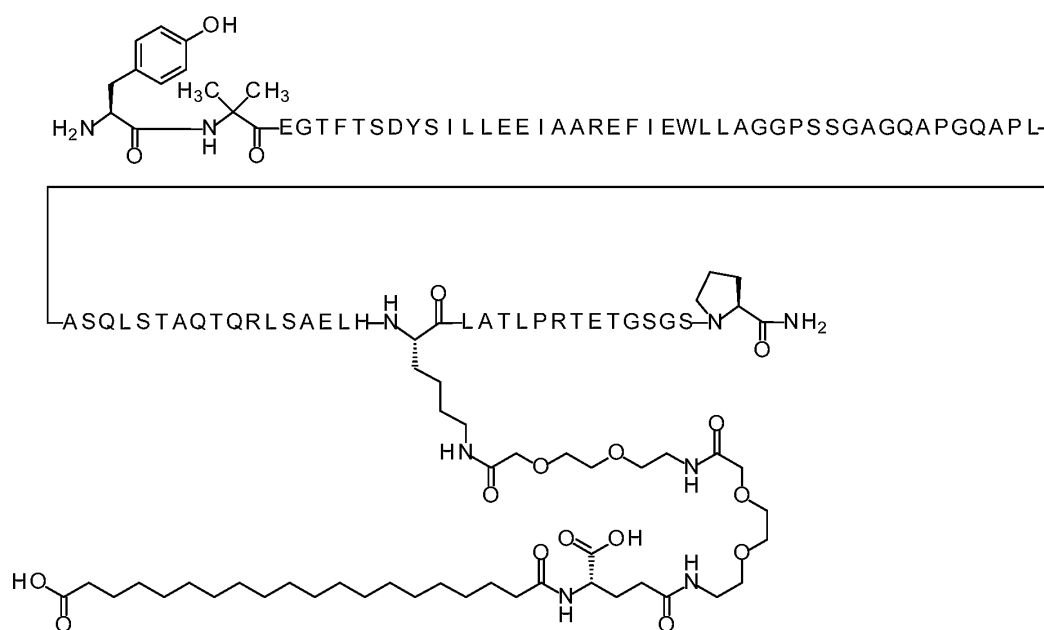
Compound 128



Compound 129

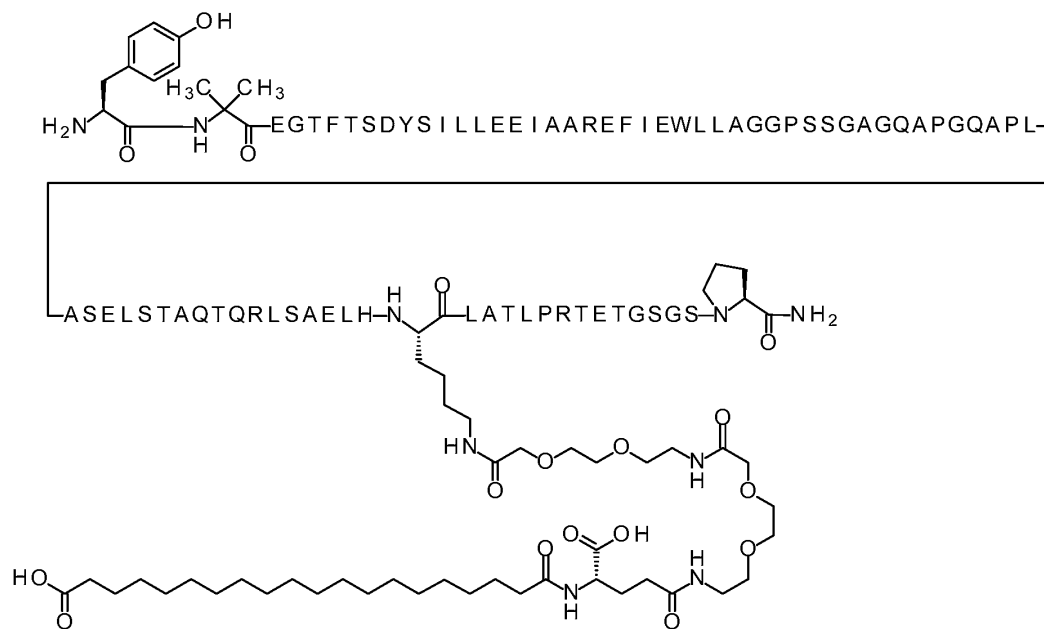


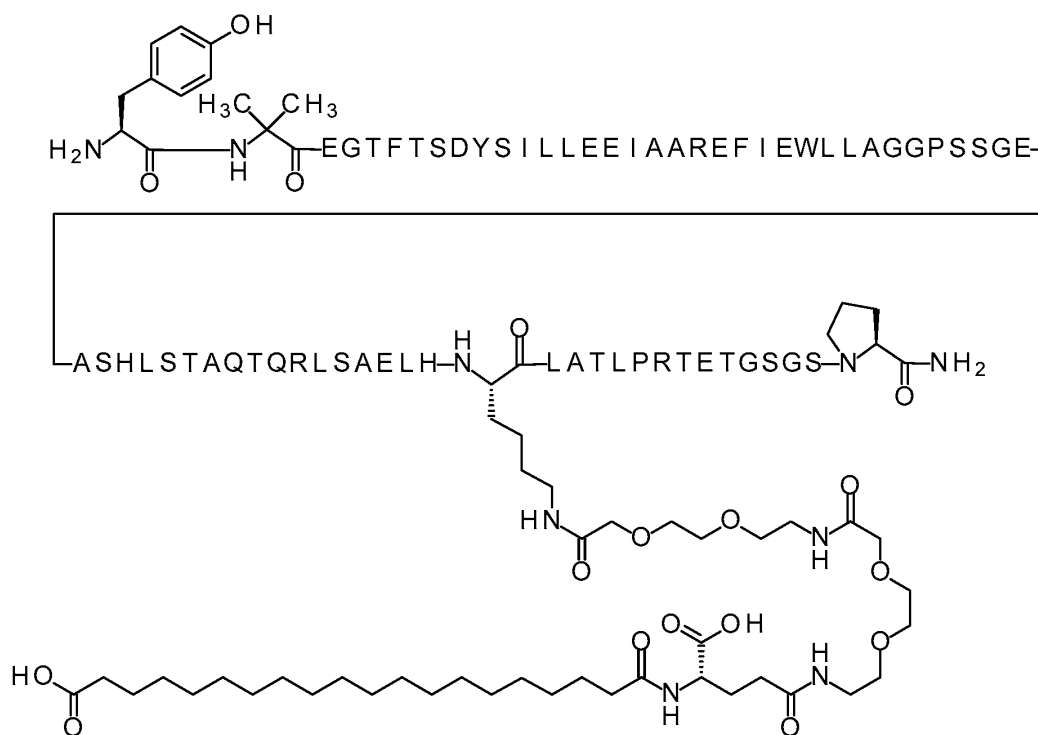
Compound 132



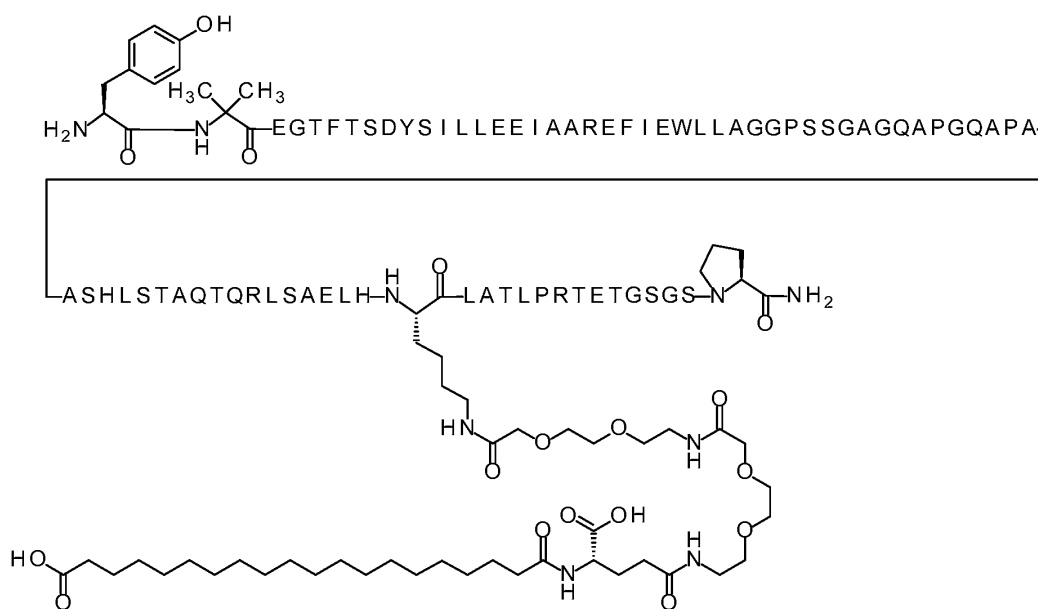
Compound 133

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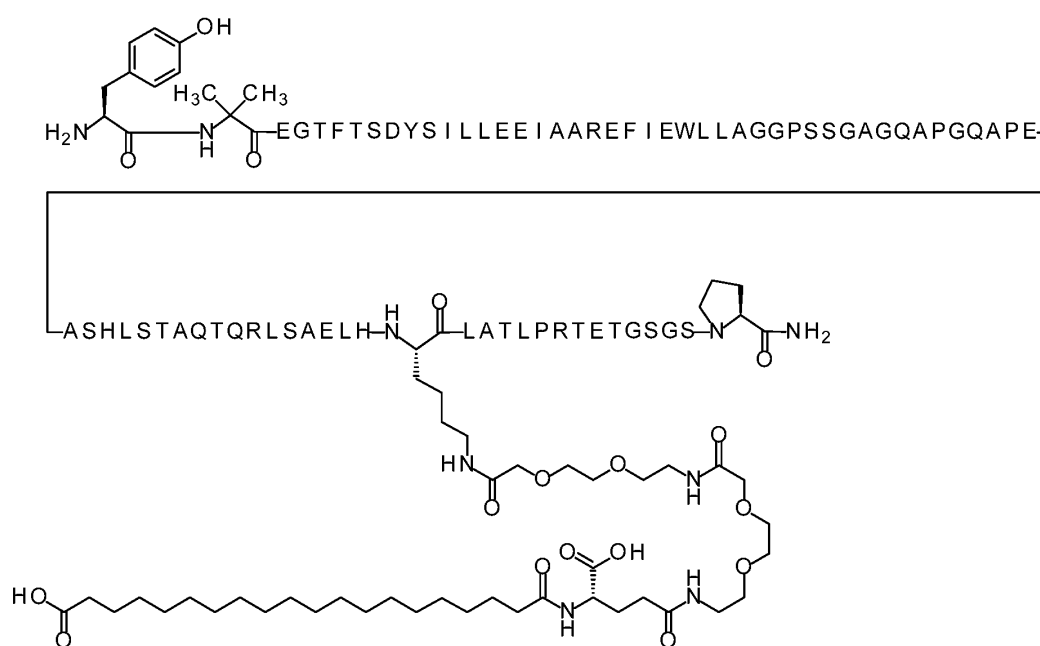




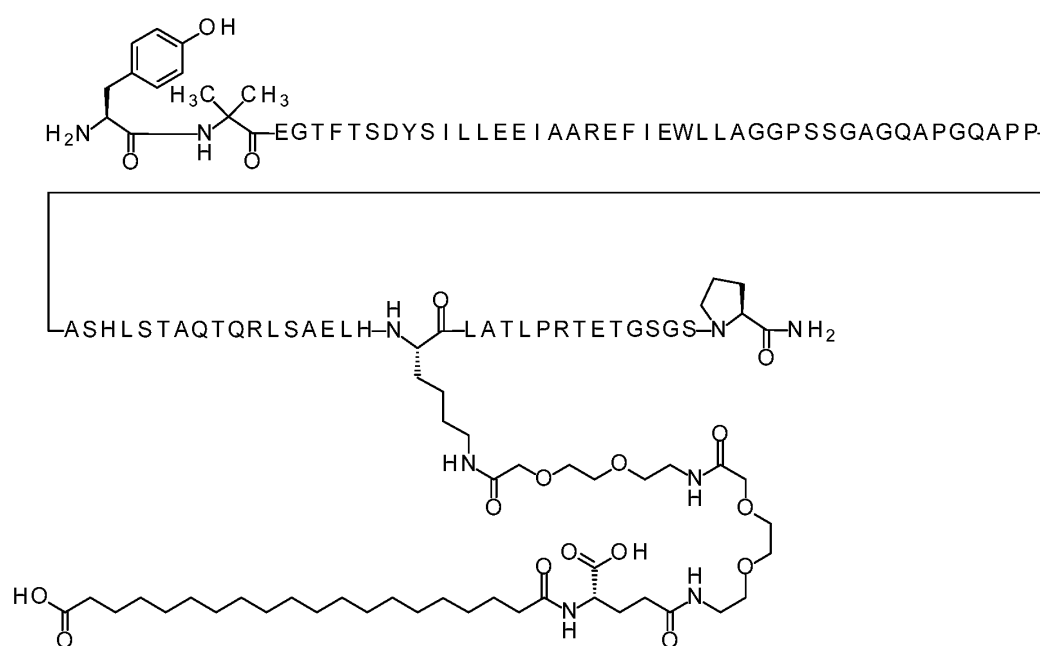
Compound 135

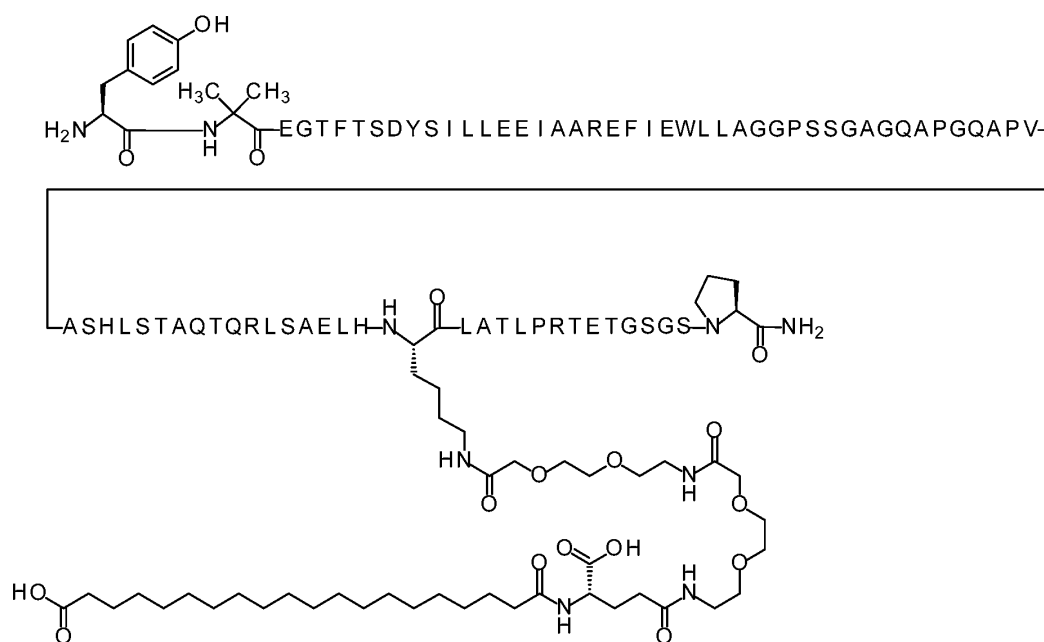


Compound 136

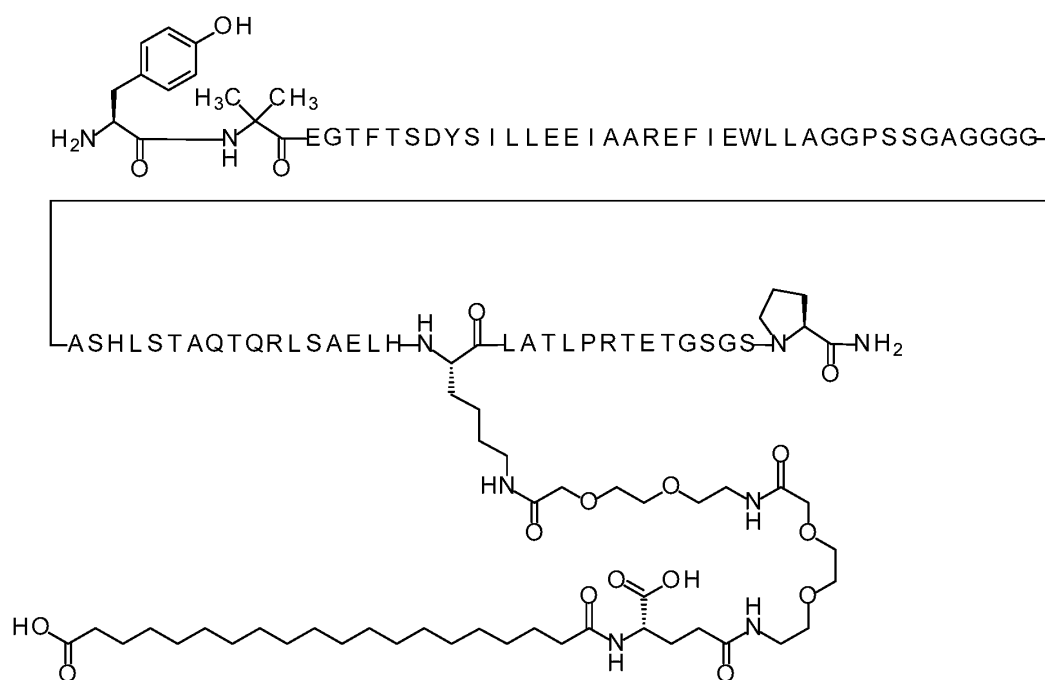


Compound 137

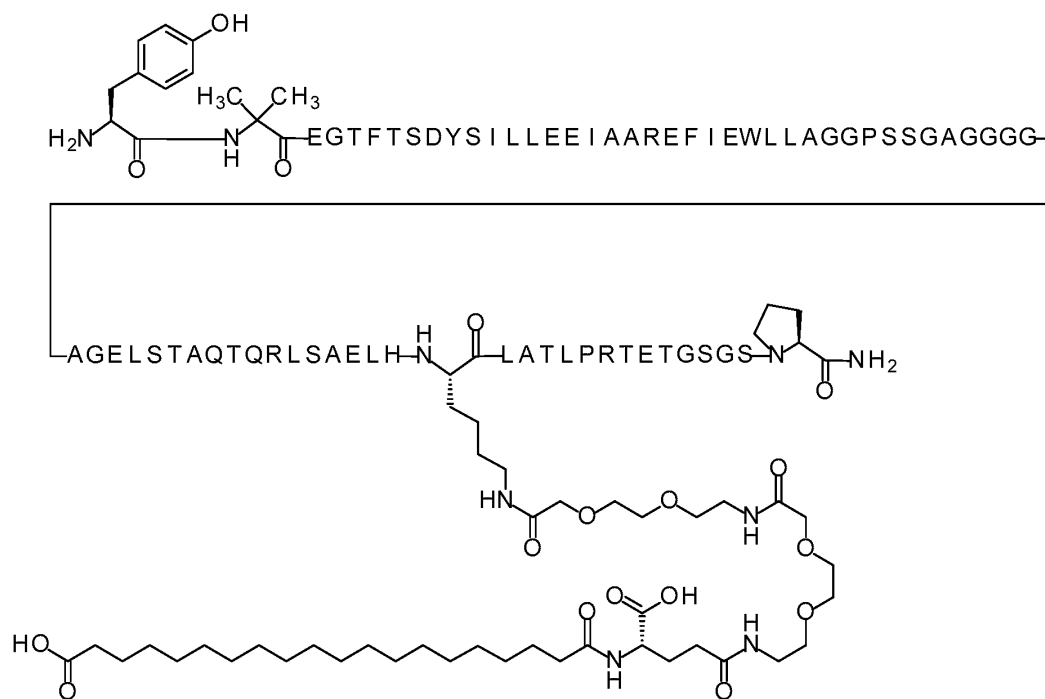


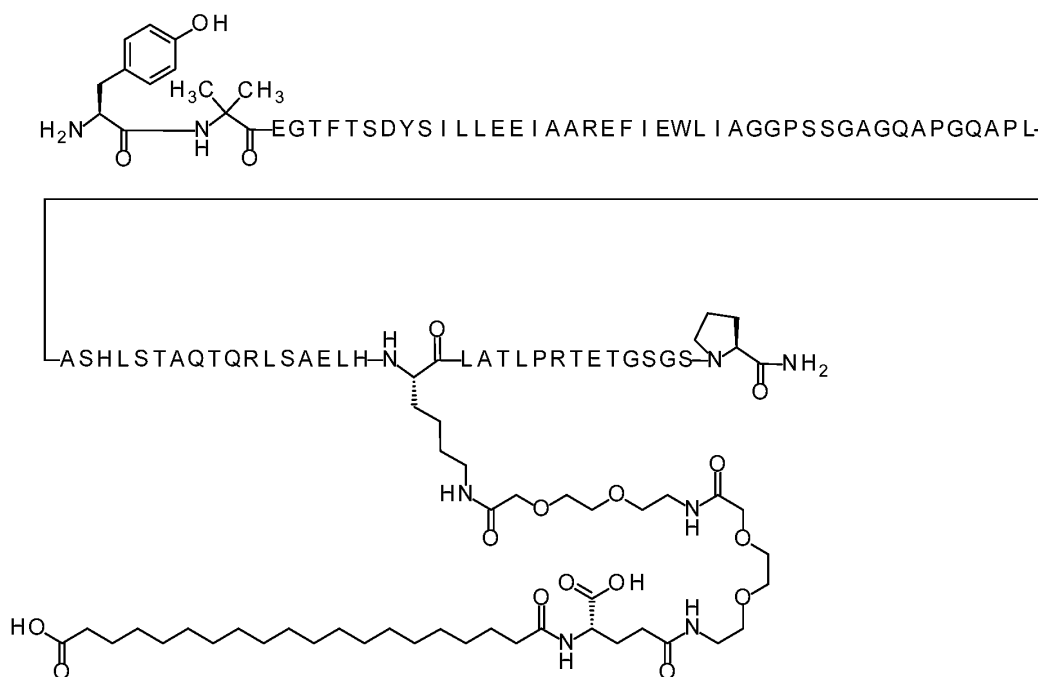


Compound 140

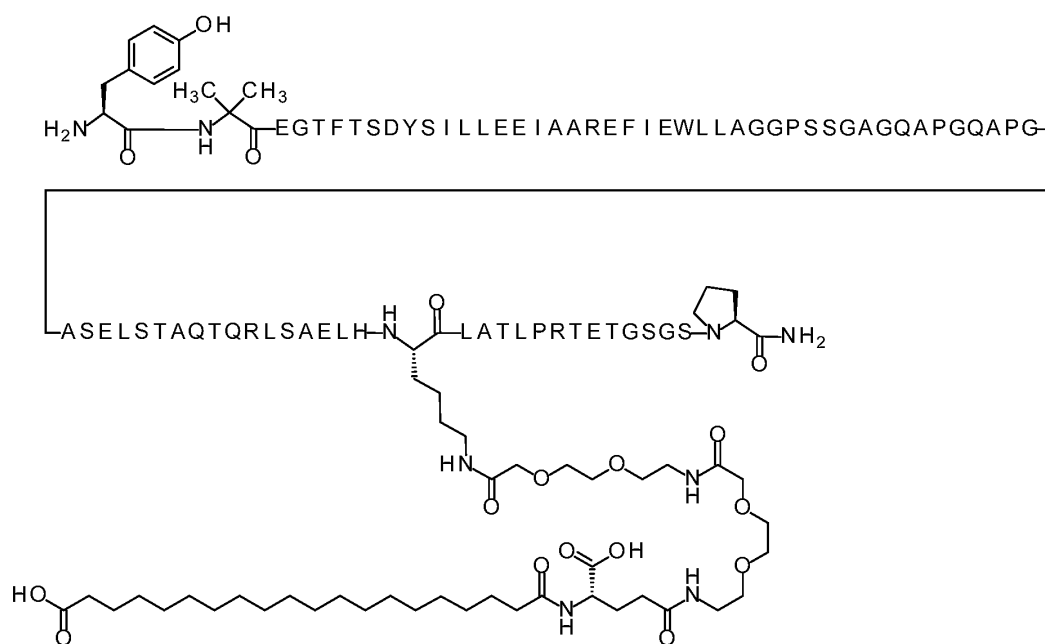


Compound 141

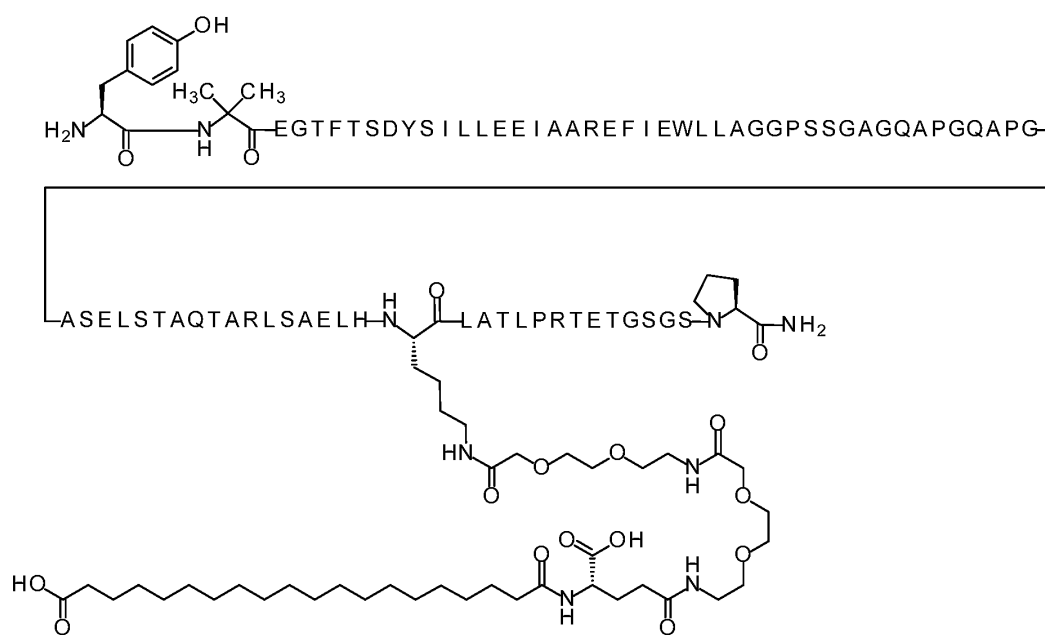




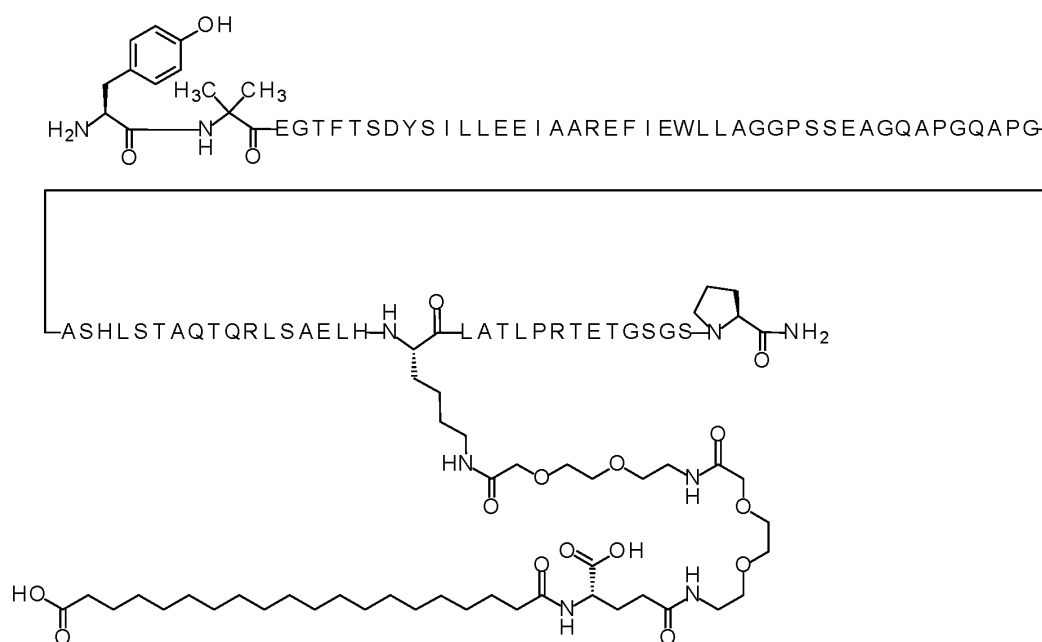
Compound 144



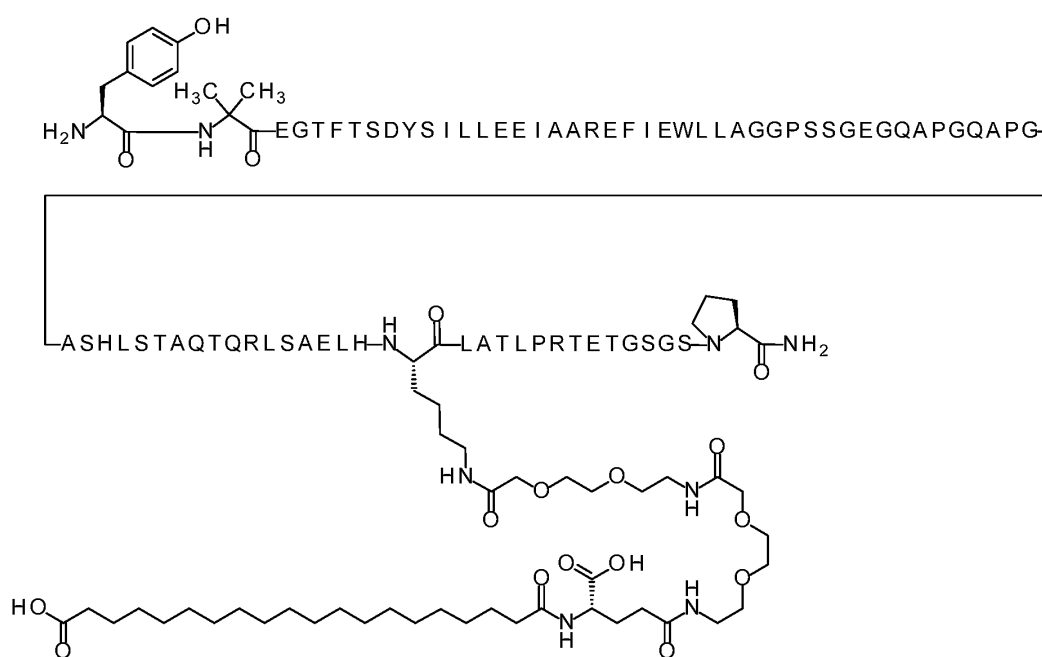
Compound 145



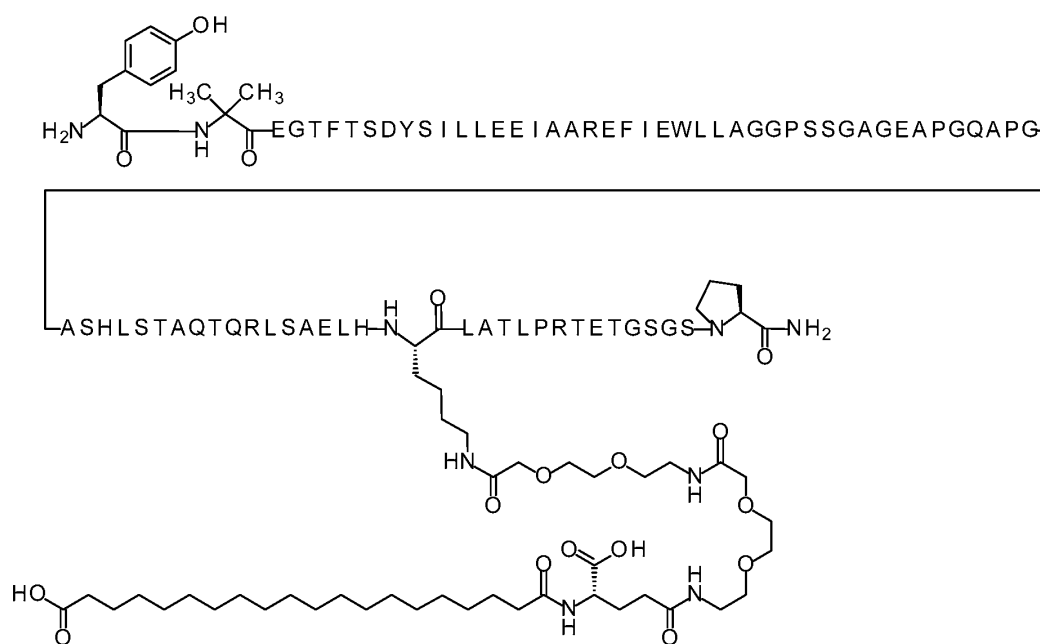
Compound 146



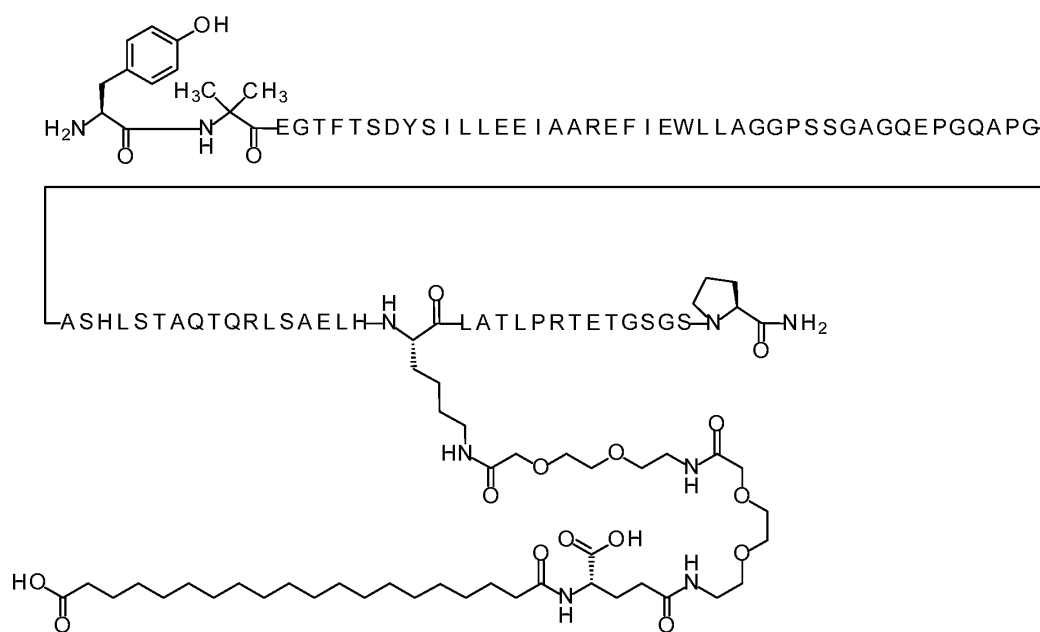
Compound 147



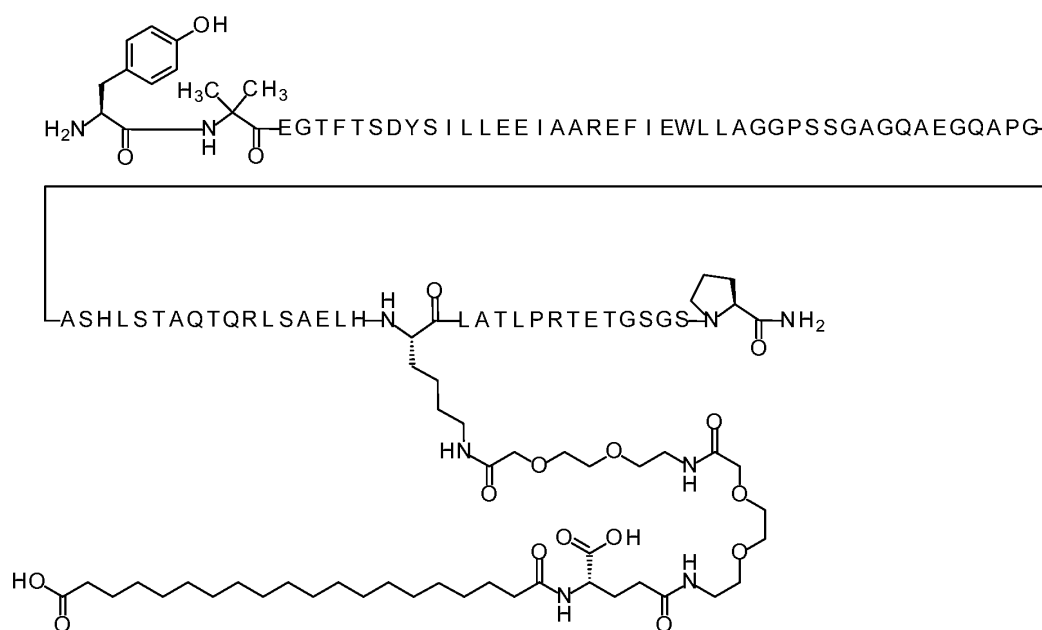
Compound 148



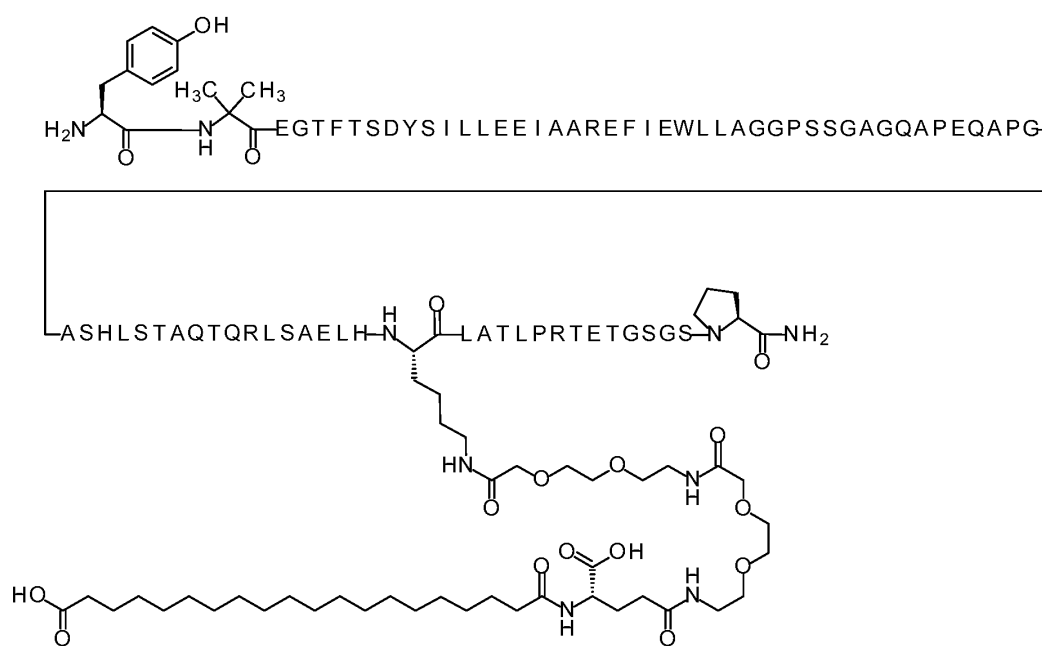
Compound 149



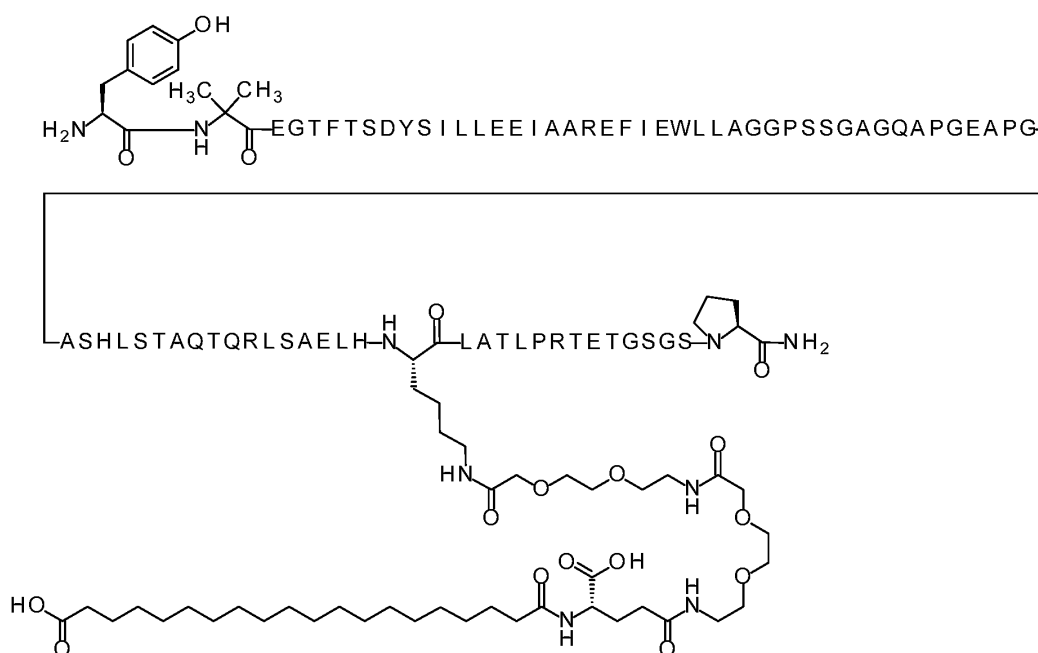
Compound 150



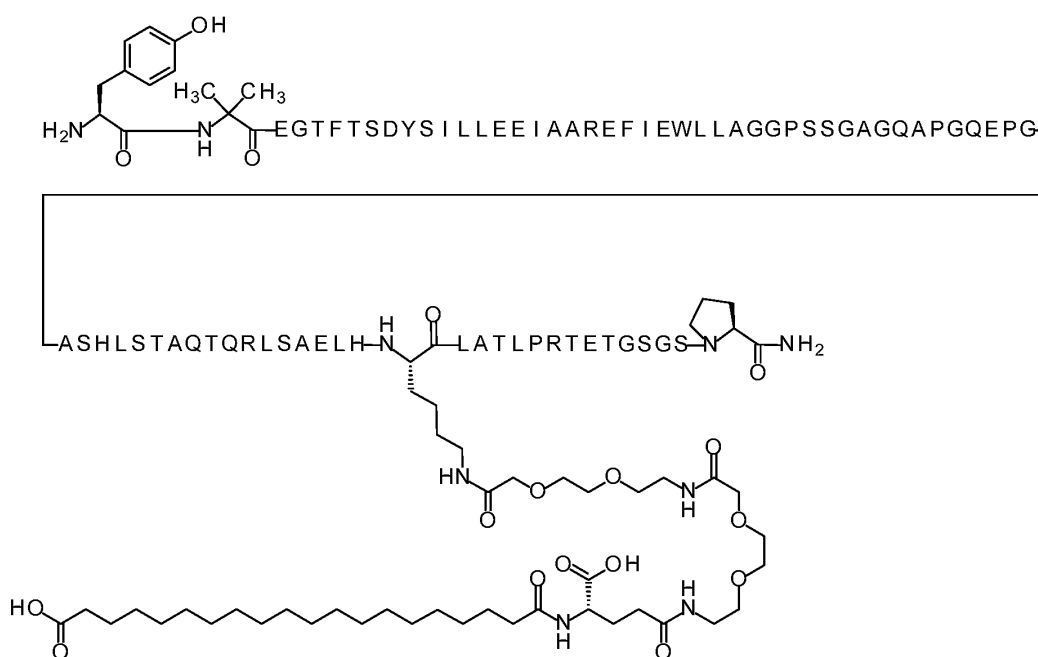
Compound 151

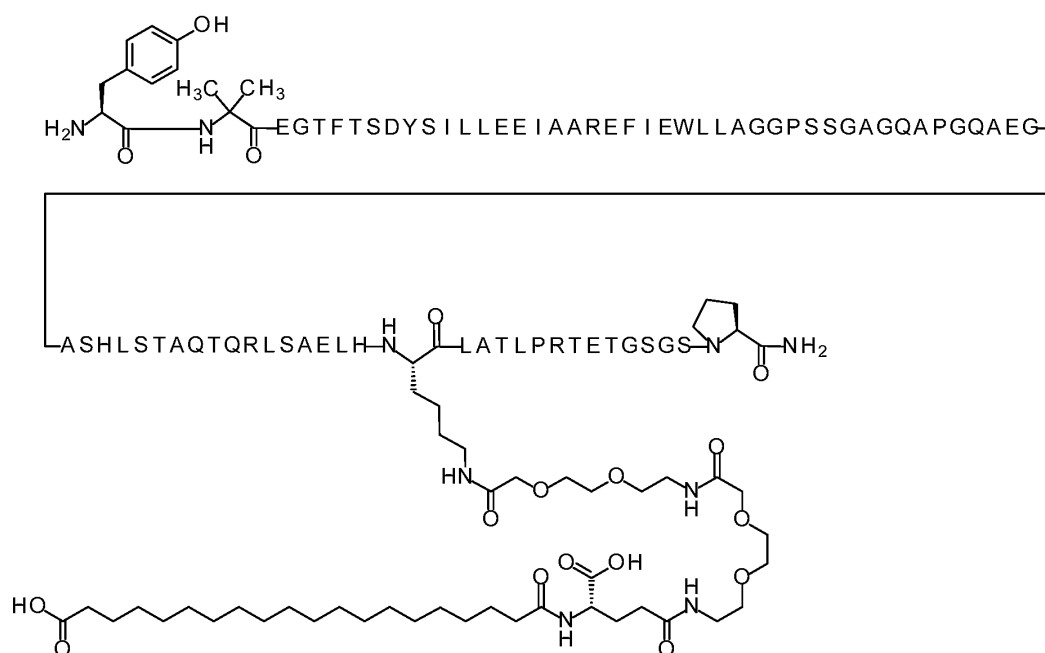


Compound 152

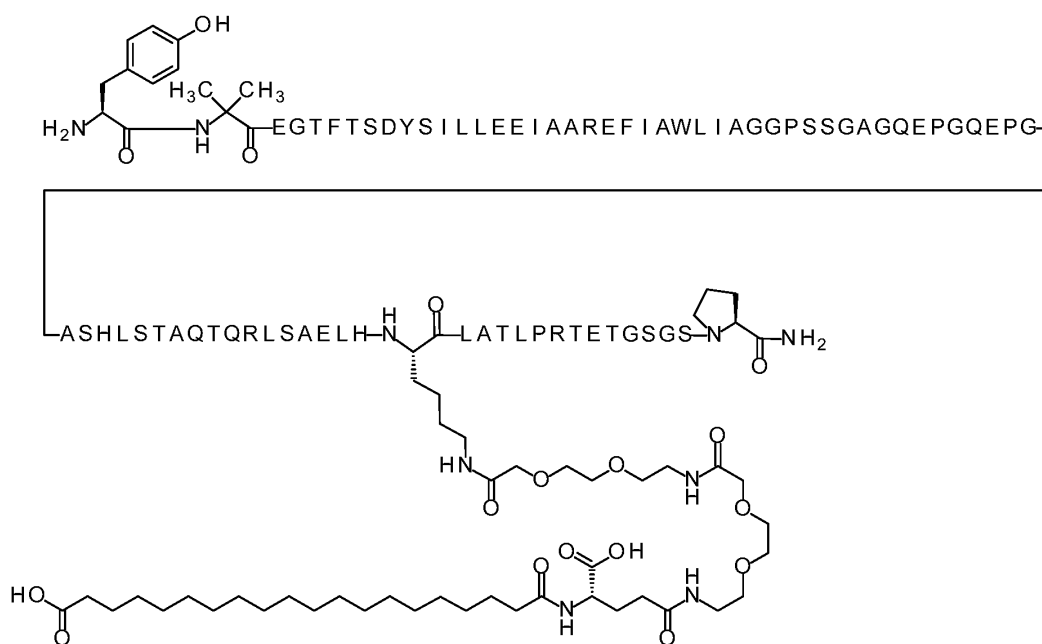


Compound 153

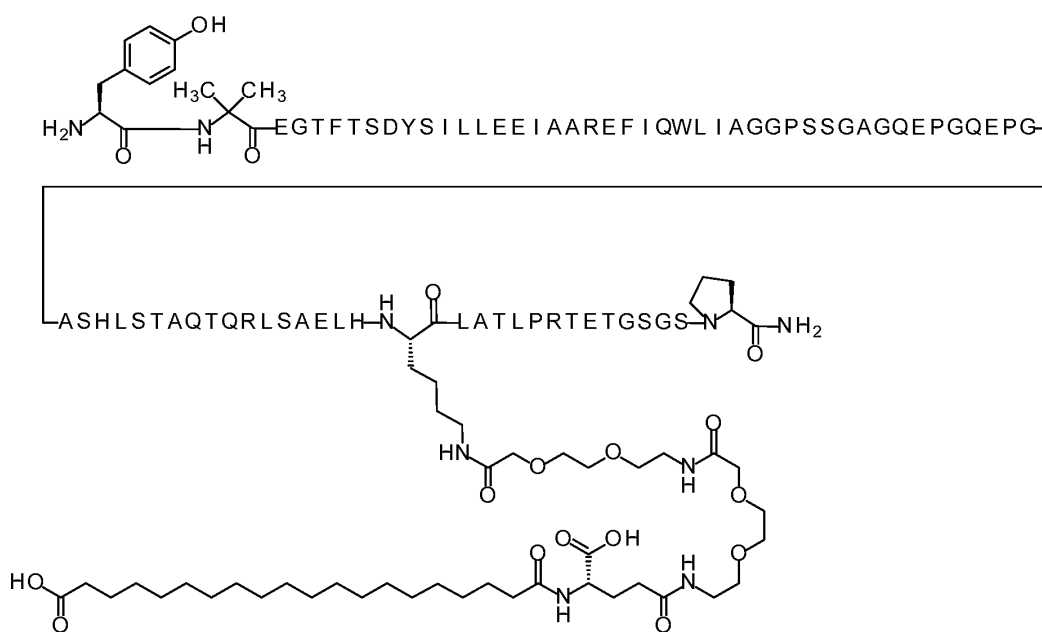


C[C@H](C)N(C(=O)[C@@H](Cc1ccc(O)cc1)C(N)=O)C(=O)EGTFTSDYSILLEEIAAREFIEWLIAAGPSSGAGQEPGPGLASHLSTAQTQRLSAELHN[C@@H](CCCCNC(=O)COCCOCCNC(=O)COCCOCCNC(=O)OCCCCCCCCCCCCCCCCCC(=O)N[C@@H](CCCNC(=O)O)C(=O)O

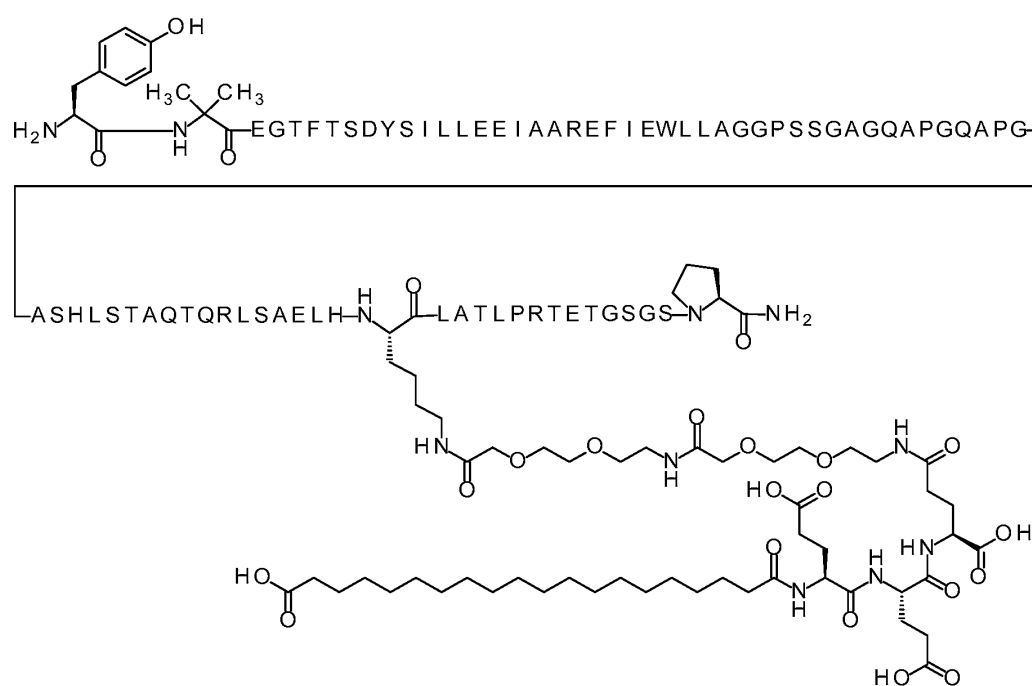
Compound 156



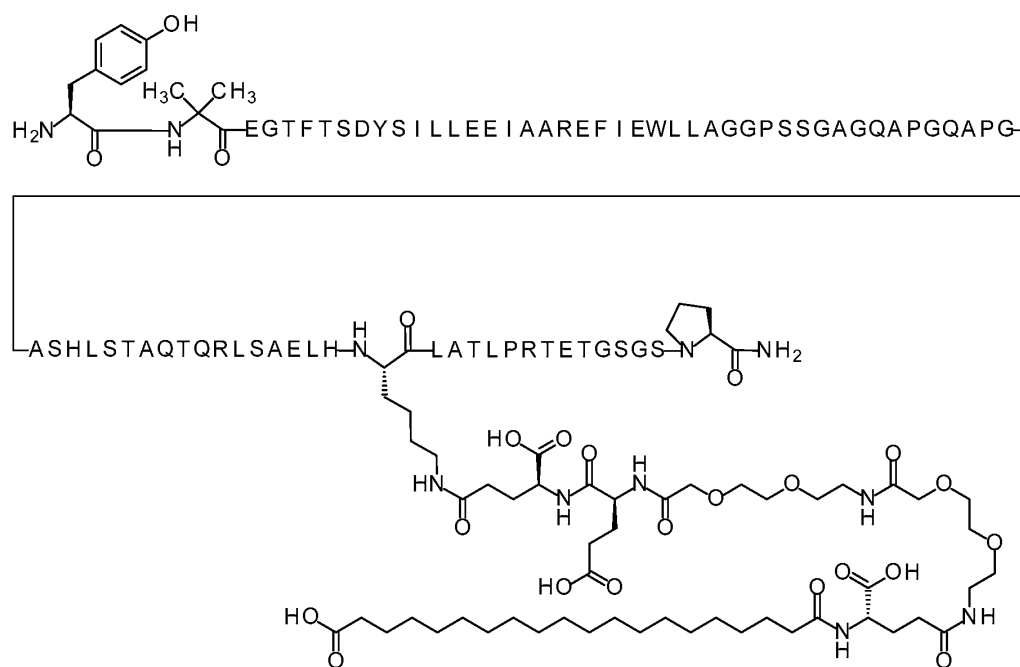
Compound 157

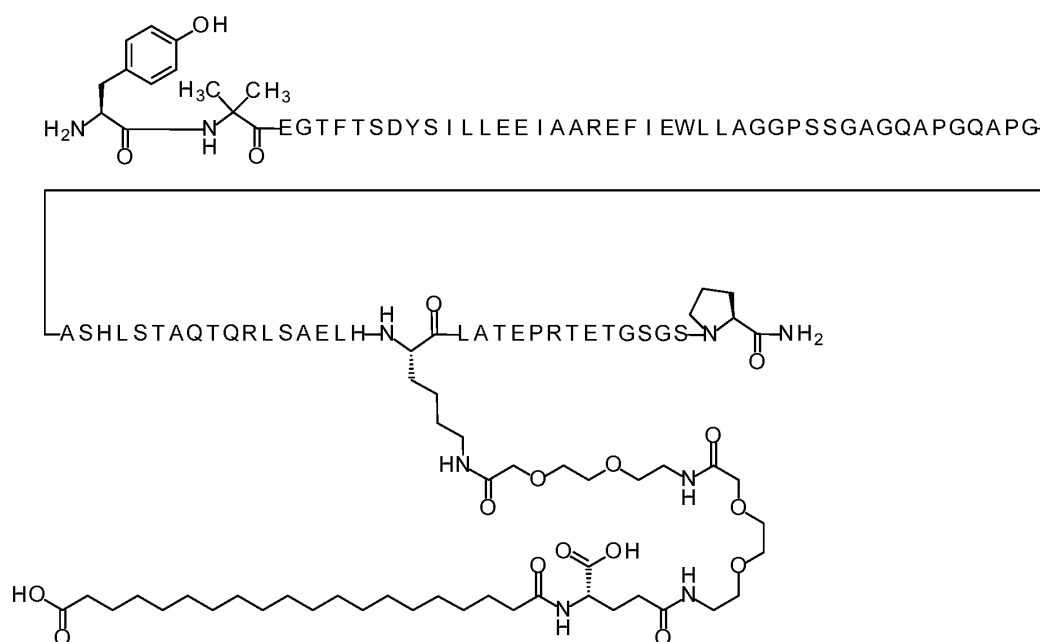


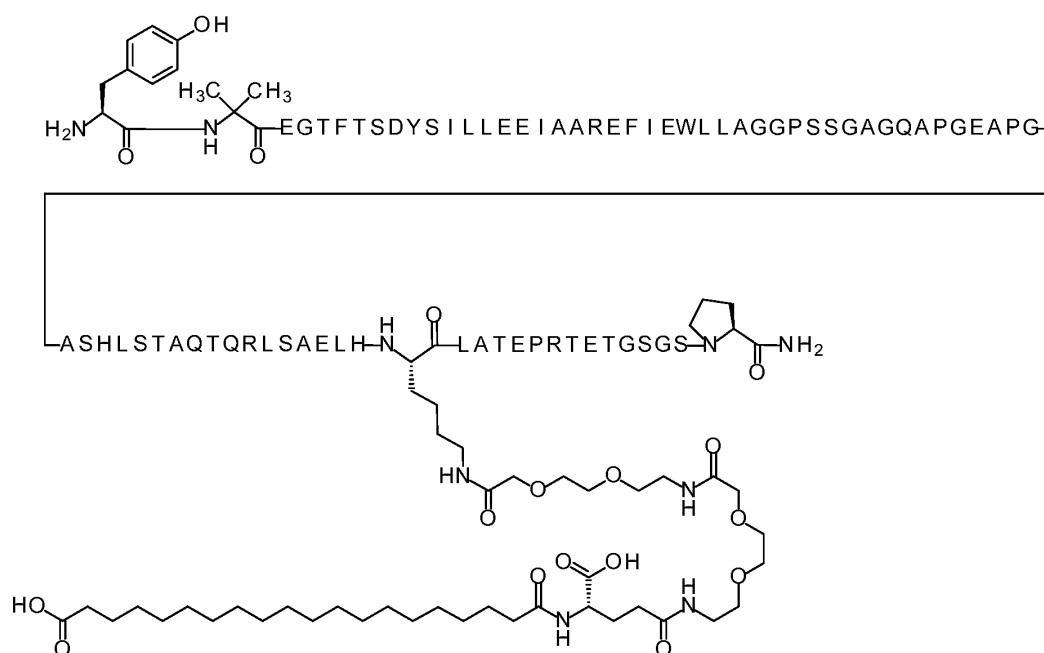
Compound 160



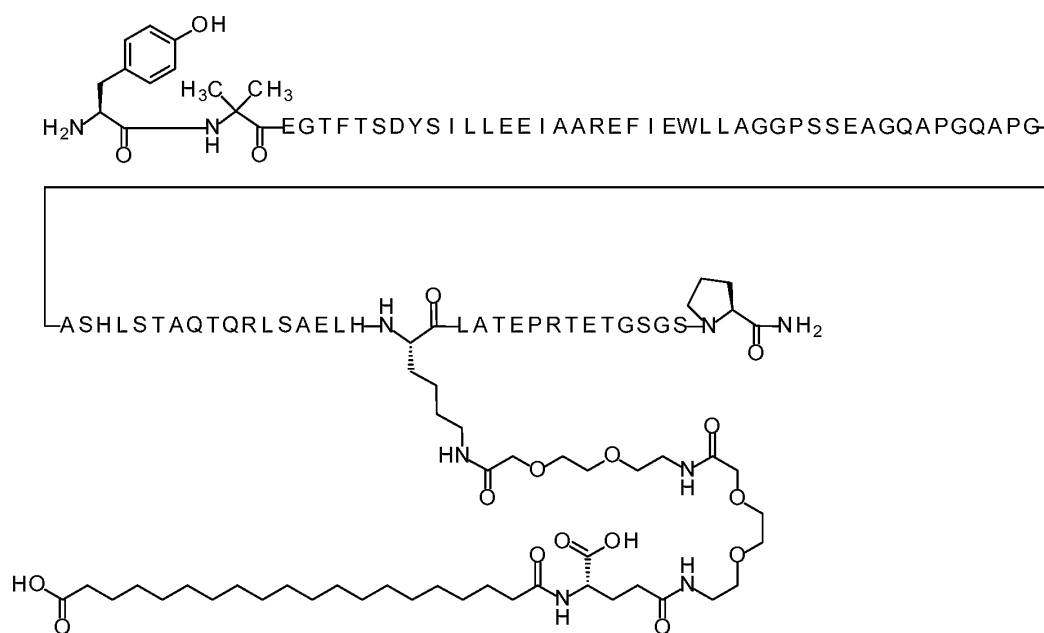
Compound 161



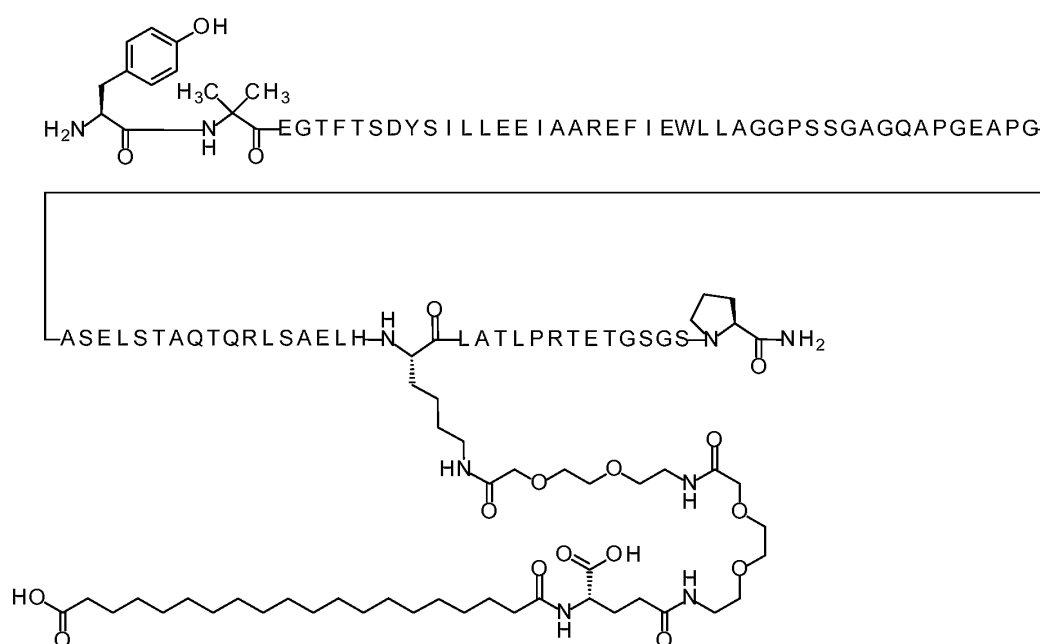
[illegible]



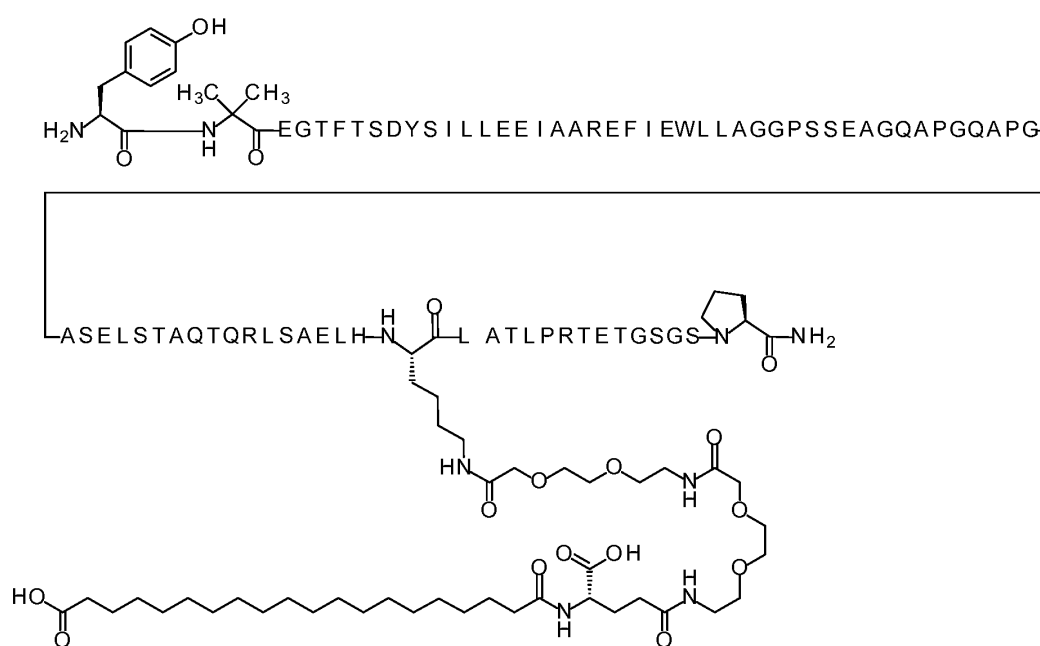
Compound 165



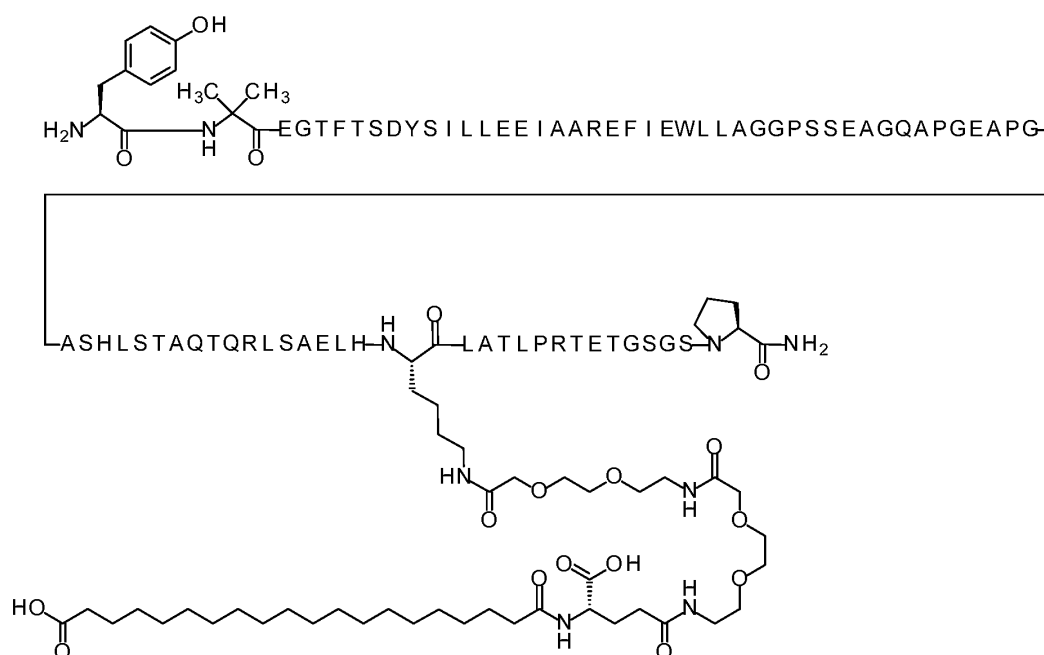
Compound 166



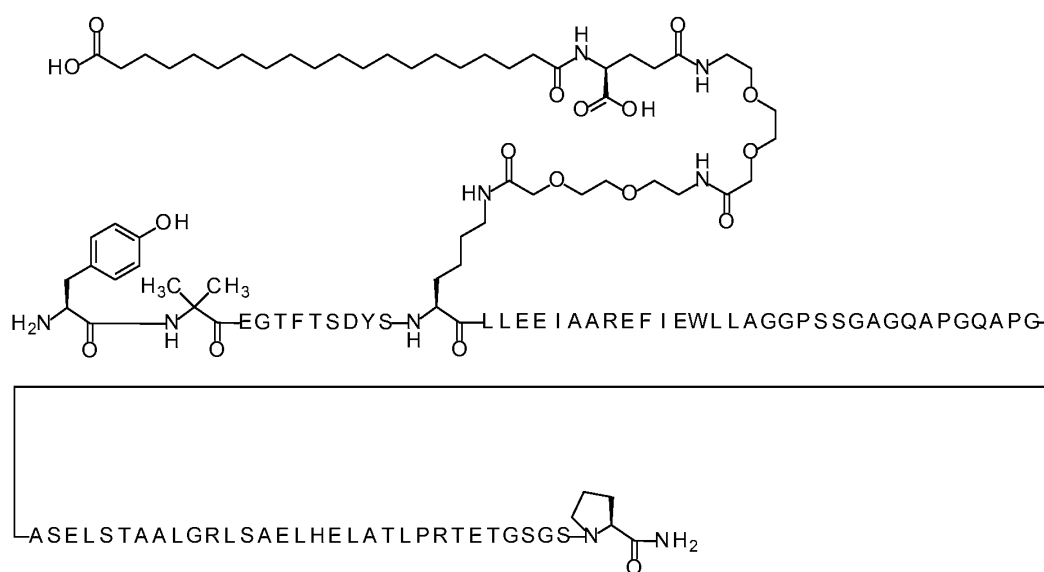
Compound 167

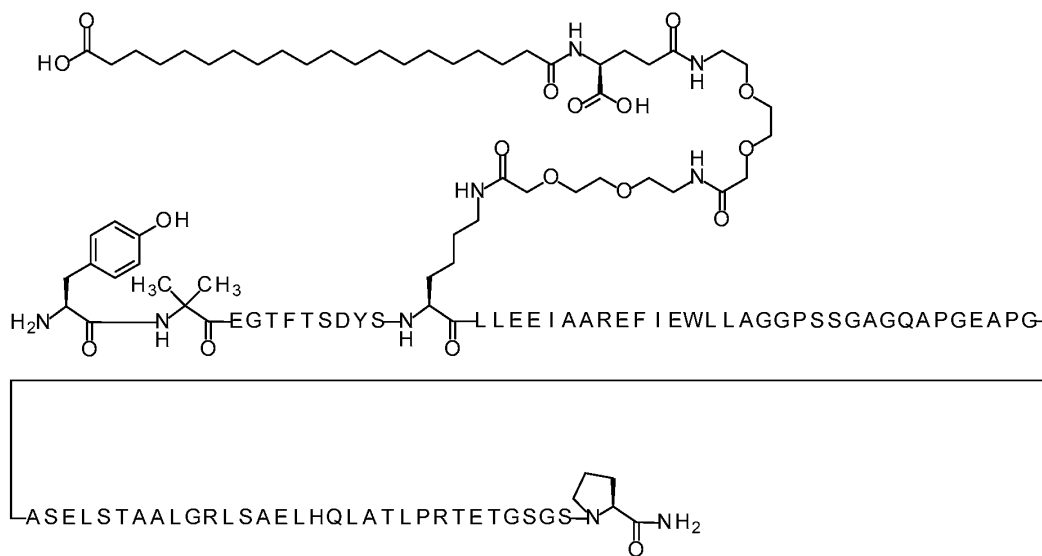


Compound 168

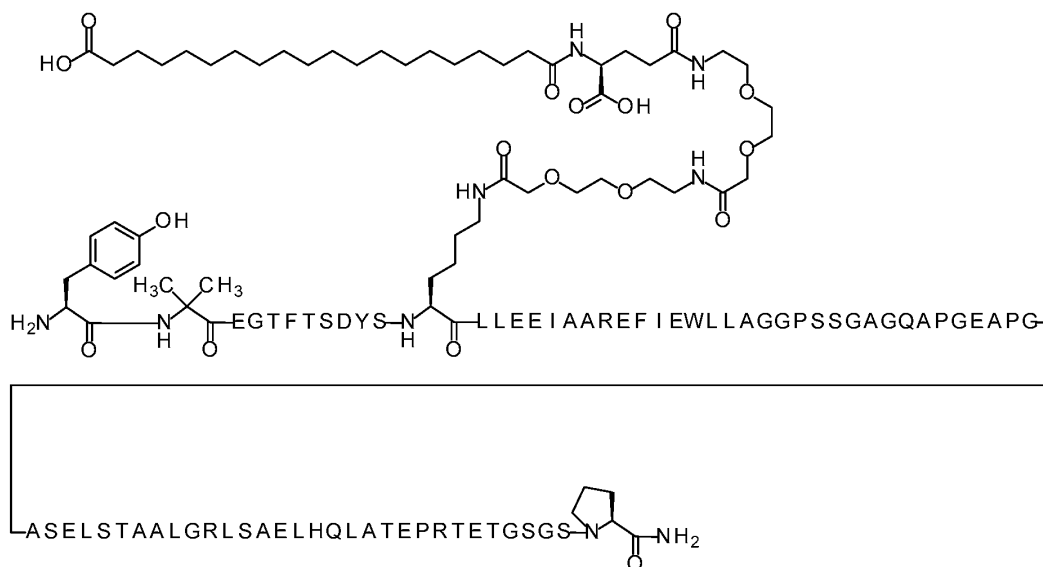


Compound 169

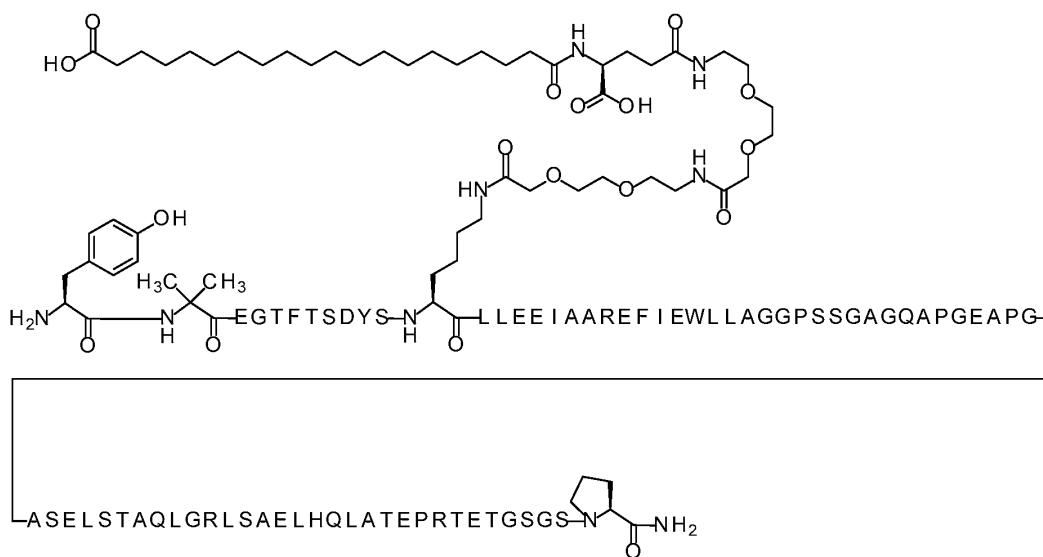




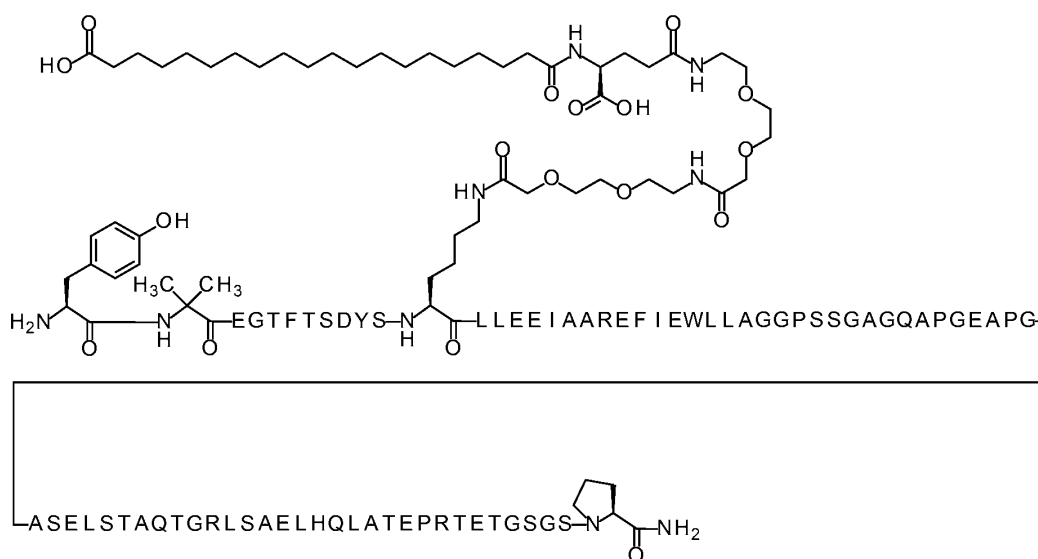
Compound 172



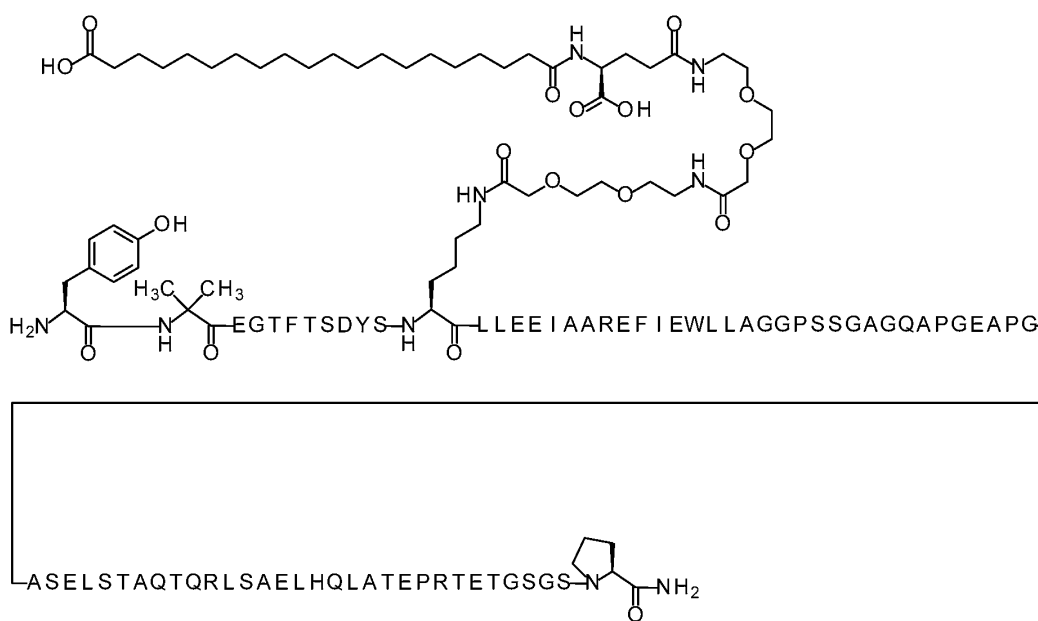
Compound 173



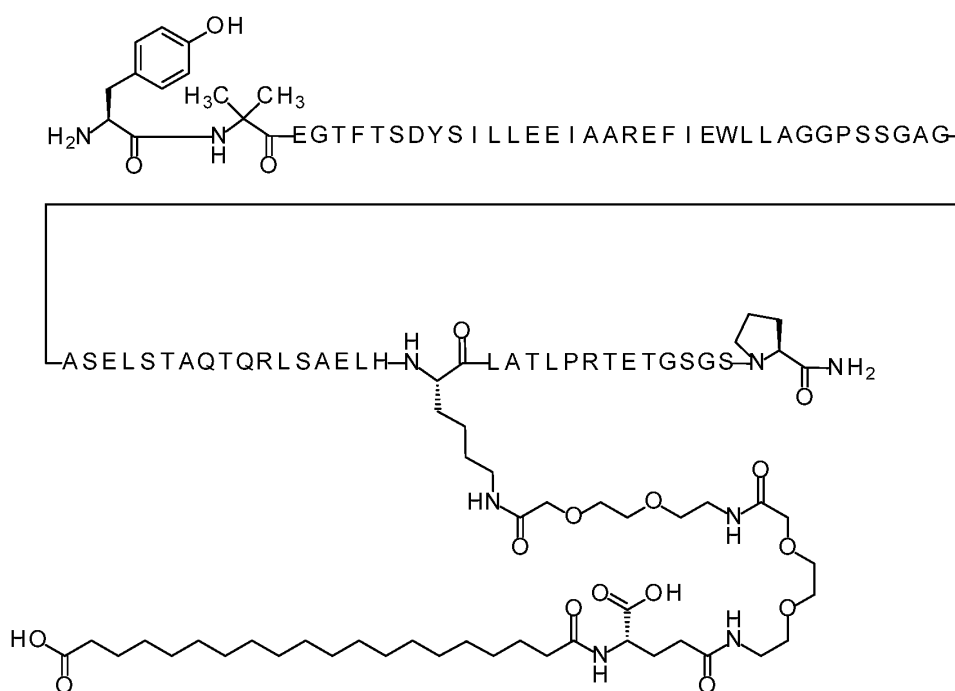
Compound 174



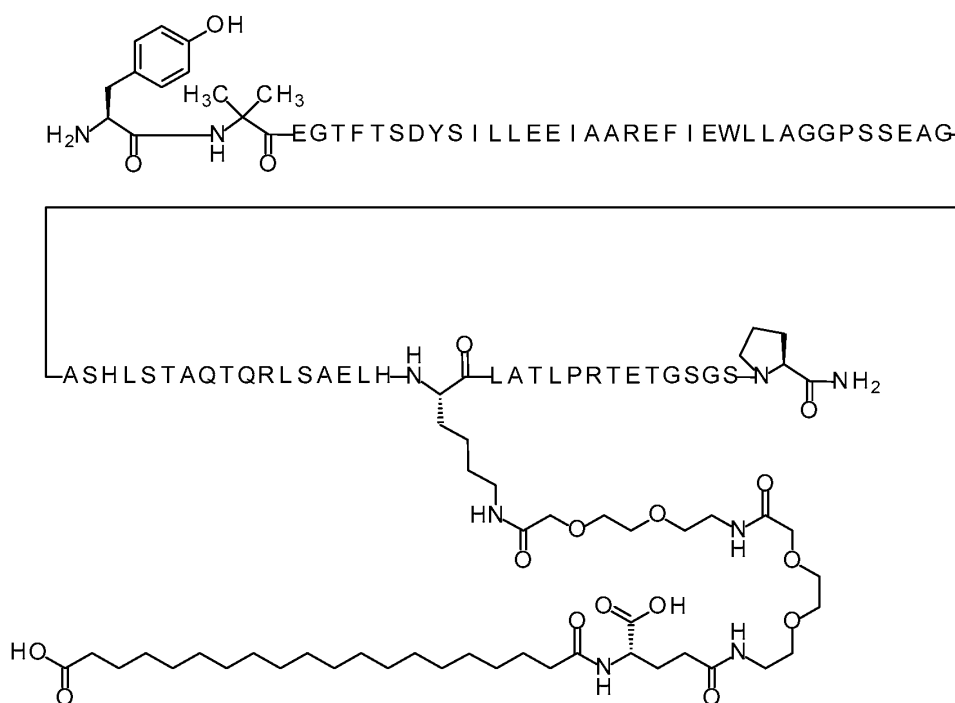
Compound 175



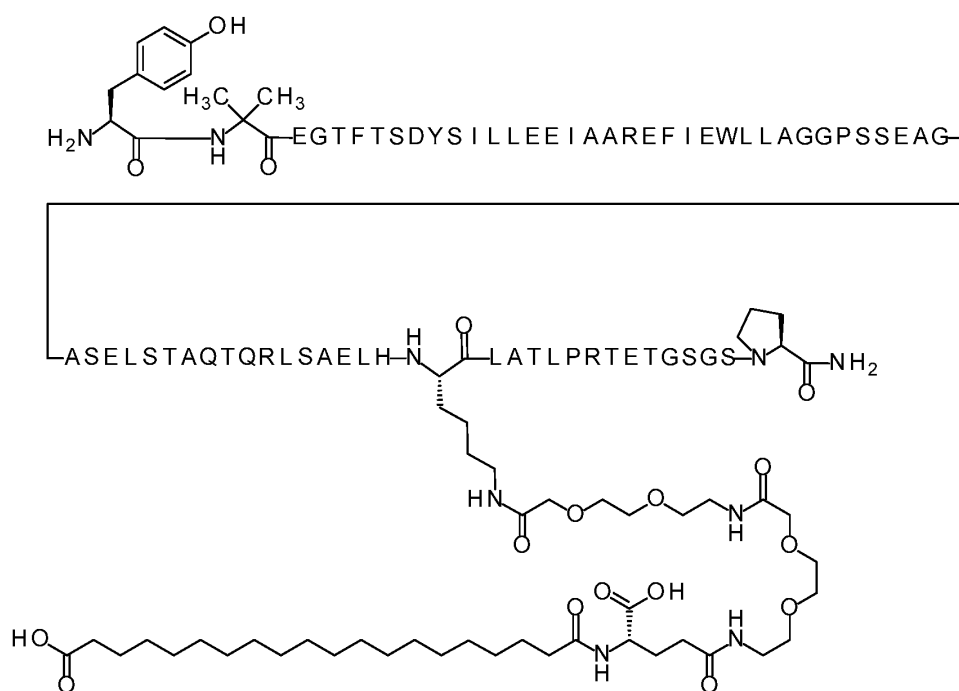
Compound 176



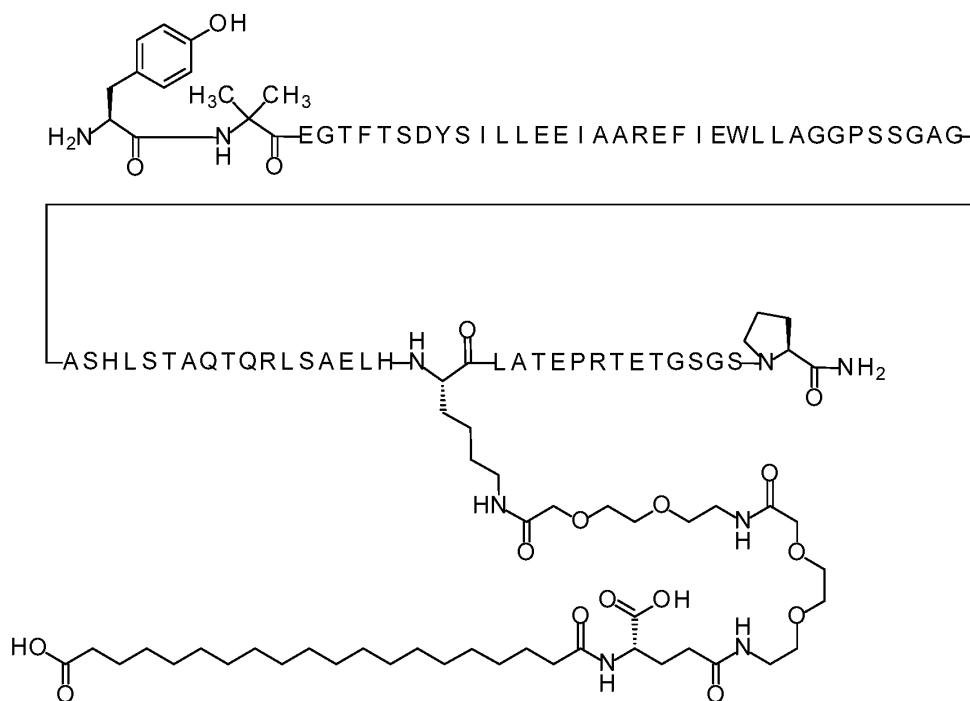
Compound 177



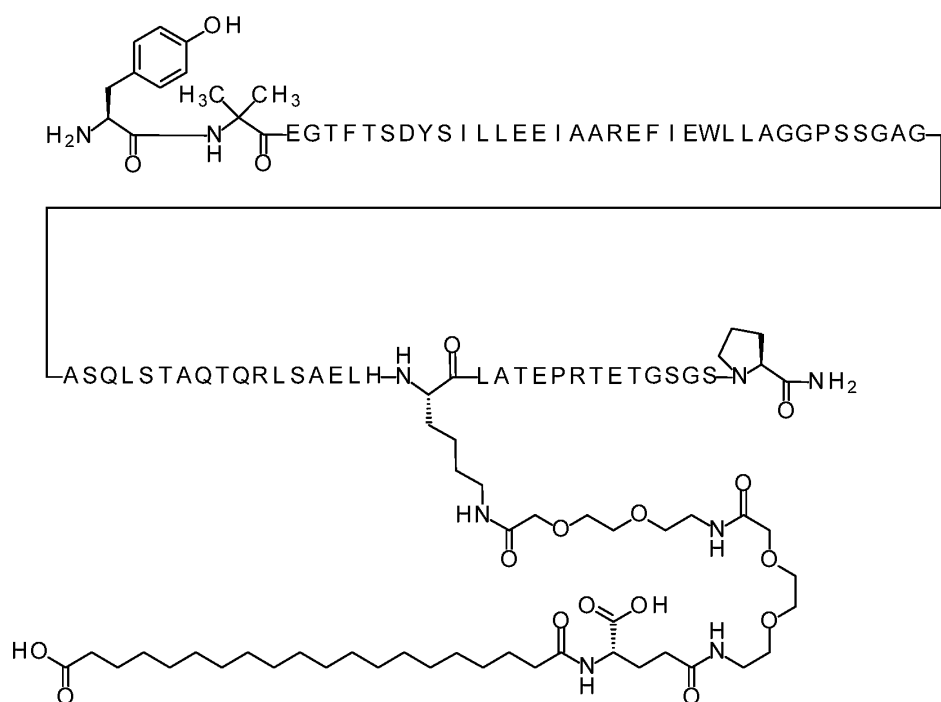
Compound 178



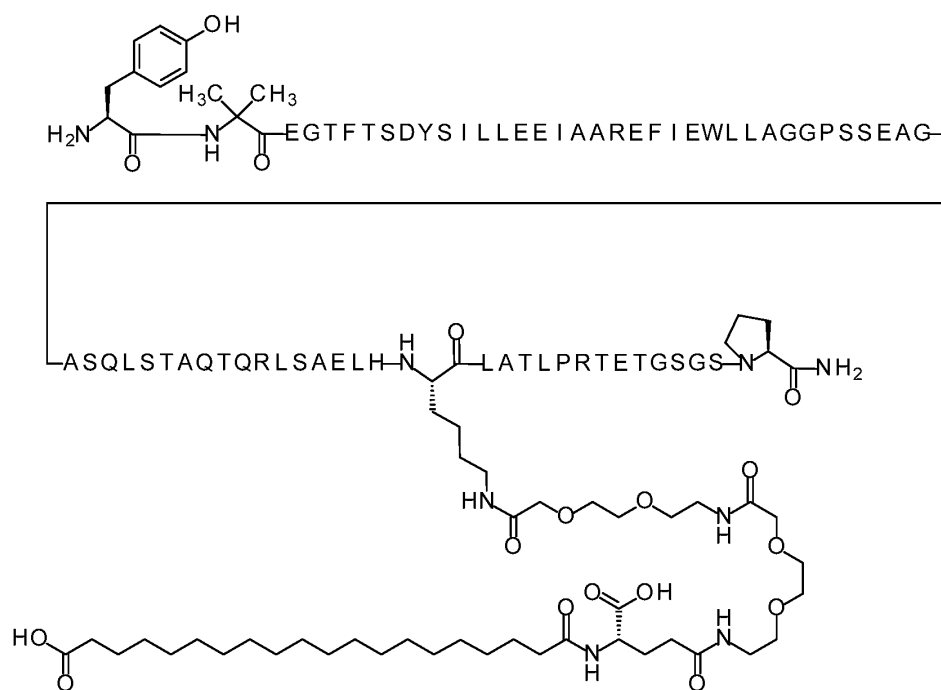
Compound 179



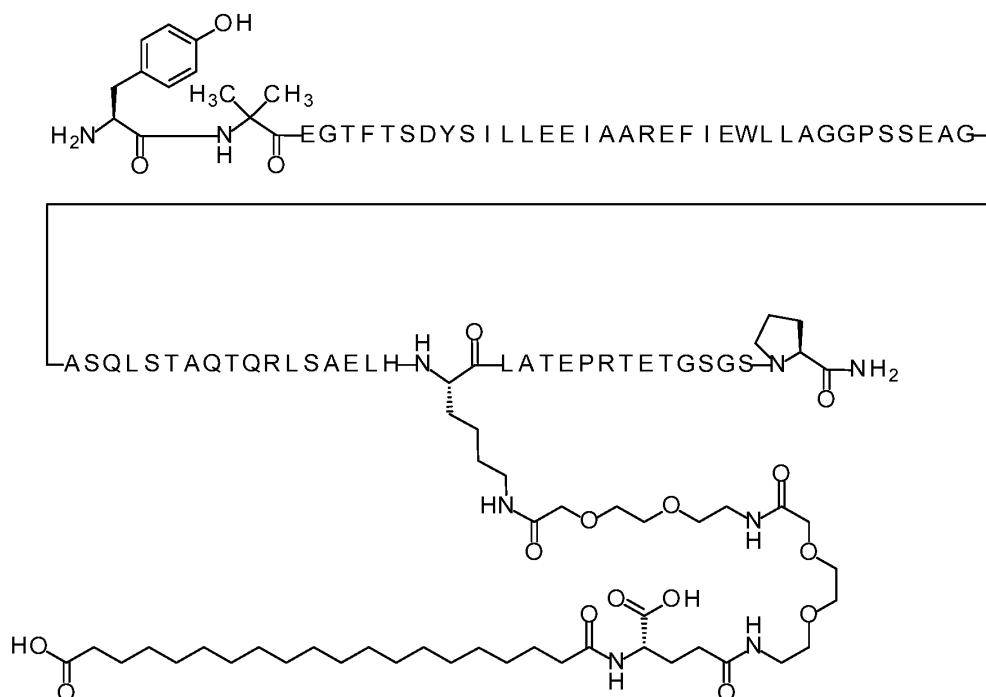
Compound 180



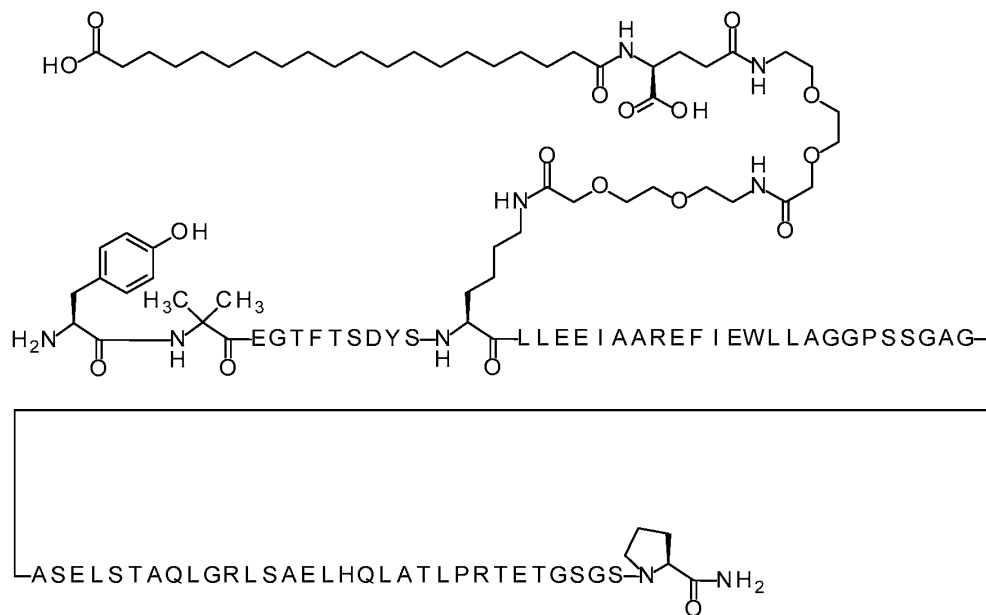
Compound 181

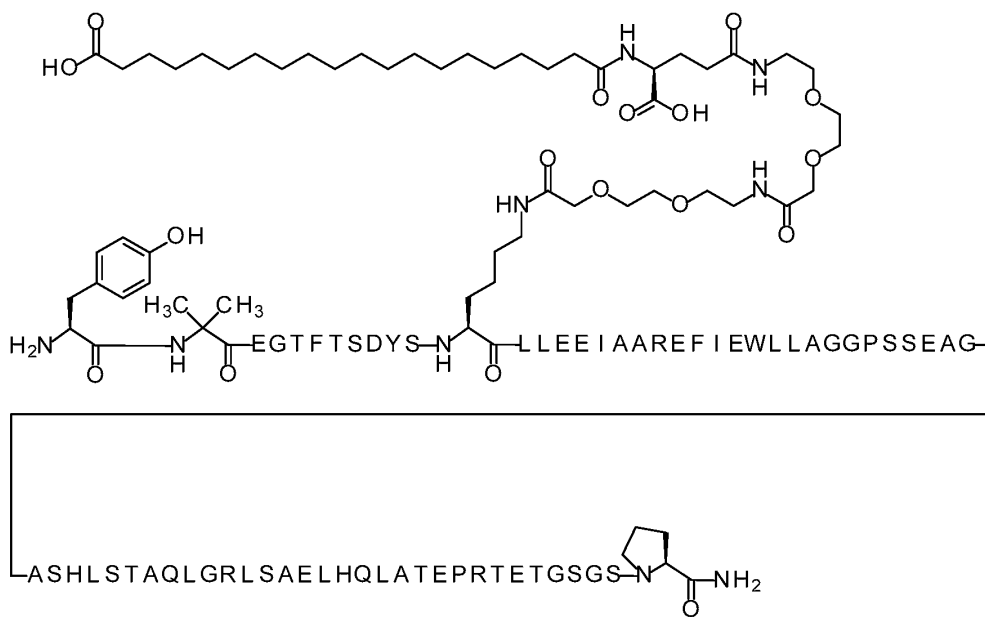


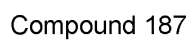
Compound 182

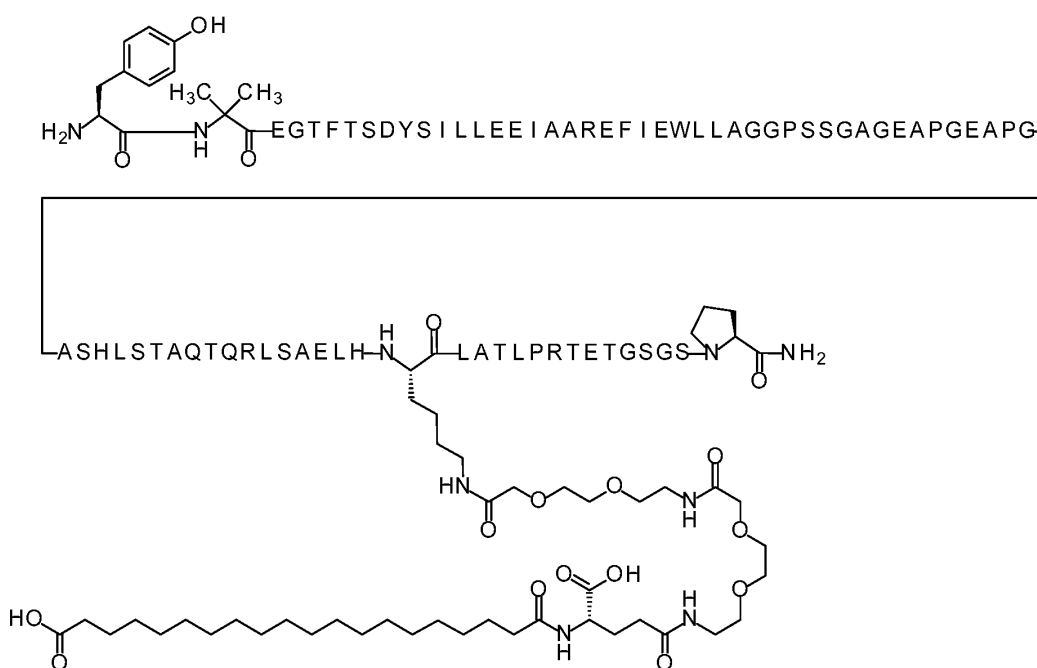


Compound 183

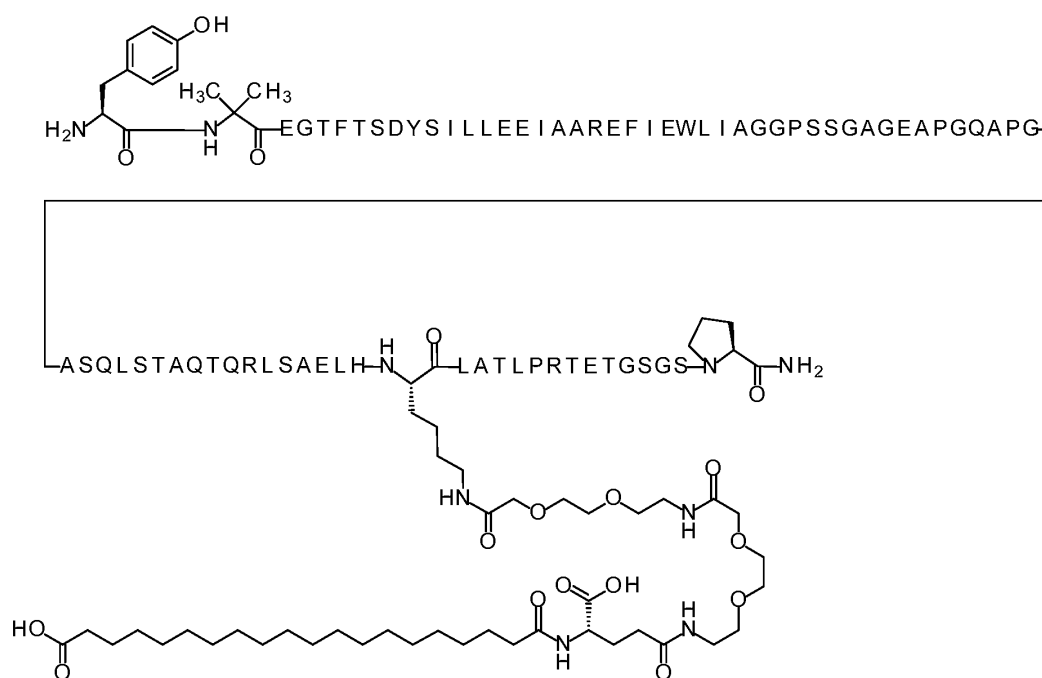




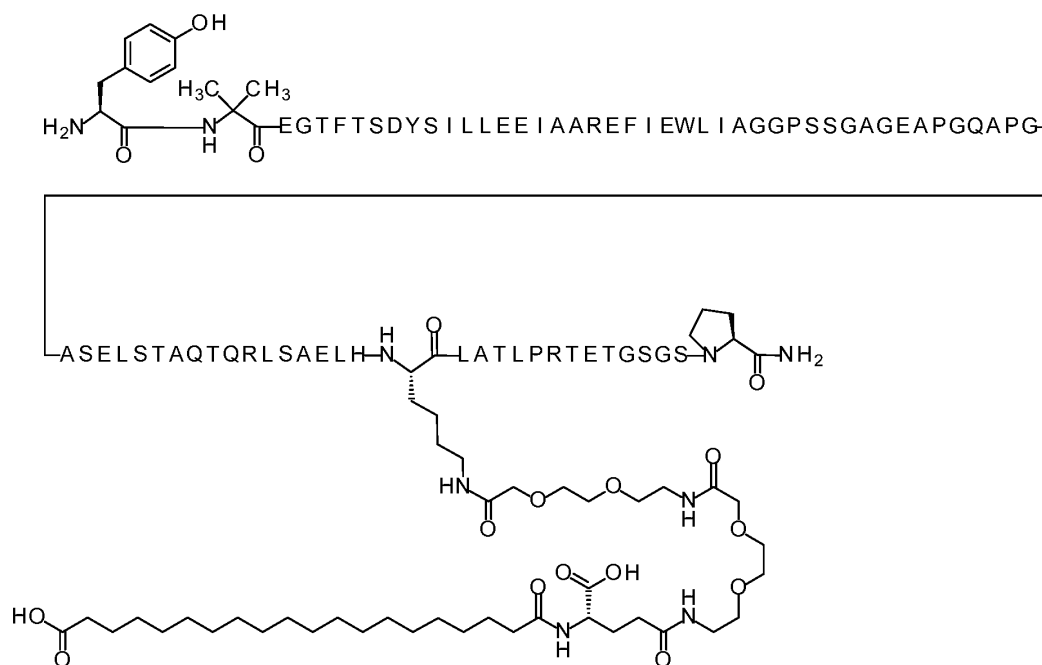




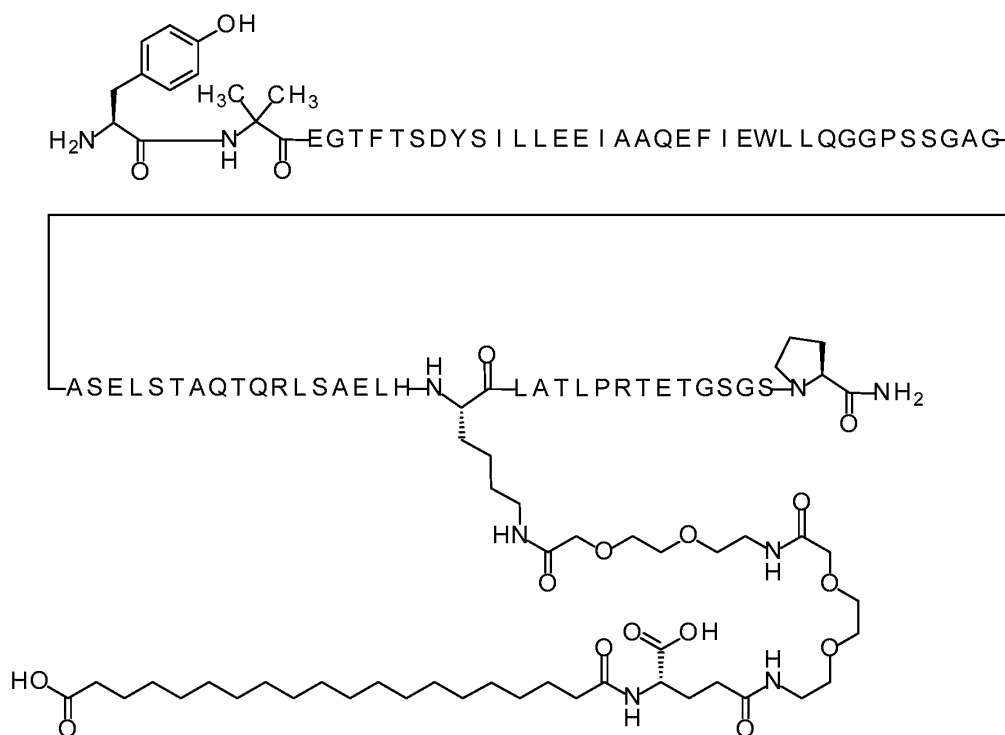
Compound 190



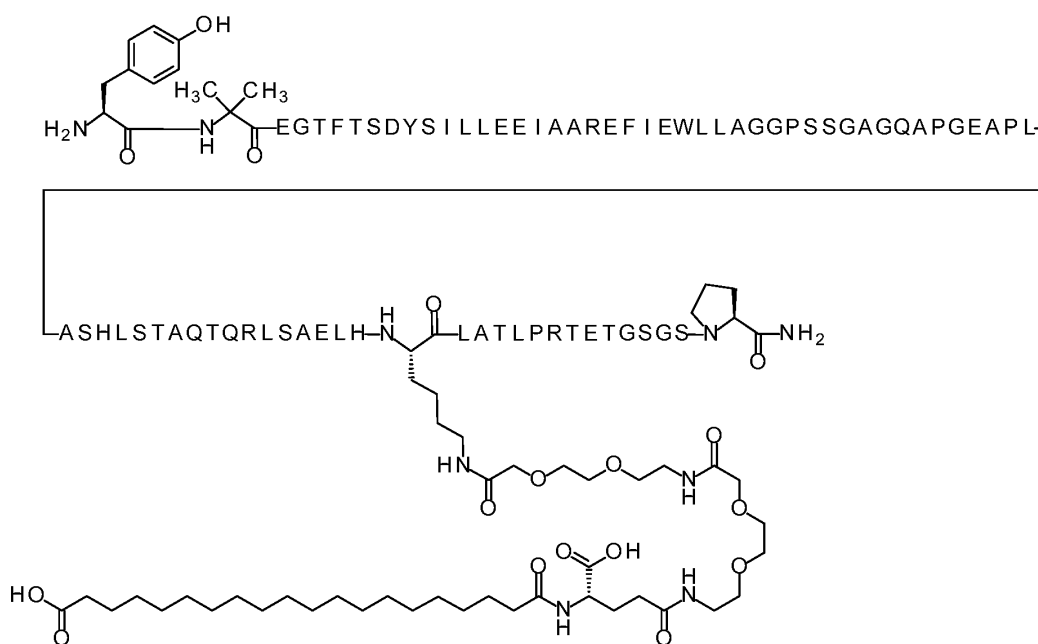
Compound 191



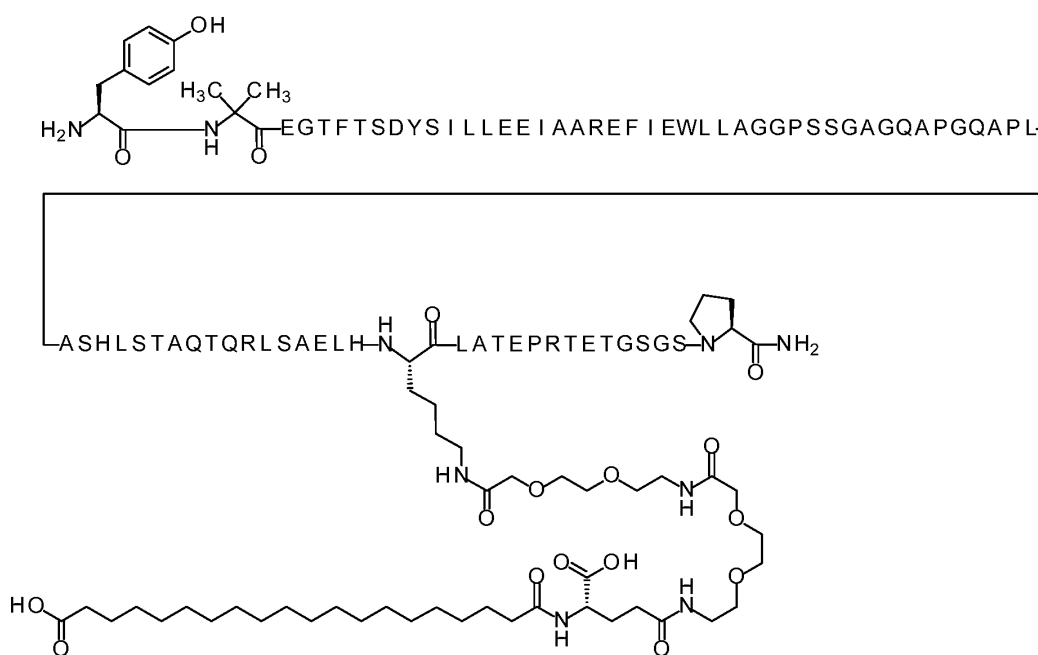


NC(=O)Cc1ccc(O)cc1NC(=O)C(C)(C)C(=O)OEGTFTSDYSILLEEIAAQEFIEWLLQGGPSSGAGEAPGQAPGASSELSTAQTQRLSAELHN[C@@H](CCCCNC(=O)COCCOCCOCCN[C@@H](CCCCNC(=O)O)C(=O)OCCCCCCCCCCCCCCCCCCCC(=O)N[C@@H](C(=O)O)C(=O)NCCOC

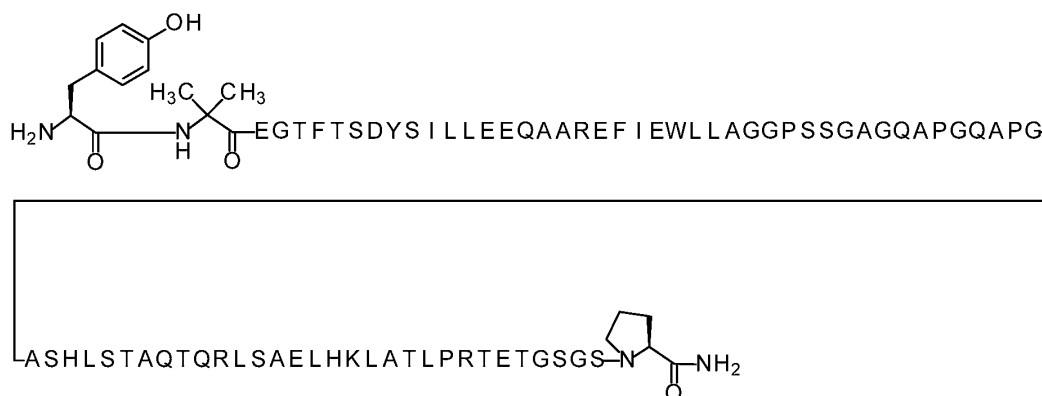
Compound 196



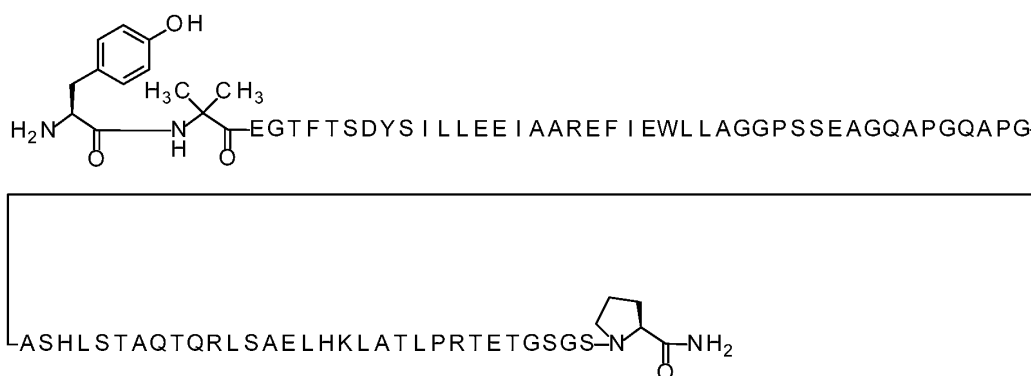
Compound 197



Compound 210

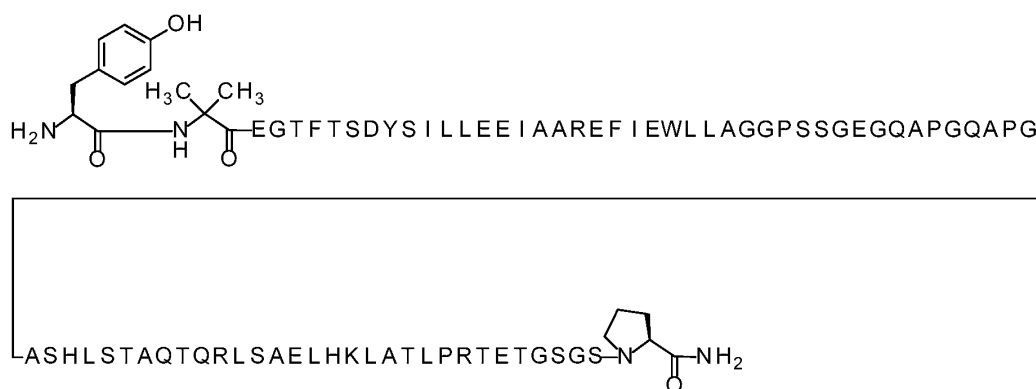


Compound 211

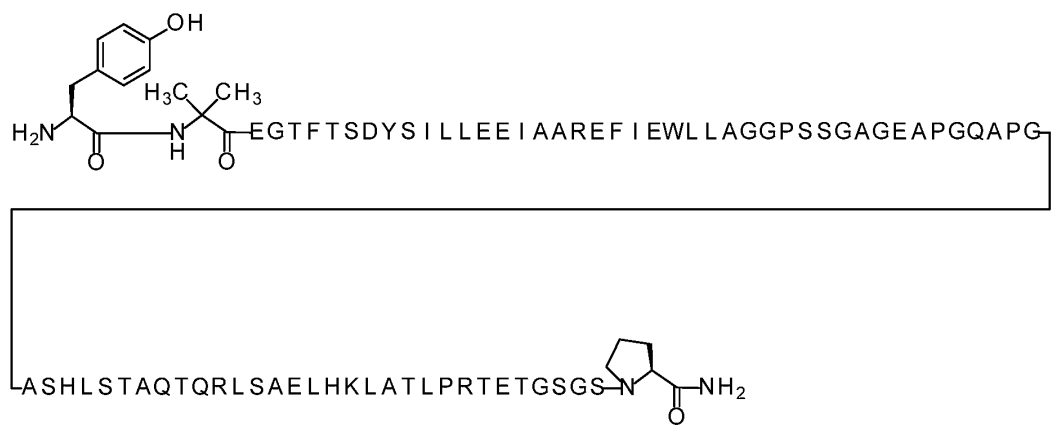


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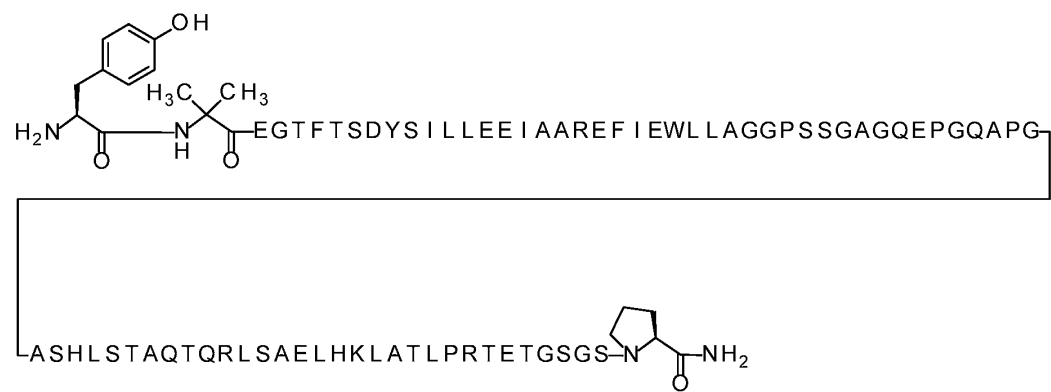
Compound 212



Compound 213

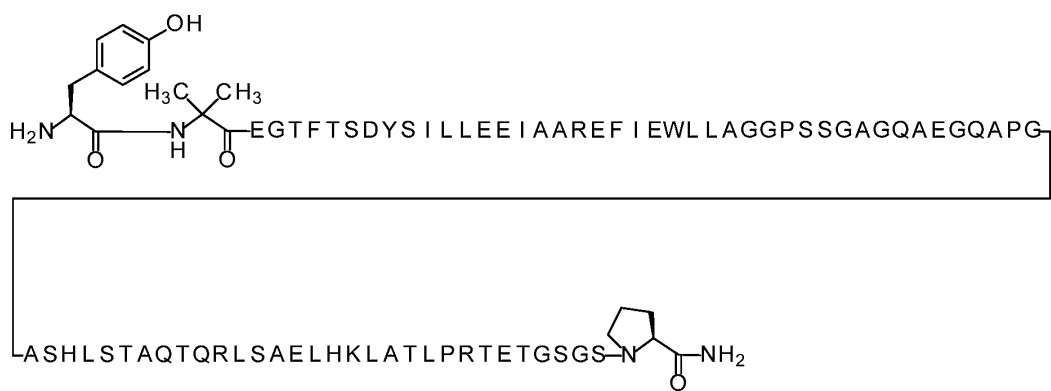


Compound 214

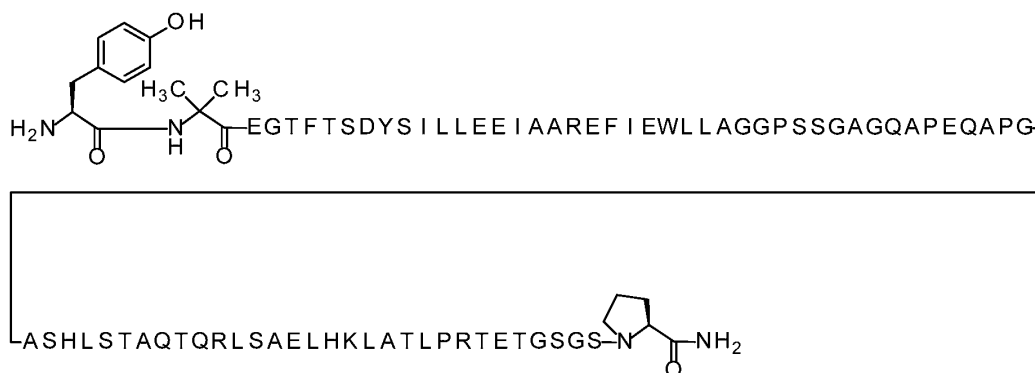


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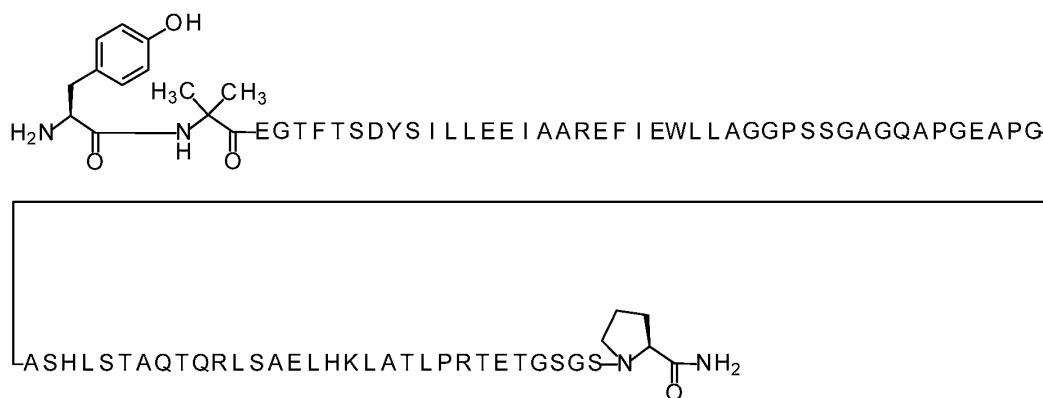
Compound 215



Compound 216

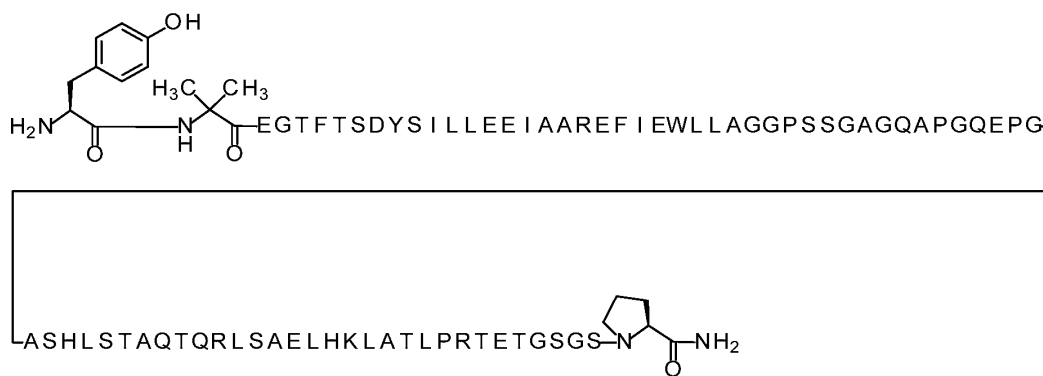


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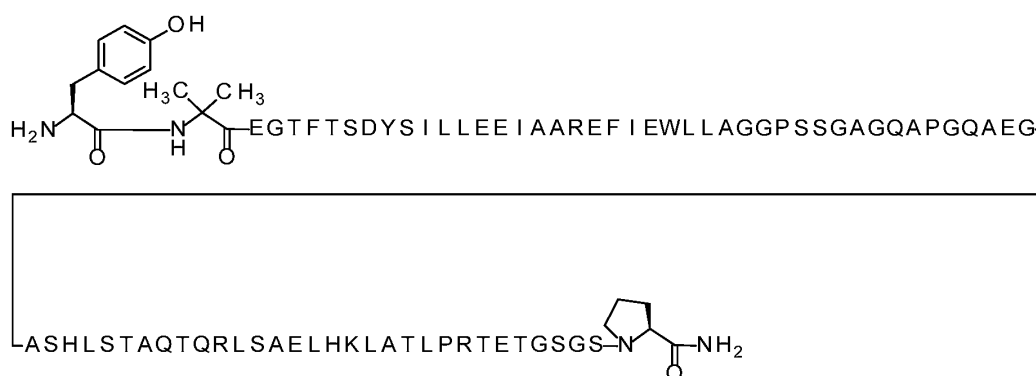


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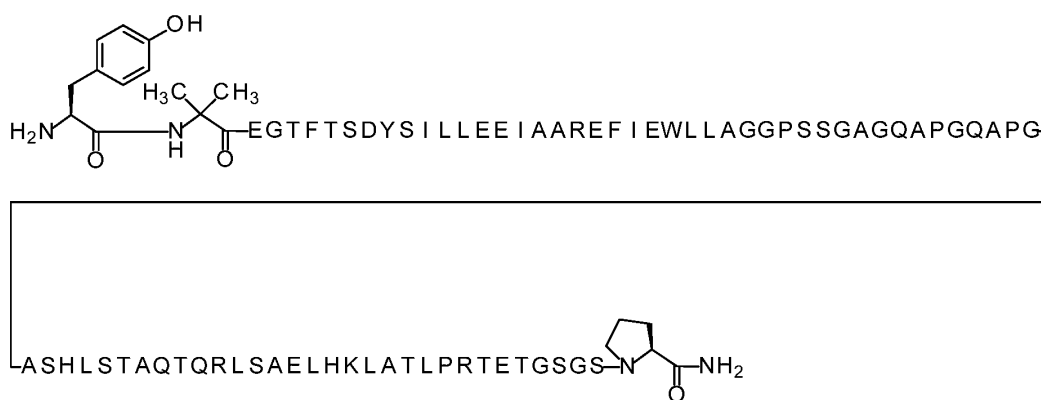
Compound 218



Compound 219



Compound 220



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Compound 221

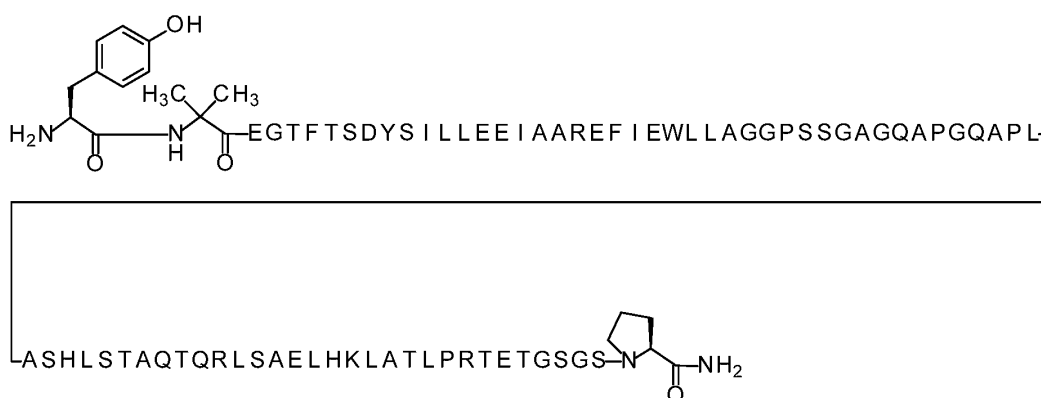


Table 17: Structure and amino acid sequences of the synthesized GLP-1-/GIP-/amylin-receptor tri-agonists according to the invention.

Compound no.	SEQ ID NO:	Amino acid sequences	Protraction moiety		
			(Table 4)	(Table 3)	(Table 5)
10	20	YXEGTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGEA SELSTAALGRLSAELHELATLPR TETGSGSP	Chem. 20	Chem. 4	Chem. 29
11	21	YXEGTFTSDYSILLEEQAAREFI EWLLAGGPSKGAEASELSTAALG RLSAELHELATLPRRTETGSGSP	Chem. 20	Chem. 4	Chem. 29
12	22	HXHGFTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGEA SELSTAALGRLSAELHELATLPR TETGSGSP	Chem. 20	Chem. 4	Chem. 29
13	23	YXEGTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGGA SELSTAALGRLSAELHRLATLPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
14	24	HXHGFTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGGA SELSTAALGRLSAELHRLATLPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
15	25	HXHGFTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGEA SELSTAALGRLSAELHELATLPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
16	26	HXHGFTFTSDYSILLEEQAAREFI EWLLAGGPSKGAPPPSGGGEA SELSTAALGRLSAELHELATLPR TETGSGSP	Chem. 20	Chem. 6	Chem. 27
17	27	HXHGFTFTSDYSILLEEQAAREFI EWLLAGGPSKGAEASELSTAALG RLSAELHRLATLPRRTETGSGSP	Chem. 20	Chem. 4	Chem. 29
18	28	YXEGTFTSDYSILLEEQAAREFI EWLLAGGPSKGAEASELSTAALG RLSAELHRLATLPRRTETGSGSP	Chem. 20	Chem. 4	Chem. 29

19	29	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALG RLSAELHRLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
20	30	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALGR LSAELHRLATLPRTETGSGSP	Chem. 20	Chem. 4	Chem. 29
21	31	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGALAQTLAQT LA SELSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
22	32	HXXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGAPPPSLAQT LA QTLASELSTAALGRLSAELHE LA TLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
23	33	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGALAQT LTGNEASELSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
24	34	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGALAQT L FVNQASELSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
25	35	HXXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALG RLSAELHRLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
26	36	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALG RLSAELHELATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
27	37	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAQL GRLSAELHRLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
28	38	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALA RLSAELHRLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
29	39	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGASELSTAALG RLSAELHYLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28

30	40	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGAAPPPSGGGEA SELSTAALGRLSAELHRLATLPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
31	41	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSKAPPPSGGGEA SELSTAALGRLSAELHRLATLPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
32	42	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGAASELSTAAL GRLSAELHELATLPR TETGSGS P	Chem. 20	Chem. 5	Chem. 28
33	43	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGAPPEASELSTA ALGRLSAELHELATLPR TETGS GSP	Chem. 20	Chem. 5	Chem. 28
34	44	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGEASHLSTAQT QRLSAELHELATLPR TETGSGS P	Chem. 20	Chem. 5	Chem. 28
35	45	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGEASHLSTAQT ARLSAELHELATLPR TETGSGS P	Chem. 20	Chem. 5	Chem. 28
36	46	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGEASHLSTAQT ARLSAELHELATLPR TETGSGS P	Chem. 20	Chem. 5	Chem. 28
37	47	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGAAPPPSGGGEA SHLSTAQTARLSAELHRLADLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
38	48	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGGQAPGQAPGE ASHLSTAQTQRLSAELHELATL PR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
39	49	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGGQEPGQEPGE ASHLSTAQTQRLSAELHELATL PR TETGSGSP	Chem. 20	Chem. 5	Chem. 28
40	50	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGGGGEGGGEG EASHLSTAQTQRLSAELHELAT LPR TETGSGSP	Chem. 20	Chem. 5	Chem. 28

41	51	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGAPPPSGGGEA SELSTAALGRLSAELHRLADLP RTETGSGSP	Chem. 18	Chem. 6	Chem. 22
42	52	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSKAPPPSGGGEA SELSTAALGRLSAELHRLATLPR TETGSGSP	Chem. 18	Chem. 6	Chem. 22
43	53	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGESQLSTAAL GRLSAELHRLADLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
44	54	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGALAQTTLGTNE ASELSTAALGRLSAELHRLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
45	55	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGALAQTTLGTNE ASHLSTAQTQRLSAELHELATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
46	56	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGAGQAPGQAPG ASHLSTAALGRLSAELHRLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
47	57	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGAGQAPGQAPG ASHLSTAQTQRLSAELHELATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
48	58	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALAQTTLAQT LA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
49	59	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAPPPSGGGEA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
50	60	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
51	61	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALAQTTLAQT LA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28

52	62	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
53	63	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAALGRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
54	64	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASKLSTAQTQRLSAELHRLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
55	65	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAKLHRLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
56	66	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLKTL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
57	67	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLATL PKTETGSGSP	Chem. 20	Chem. 5	Chem. 28
58	68	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPL ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
59	69	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALAQTLAQTLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 4	Chem. 29
60	70	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALAQTLAQTLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
61	71	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGAPPPSGGGEA SHLSTAQTARLSAELHRLADLP RTETGSGSP	Chem. 20	Chem. 4	Chem. 29
62	72	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSKGESQLSTAAL GRLSAELHRLADLPRTETGSGS P	Chem. 20	Chem. 4	Chem. 29

63	73	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGEASQLSTAAL GRLSAELHTLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
64	74	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGEASQLSTAAL GRLSAELHQLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
65	75	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGEASQLSTAAL GRLSAELHGLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
66	76	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGEASQLSTAAL GRLSAELHHLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
67	77	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGEASELSTAALG RLSAELHKLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
68	78	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
69	79	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
70	80	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGAPPPSGGGEA SELSTAALGRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
71	81	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGAPPPSGGGEA SHLSTQTQGRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
72	82	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSRGAPPPSGGGEA SHLSTQTQGRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
73	83	HXHGTFTSDYSILLEEQAAAREFI EWLLAGGPSKGEASQLSTAAL GRLSAELHRLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28

74	84	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
75	85	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
76	86	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLATL PKTETGSGSP	Chem. 20	Chem. 6	Chem. 27
77	87	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPL ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
78	88	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAKLHRLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
79	89	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAKLHRLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
80	90	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHRLKTLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
81	91	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHRLATLPKTETGSGS P	Chem. 20	Chem. 5	Chem. 28
82	92	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAKLHRLATLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
83	93	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHRLKTLPRTETGSGS P	Chem. 20	Chem. 5	Chem. 28
84	94	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHRLATLPKTETGSGS P	Chem. 20	Chem. 5	Chem. 28

85	95	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAQT QRLSAELHKLATLPRTETGSGA P	Chem. 20	Chem. 5	Chem. 28
86	96	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSKGAPPPSGGGAS ELSTAALGRLSAELHRLATLPRT ETGSGSP	Chem. 20	Chem. 5	Chem. 28
87	97	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAALG RLSAKLHRLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
88	98	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAALG RLSAELHKLATLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
89	99	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAALG RLSAELHRLKTLPRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
90	100	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGEASHLSTAALG RLSAELHRLATLPKTETGSGSP	Chem. 20	Chem. 5	Chem. 28
91	101	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLKTL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
92	102	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLATL PKTETGSGSP	Chem. 20	Chem. 5	Chem. 28
93	103	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPL ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
94	104	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSRGEASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
95	105	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGGGEASHLSTA QTQRLSAELHKLATLPRTETGS GSP	Chem. 20	Chem. 5	Chem. 28

96	106	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGGGEASHLSTA QTQRLSAELHKLATLPRTETGS GSP	Chem. 20	Chem. 6	Chem. 27
97	107	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGASHL STAQTQRLSAELHKLATLP RTE TGSGSP	Chem. 20	Chem. 5	Chem. 28
98	108	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPL ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
99	109	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALQAPGQAPL ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
100	110	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSKGAPPPSGGGEA SHLSTAQTARLSAELHRLADLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
101	111	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSKGAPPPSGGGEA SHLSTAQTARLSAELHQLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
102	112	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHQLKTL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28
103	113	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
104	114	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
105	115	YXEGTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALQAPGQAPL ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
106	116	HXHGFTFTSDYSILLEEQAAAREFI EWLLAGGPSSGALQAPGQAPG ASHLSTAQTARLSAELHRLKTL PRTETGSGSP	Chem. 20	Chem. 5	Chem. 28

107	117	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGALQAPGQAPG ASHLSTAQTARLSAELHRLATLP KTETGSGSP	Chem. 20	Chem. 5	Chem. 28
108	118	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGALQAPGQAPL ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
109	119	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAPPPSGGGEA SHLSTAQTQRLSAKHLRLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
110	120	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAPPPSGGGEA SHLSTAQTQRLSAKHLRLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
111	121	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHRLKTL PRTETGSGSP	Chem. 21	Chem. 5	Chem. 31
112	122	HXHGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSKGAPPPSGGGEA SHLSTAQTARLSAELHRLADLP RTETGSGSP	Chem. 18	Chem. 5	Chem. 30
113	123	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
114	124	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAALARLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 5	Chem. 28
115	62	YXEGTFSTSDYSILLEEQAAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHKLATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
120	170	YXEGTFSTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
121	171	YXEGTFSTSDYSILLEEIAAREFIE WLIAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

122	172	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQLGRLSAELHELATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
123	173	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQLGRLSAELHQLATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
124	174	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAQTQRLSAELHELATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
125	175	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
126	176	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASHLSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
127	177	YXEGTFTSDYSKLLLEEIAAREFI EWLIAGGPSSGAGQAPGQAPG ASHLSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
128	178	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SHLSTAQTARLSAELHKLATLPR TETGSGSP	Chem. 20	Chem. 6	Chem. 27
129	179	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPL ASHLSTAQLGRLSAELHQLATL PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
130	180	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGASHLSTAQ LGRLSAELHQLATLPRTETGSG SP	Chem. 20	Chem. 6	Chem. 27
131	181	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
132	182	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SQLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

133	183	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
134	184	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGEASHLSTAQTQ RLSAELHKLATLPRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
135	185	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPAA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
136	186	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPEA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
137	187	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPPA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
138	188	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPSA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
139	189	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPVA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
140	190	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGGGGASHLST AQTQRLSAELHKLATLPRTETG SGSP	Chem. 20	Chem. 6	Chem. 27
141	191	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGGGGAGELST AQTQRLSAELHKLATLPRTETG SGSP	Chem. 20	Chem. 6	Chem. 27
142	175	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 19	Chem. 6	Chem. 27
143	192	YXEGTFTSDYSILLEEIAAREFIE WLIAGGPSSGAGQAPGQAPLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

144	193	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
145	194	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SELSTAQTARLSAELHKLATLPR TETGSGSP	Chem. 20	Chem. 6	Chem. 27
146	195	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
147	196	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGEGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
148	197	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGEAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
149	198	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQEPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
150	199	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAEQGAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
151	200	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPEQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
152	201	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGEAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
153	202	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQEPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
154	203	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAEGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

155	204	YXEGTFTSDYSILLEEIAAREFIE WLIAGGPSSGAGQEPGQEPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
156	205	YXEGTFTSDYSILLEEIAAREFIA WLIAGGPSSGAGQEPGQEPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
157	206	YXEGTFTSDYSILLEEIAAREFIQ WLIAGGPSSGAGQEPGQEPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
158	207	YXEGTFTSDYSILLEEIAAREFIE WLIAGGPSSGAGASHLSTAQTQ RLSAELHKLATLPRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
159	208	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPAS HLSTAQTQRLSAELHKLATLPR TETGSGSP	Chem. 20	Chem. 6	Chem. 27
160	170	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 33	Chem. 6	Chem. 36
161	170	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 34	Chem. 6	Chem. 37
162	209	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATEP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
163	210	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SELSTAQTQRLSAELHKLATEP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
164	211	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGEAPGA SHLSTAQTQRLSAELHKLATEP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
165	212	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGQAPGQAPGA SHLSTAQTQRLSAELHKLATEP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

166	213	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGEAPGA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
167	214	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSSEAGQAPGQAPGA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
168	215	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSSEAGQAPGEAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
169	216	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASELSTAALGRLSAELHELATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
170	217	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGQAPG ASELSTAALGRLSAELHQLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
171	218	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGEAPG ASELSTAALGRLSAELHQLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
172	219	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGEAPG ASELSTAALGRLSAELHQLATE PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
173	220	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGEAPG ASELSTAQLGRLSAELHQLATE PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
174	221	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGEAPG ASELSTAQTGRLSAELHQLATE PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
175	222	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGQAPGEAPG ASELSTAQTQRLSAELHQLATE PRTETGSGSP	Chem. 20	Chem. 6	Chem. 27
176	223	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGASELSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27

177	224	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
178	225	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGASELSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
179	226	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGASHLSTAQT QRLSAELHKLATEPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
180	227	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGASQLSTAQT QRLSAELHKLATEPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
181	228	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGASQLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
182	229	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSEAGASQLSTAQT QRLSAELHKLATEPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
183	230	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGASELSTAQ LGRLSAELHQLATLPRTETGSG SP	Chem. 20	Chem. 6	Chem. 27
184	231	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGASHLSTAQ LGRLSAELHQLATEPRTETGSG SP	Chem. 20	Chem. 6	Chem. 27
185	232	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSEAGASHLSTAQL GRLSAELHQLATEPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
186	233	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSGAGASELSTAQ LGRLSAELHQLATEPRTETGSG SP	Chem. 20	Chem. 6	Chem. 27
187	234	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSEAGASELSTAQL GRLSAELHQLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27

188	235	YXEGTFTSDYSKLLLEEIAAREFI EWLLAGGPSSEAGASELSTAQL GRLSAELHQLATEPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
189	236	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGEAPGEAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
190	237	YXEGTFTSDYSILLEEIAAREFIE WLIAGGPSSGAGEAPGQAPGA SQLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
191	238	YXEGTFTSDYSILLEEIAAREFIE WLIAGGPSSGAGEAPGQAPGA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
192	170	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPGA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 32	Chem. 35
193	181	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGASHLSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 32	Chem. 35
194	239	YXEGTFTSDYSILLEEIAAQEFIE WLLQGGPSSGAGASELSTAQT QRLSAELHKLATLPRTETGSGS P	Chem. 20	Chem. 6	Chem. 27
195	240	YXEGTFTSDYSILLEEIAAQEFIE WLLQGGPSSGAGEAPGQAPGA SELSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
196	241	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGEAPLA SHLSTAQTQRLSAELHKLATLP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27
197	242	YXEGTFTSDYSILLEEIAAREFIE WLLAGGPSSGAGQAPGQAPLA SHLSTAQTQRLSAELHKLATEP RTETGSGSP	Chem. 20	Chem. 6	Chem. 27

wherein X represents always Aib.

Example 3: LCMS characterization of the synthesized compoundsLCMS characterization method

- LCMS analysis was performed on a set up consisting of Waters Acquity UPLC H Class system and Waters Xevo G2-XS QTof. Eluents: A: Milli-Q water; B: MeCN; C: 2% formic acid + 0.1% TFA in Milli-Q water.

The analysis was performed at RT (column temperature 60 °C) by injecting an appropriate volume of the sample onto the column. The sample was eluted with a linear gradient of 5-95% B in A, and constant 5% C.

- The UPLC conditions, detector settings, and mass spectrometer settings were: Column: Waters Acquity BEH Shield, C-18, 1.7 µm, 2.1 mm x 50 mm. Gradient: Linear 5% - 95% B, and constant 5% C during 4.0 min at 0.4 ml/min. Total run-time: 7.0 min. Detection: MS sensitivity mode, ionisation method: ES. Scan: 50-5000 amu.

- The monoisotopic mass was recorded for the synthesized compounds and their found and calculated values are depicted in Table 6.

Table 6: Measured and calculated MS species for all synthesized compounds

	Species	m/z found	m/z calc
Reference compound 1	[M+5H] ⁵⁺	1385.469	1385.485
	[M+6H] ⁶⁺	1154.892	1154.739
Reference compound 2	[M+5H] ⁵⁺	1796.108	1795.913
	[M+6H] ⁶⁺	1496.760	1496.762
Reference compound 3	[M+5H] ⁵⁺	1675.279	1675.266
	[M+6H] ⁶⁺	1396.379	1396.223
Reference compound 4	[M+3H] ³⁺	1517.136	1517.156
	[M+4H] ⁴⁺	1138.116	1138.119
Reference compound 5	[M+3H] ³⁺	1604.490	1604.516
	[M+4H] ⁴⁺	1203.626	1203.639
Reference compound 6	[M+3H] ³⁺	1469.738	1469.758
	[M+4H] ⁴⁺	1102.568	1102.570
Reference compound 7	[M+3H] ³⁺	1371.374	1371.379
	[M+4H] ⁴⁺	1028.789	1028.786
Compound 10	[M+5H] ⁵⁺	1681.268	1681.254
	[M+6H] ⁶⁺	1401.225	1401.213

Compound 11	[M+5H] ⁵⁺	1557.203	1557.196
	[M+6H] ⁶⁺	1297.849	1297.831
Compound 12	[M+5H] ⁵⁺	1677.680	1677.656
	[M+6H] ⁶⁺	1398.225	1398.215
Compound 13	[M+5H] ⁵⁺	1677.881	1677.868
	[M+6H] ⁶⁺	1398.408	1398.391
Compound 14	[M+5H] ⁵⁺	1674.498	1674.270
	[M+6H] ⁶⁺	1395.427	1395.393
Compound 15	[M+5H] ⁵⁺	1683.281	1683.263
	[M+6H] ⁶⁺	1402.894	1402.887
Compound 16	[M+5H] ⁵⁺	1688.892	1688.869
	[M+6H] ⁶⁺	1407.740	1407.559
Compound 17	[M+5H] ⁵⁺	1559.027	1559.010
	[M+6H] ⁶⁺	1299.352	1299.343
Compound 18	[M+5H] ⁵⁺	1562.615	1562.607
	[M+6H] ⁶⁺	1302.348	1302.341
Compound 19	[M+5H] ⁵⁺	1568.232	1568.214
	[M+6H] ⁶⁺	1307.017	1307.013
Compound 20	[M+5H] ⁵⁺	1536.816	1536.799
	[M+6H] ⁶⁺	1280.839	1280.834
Compound 21	[M+5H] ⁵⁺	1739.141	1739.109
	[M+6H] ⁶⁺	1449.597	1449.425
Compound 22	[M+5H] ⁵⁺	1811.376	1811.149
	[M+6H] ⁶⁺	1509.467	1509.459
Compound 23	[M+5H] ⁵⁺	1736.720	1736.694
	[M+6H] ⁶⁺	1447.432	1447.413
Compound 24	[M+5H] ⁵⁺	1754.324	1754.111
	[M+6H] ⁶⁺	1461.943	1461.927
Compound 25	[M+5H] ⁵⁺	1564.734	1564.616
	[M+6H] ⁶⁺	1304.031	1304.015
Compound 26	[M+5H] ⁵⁺	1562.809	1562.802
	[M+6H] ⁶⁺	1302.510	1302.503
Compound 27	[M+5H] ⁵⁺	1579.625	1579.618
	[M+6H] ⁶⁺	1316.529	1316.516

Compound 28	[M+5H]5+	1571.036	1571.017
	[M+6H]6+	1309.355	1309.349
Compound 29	[M+5H]5+	1569.625	1569.606
	[M+6H]6+	1308.186	1308.173
Compound 30	[M+5H]5+	1688.690	1688.674
	[M+6H]6+	1407.403	1407.397
Compound 31	[M+5H]5+	1698.488	1698.274
	[M+6H]6+	1415.410	1415.396
Compound 32	[M+5H]5+	1577.025	1577.009
	[M+6H]6+	1314.348	1314.342
Compound 33	[M+5H]5+	1615.844	1615.830
	[M+6H]6+	1346.699	1346.693
Compound 34	[M+5H]5+	1587.635	1587.610
	[M+6H]6+	1323.187	1323.176
Compound 35	[M+5H]5+	1576.229	1576.205
	[M+6H]6+	1313.681	1313.672
Compound 36	[M+5H]5+	1572.625	1572.608
	[M+6H]6+	1310.687	1310.674
Compound 37	[M+5H]5+	1704.884	1704.874
	[M+6H]6+	1420.910	1420.896
Compound 38	[M+5H]5+	1740.302	1740.282
	[M+6H]6+	1450.578	1450.403
Compound 39	[M+5H]5+	1763.503	1763.484
	[M+6H]6+	1469.759	1469.738
Compound 40	[M+5H]5+	1719.068	1719.057
	[M+6H]6+	1432.731	1432.715
Compound 41	[M+5H]5+	1642.859	1642.645
	[M+6H]6+	1369.054	1369.038
Compound 42	[M+5H]5+	1645.862	1645.851
	[M+6H]6+	1371.719	1371.710
Compound 43	[M+5H]5+	1567.228	1567.215
	[M+6H]6+	1306.189	1306.180
Compound 44	[M+5H]5+	1738.732	1738.508
	[M+6H]6+	1449.098	1448.925

Compound 45	[M+5H] ⁵⁺	1757.924	1757.904
	[M+6H] ⁶⁺	1465.104	1465.088
Compound 46	[M+5H] ⁵⁺	1707.503	1707.290
	[M+6H] ⁶⁺	1423.085	1422.910
Compound 47	[M+5H] ⁵⁺	1725.299	1725.083
	[M+6H] ⁶⁺	1437.908	1437.737
Compound 48	[M+5H] ⁵⁺	1755.541	1755.514
	[M+6H] ⁶⁺	1463.116	1463.096
Compound 49	[M+5H] ⁵⁺	1703.280	1703.266
	[M+6H] ⁶⁺	1419.569	1419.556
Compound 50	[M+5H] ⁵⁺	1579.219	1579.208
	[M+6H] ⁶⁺	1316.188	1316.174
Compound 51	[M+5H] ⁵⁺	1752.131	1751.917
	[M+6H] ⁶⁺	1460.270	1460.098
Compound 52	[M+5H] ⁵⁺	1720.290	1720.279
	[M+6H] ⁶⁺	1433.738	1433.733
Compound 53	[M+5H] ⁵⁺	1697.089	1697.074
	[M+6H] ⁶⁺	1414.410	1414.396
Compound 54	[M+5H] ⁵⁺	1724.110	1724.087
	[M+6H] ⁶⁺	1436.915	1436.907
Compound 55	[M+5H] ⁵⁺	1725.707	1725.690
	[M+6H] ⁶⁺	1438.420	1438.243
Compound 56	[M+5H] ⁵⁺	1737.316	1737.291
	[M+6H] ⁶⁺	1447.931	1447.911
Compound 57	[M+5H] ⁵⁺	1720.494	1720.279
	[M+6H] ⁶⁺	1433.908	1433.733
Compound 58	[M+5H] ⁵⁺	1731.507	1731.491
	[M+6H] ⁶⁺	1443.094	1443.077
Compound 59	[M+5H] ⁵⁺	1749.923	1749.908
	[M+6H] ⁶⁺	1458.442	1458.425
Compound 60	[M+5H] ⁵⁺	1761.133	1761.120
	[M+6H] ⁶⁺	1467.783	1467.768
Compound 61	[M+5H] ⁵⁺	1699.483	1699.267
	[M+6H] ⁶⁺	1416.241	1416.224

Compound 62	[M+5H] ⁵⁺	1561.823	1561.609
	[M+6H] ⁶⁺	1301.521	1301.509
Compound 63	[M+5H] ⁵⁺	1553.427	1553.409
	[M+6H] ⁶⁺	1295.021	1294.675
Compound 64	[M+5H] ⁵⁺	1559.027	1558.811
	[M+6H] ⁶⁺	1299.190	1299.177
Compound 65	[M+5H] ⁵⁺	1544.620	1544.603
	[M+6H] ⁶⁺	1287.347	1287.337
Compound 66	[M+5H] ⁵⁺	1560.627	1560.611
	[M+6H] ⁶⁺	1300.680	1300.677
Compound 67	[M+5H] ⁵⁺	1568.426	1568.214
	[M+6H] ⁶⁺	1307.017	1307.013
Compound 68	[M+5H] ⁵⁺	1593.035	1593.021
	[M+6H] ⁶⁺	1327.700	1327.686
Compound 69	[M+5H] ⁵⁺	1589.444	1589.424
	[M+6H] ⁶⁺	1324.870	1324.688
Compound 70	[M+5H] ⁵⁺	1688.690	1688.674
	[M+6H] ⁶⁺	1407.572	1407.397
Compound 71	[M+5H] ⁵⁺	1710.700	1710.679
	[M+6H] ⁶⁺	1425.741	1425.734
Compound 72	[M+5H] ⁵⁺	1714.492	1714.277
	[M+6H] ⁶⁺	1428.739	1428.732
Compound 73	[M+5H] ⁵⁺	1564.427	1564.419
	[M+6H] ⁶⁺	1303.854	1303.851
Compound 74	[M+5H] ⁵⁺	1573.420	1573.205
	[M+6H] ⁶⁺	1311.176	1311.172
Compound 75	[M+5H] ⁵⁺	1569.625	1569.608
	[M+6H] ⁶⁺	1308.186	1308.174
Compound 76	[M+5H] ⁵⁺	1725.911	1725.885
	[M+6H] ⁶⁺	1438.420	1438.405
Compound 77	[M+5H] ⁵⁺	1737.112	1737.097
	[M+6H] ⁶⁺	1447.759	1447.749
Compound 78	[M+5H] ⁵⁺	1722.310	1722.093
	[M+6H] ⁶⁺	1435.256	1435.245

Compound 79	[M+5H] ⁵⁺	1584.637	1584.619
	[M+6H] ⁶⁺	1320.689	1320.684
Compound 80	[M+5H] ⁵⁺	1596.236	1596.220
	[M+6H] ⁶⁺	1330.355	1330.351
Compound 81	[M+5H] ⁵⁺	1579.219	1579.208
	[M+6H] ⁶⁺	1316.188	1316.174
Compound 82	[M+5H] ⁵⁺	1581.235	1581.022
	[M+6H] ⁶⁺	1317.851	1317.686
Compound 83	[M+5H] ⁵⁺	1592.839	1592.623
	[M+6H] ⁶⁺	1327.357	1327.353
Compound 84	[M+5H] ⁵⁺	1575.628	1575.610
	[M+6H] ⁶⁺	1313.192	1313.176
Compound 85	[M+5H] ⁵⁺	1572.431	1572.411
	[M+6H] ⁶⁺	1310.524	1310.510
Compound 86	[M+5H] ⁵⁺	1662.884	1662.866
	[M+6H] ⁶⁺	1386.060	1385.889
Compound 87	[M+5H] ⁵⁺	1557.832	1557.817
	[M+6H] ⁶⁺	1298.527	1298.349
Compound 88	[M+5H] ⁵⁺	1552.412	1552.405
	[M+6H] ⁶⁺	1293.843	1293.839
Compound 89	[M+5H] ⁵⁺	1569.431	1569.418
	[M+6H] ⁶⁺	1308.023	1308.016
Compound 90	[M+5H] ⁵⁺	1552.412	1552.405
	[M+6H] ⁶⁺	1293.843	1293.839
Compound 91	[M+5H] ⁵⁺	1733.908	1733.694
	[M+6H] ⁶⁺	1444.928	1444.913
Compound 92	[M+5H] ⁵⁺	1716.898	1716.681
	[M+6H] ⁶⁺	1430.750	1430.735
Compound 93	[M+5H] ⁵⁺	1727.917	1727.894
	[M+6H] ⁶⁺	1440.096	1440.079
Compound 94	[M+5H] ⁵⁺	1598.656	1598.628
	[M+6H] ⁶⁺	1332.534	1332.358
Compound 95	[M+5H] ⁵⁺	1596.024	1596.014
	[M+6H] ⁶⁺	1330.191	1330.180

Compound 96	[M+5H]5+	1601.635	1601.620
	[M+6H]6+	1334.865	1334.851
Compound 97	[M+5H]5+	1649.665	1649.645
	[M+6H]6+	1375.040	1374.872
Compound 98	[M+5H]5+	1720.120	1720.087
	[M+6H]6+	1433.598	1433.574
Compound 99	[M+5H]5+	1742.914	1742.704
	[M+6H]6+	1452.433	1452.421
Compound 100	[M+5H]5+	1710.497	1710.480
	[M+6H]6+	1425.741	1425.568
Compound 101	[M+5H]5+	1696.685	1696.469
	[M+6H]6+	1414.071	1413.892
Compound 102	[M+5H]5+	1731.898	1731.683
	[M+6H]6+	1443.249	1443.237
Compound 103	[M+5H]5+	1705.492	1705.277
	[M+6H]6+	1421.403	1421.232
Compound 104	[M+5H]5+	1711.106	1710.883
	[M+6H]6+	1425.926	1425.904
Compound 105	[M+5H]5+	1731.320	1731.299
	[M+6H]6+	1442.923	1442.917
Compound 106	[M+5H]5+	1733.533	1733.502
	[M+6H]6+	1444.772	1444.753
Compound 107	[M+5H]5+	1716.508	1716.489
	[M+6H]6+	1430.595	1430.576
Compound 108	[M+5H]5+	1727.917	1727.702
	[M+6H]6+	1440.096	1439.919
Compound 109	[M+5H]5+	1708.890	1708.678
	[M+6H]6+	1424.243	1424.066
Compound 110	[M+5H]5+	1714.289	1714.284
	[M+6H]6+	1428.909	1428.738
Compound 111	[M+5H]5+	1766.339	1766.306
	[M+6H]6+	1472.112	1472.090
Compound 112	[M+5H]5+	1646.858	1646.844
	[M+6H]6+	1372.553	1372.538

Compound 113	[M+5H]5+	1708.890	1708.874
	[M+6H]6+	1424.243	1424.230
Compound 114	[M+5H]5+	1700.090	1699.877
	[M+6H]6+	1416.903	1416.732
Compound 115	[M+5H]5+	1725.881	1725.885
	[M+6H]6+	1438.401	1438.405
Compound 120	[M+5H]5+	1723.077	1722.890
	[M+6H]6+	1435.903	1435.910
Compound 121	[M+5H]5+	1723.077	1722.890
	[M+6H]6+	1435.903	1435.910
Compound 122	[M+5H]5+	1714.270	1714.282
	[M+6H]6+	1428.731	1428.736
Compound 123	[M+5H]5+	1714.287	1714.085
	[M+6H]6+	1428.731	1428.572
Compound 124	[M+5H]5+	1726.068	1726.082
	[M+6H]6+	1438.557	1438.569
Compound 125	[M+5H]5+	1734.292	1734.103
	[M+6H]6+	1445.255	1445.253
Compound 126	[M+5H]5+	1702.867	1702.877
	[M+6H]6+	1419.232	1419.232
Compound 127	[M+5H]5+	1702.867	1702.877
	[M+6H]6+	1419.232	1419.232
Compound 128	[M+5H]5+	1722.686	1722.698
	[M+6H]6+	1435.748	1435.750
Compound 129	[M+5H]5+	1725.286	1725.297
	[M+6H]6+	1437.905	1437.916
Compound 130	[M+5H]5+	1572.809	1572.817
	[M+6H]6+	1310.841	1310.849
Compound 131	[M+5H]5+	1581.620	1581.622
	[M+6H]6+	1318.171	1318.186
Compound 132	[M+5H]5+	1732.298	1732.302
	[M+6H]6+	1443.747	1443.753
Compound 133	[M+5H]5+	1732.485	1732.499
	[M+6H]6+	1443.918	1443.917

Compound 134	[M+5H] ⁵⁺	1581.815	1581.819
	[M+6H] ⁶⁺	1318.513	1318.350
Compound 135	[M+5H] ⁵⁺	1725.677	1725.693
	[M+6H] ⁶⁺	1438.401	1438.246
Compound 136	[M+5H] ⁵⁺	1737.481	1737.294
	[M+6H] ⁶⁺	1447.902	1447.913
Compound 137	[M+5H] ⁵⁺	1730.884	1730.896
	[M+6H] ⁶⁺	1442.580	1442.581
Compound 138	[M+5H] ⁵⁺	1729.080	1728.892
	[M+6H] ⁶⁺	1440.902	1440.911
Compound 139	[M+5H] ⁵⁺	1731.293	1731.299
	[M+6H] ⁶⁺	1442.907	1442.917
Compound 140	[M+5H] ⁵⁺	1615.824	1615.835
	[M+6H] ⁶⁺	1346.693	1346.697
Compound 141	[M+5H] ⁵⁺	1608.428	1608.230
	[M+6H] ⁶⁺	1340.347	1340.359
Compound 142	[M+5H] ⁵⁺	1705.080	1705.088
	[M+6H] ⁶⁺	1421.068	1421.074
Compound 143	[M+5H] ⁵⁺	1734.087	1734.103
	[M+6H] ⁶⁺	1445.240	1445.253
Compound 144	[M+5H] ⁵⁺	1721.276	1721.287
	[M+6H] ⁶⁺	1434.570	1434.574
Compound 145	[M+5H] ⁵⁺	1709.867	1709.882
	[M+6H] ⁶⁺	1425.067	1425.070
Compound 146	[M+5H] ⁵⁺	1737.293	1737.294
	[M+6H] ⁶⁺	1448.073	1447.913
Compound 147	[M+5H] ⁵⁺	1734.479	1734.491
	[M+6H] ⁶⁺	1445.567	1445.577
Compound 148	[M+5H] ⁵⁺	1723.077	1723.087
	[M+6H] ⁶⁺	1436.229	1436.074
Compound 149	[M+5H] ⁵⁺	1734.479	1734.491
	[M+6H] ⁶⁺	1445.567	1445.577
Compound 150	[M+5H] ⁵⁺	1729.284	1729.288
	[M+6H] ⁶⁺	1441.399	1441.241

Compound 151	[M+5H]5+	1737.481	1737.294
	[M+6H]6+	1447.902	1447.913
Compound 152	[M+5H]5+	1723.077	1723.087
	[M+6H]6+	1436.074	1436.074
Compound 153	[M+5H]5+	1734.479	1734.491
	[M+6H]6+	1445.567	1445.577
Compound 154	[M+5H]5+	1729.284	1729.288
	[M+6H]6+	1441.244	1441.241
Compound 155	[M+5H]5+	1746.278	1746.092
	[M+6H]6+	1455.231	1455.245
Compound 156	[M+5H]5+	1734.479	1734.491
	[M+6H]6+	1445.567	1445.577
Compound 157	[M+5H]5+	1745.885	1745.895
	[M+6H]6+	1455.075	1455.081
Compound 158	[M+5H]5+	1581.620	1581.622
	[M+6H]6+	1318.350	1318.186
Compound 159	[M+5H]5+	1711.475	1711.486
	[M+6H]6+	1426.396	1426.406
Compound 160	[M+5H]5+	1774.701	1774.507
	[M+6H]6+	1478.920	1478.924
Compound 161	[M+5H]5+	1774.494	1774.507
	[M+6H]6+	1478.920	1478.924
Compound 162	[M+5H]5+	1726.068	1726.082
	[M+6H]6+	1438.557	1438.569
Compound 163	[M+5H]5+	1724.470	1724.478
	[M+6H]6+	1437.222	1437.233
Compound 164	[M+5H]5+	1726.272	1726.279
	[M+6H]6+	1438.728	1438.733
Compound 165	[M+5H]5+	1740.673	1740.486
	[M+6H]6+	1450.567	1450.573
Compound 166	[M+5H]5+	1721.480	1721.484
	[M+6H]6+	1434.895	1434.738
Compound 167	[M+5H]5+	1735.673	1735.691
	[M+6H]6+	1446.563	1446.577

Compound 168	[M+5H]5+	1737.481	1737.491
	[M+6H]6+	1448.073	1448.077
Compound 169	[M+5H]5+	1701.263	1701.274
	[M+6H]6+	1417.891	1417.896
Compound 170	[M+5H]5+	1701.263	1701.077
	[M+6H]6+	1417.721	1417.732
Compound 171	[M+5H]5+	1701.263	1701.274
	[M+6H]6+	1417.891	1417.896
Compound 172	[M+5H]5+	1704.455	1704.466
	[M+6H]6+	1420.543	1420.556
Compound 173	[M+5H]5+	1715.864	1715.870
	[M+6H]6+	1430.046	1430.060
Compound 174	[M+5H]5+	1713.457	1713.463
	[M+6H]6+	1428.051	1428.054
Compound 175	[M+5H]5+	1727.854	1727.670
	[M+6H]6+	1439.892	1439.893
Compound 176	[M+5H]5+	1580.009	1580.019
	[M+6H]6+	1317.013	1316.850
Compound 177	[M+5H]5+	1596.026	1596.026
	[M+6H]6+	1330.176	1330.190
Compound 178	[M+5H]5+	1594.424	1594.423
	[M+6H]6+	1328.848	1328.854
Compound 179	[M+5H]5+	1584.812	1584.814
	[M+6H]6+	1320.833	1320.846
Compound 180	[M+5H]5+	1583.004	1583.014
	[M+6H]6+	1319.509	1319.346
Compound 181	[M+5H]5+	1594.228	1594.226
	[M+6H]6+	1328.684	1328.690
Compound 182	[M+5H]5+	1597.416	1597.418
	[M+6H]6+	1331.341	1331.350
Compound 183	[M+5H]5+	1571.203	1571.214
	[M+6H]6+	1309.507	1309.513
Compound 184	[M+5H]5+	1576.009	1576.009
	[M+6H]6+	1313.495	1313.508

Compound 185	[M+5H]5+	1590.406	1590.413
	[M+6H]6+	1325.508	1325.512
Compound 186	[M+5H]5+	1574.401	1574.405
	[M+6H]6+	1312.160	1312.172
Compound 187	[M+5H]5+	1585.610	1585.618
	[M+6H]6+	1321.502	1321.516
Compound 188	[M+5H]5+	1588.806	1588.810
	[M+6H]6+	1324.167	1324.176
Compound 189	[M+5H]5+	1723.485	1723.284
	[M+6H]6+	1436.229	1436.238
Compound 190	[M+5H]5+	1721.276	1721.287
	[M+6H]6+	1434.570	1434.574
Compound 191	[M+5H]5+	1721.480	1721.484
	[M+6H]6+	1434.895	1434.738
Compound 192	[M+5H]5+	1730.271	1730.085
	[M+6H]6+	1441.896	1441.906
Compound 193	[M+5H]5+	1588.806	1588.817
	[M+6H]6+	1324.182	1324.182
Compound 194	[M+5H]5+	1585.806	1585.815
	[M+6H]6+	1321.845	1321.680
Compound 195	[M+5H]5+	1727.276	1727.279
	[M+6H]6+	1439.566	1439.567
Compound 196	[M+5H]5+	1734.292	1734.299
	[M+6H]6+	1445.411	1445.417
Compound 197	[M+5H]5+	1737.481	1737.294
	[M+6H]6+	1447.902	1447.913
Compound 210	[M+5H]5+	1577.390	1577.194
	[M+6H]6+	1314.489	1314.496
Compound 211	[M+5H]5+	1588.594	1588.603
	[M+6H]6+	1324.167	1324.004
Compound 212	[M+5H]5+	1585.790	1585.800
	[M+6H]6+	1321.666	1321.668
Compound 213	[M+5H]5+	1574.384	1574.395
	[M+6H]6+	1312.160	1312.164

Compound 214	[M+5H]5+	1585.790	1585.800
	[M+6H]6+	1321.830	1321.668
Compound 215	[M+5H]5+	1580.594	1580.597
	[M+6H]6+	1317.325	1317.332
Compound 216	[M+5H]5+	1588.594	1588.603
	[M+6H]6+	1324.167	1324.004
Compound 217	[M+5H]5+	1574.384	1574.395
	[M+6H]6+	1312.160	1312.164
Compound 218	[M+5H]5+	1585.790	1585.800
	[M+6H]6+	1321.666	1321.668
Compound 219	[M+5H]5+	1580.594	1580.597
	[M+6H]6+	1317.325	1317.332
Compound 220	[M+5H]5+	1574.189	1574.199
	[M+6H]6+	1311.997	1312.000
Compound 221	[M+5H]5+	1585.610	1585.411
	[M+6H]6+	1321.339	1321.344

**Example 4: Human GLP-1-, GIP-, and Amylin-receptors *in vitro* potency assays
(High throughput assay)**

5 GLP-1 receptor assay

To determine the ability of compounds to activate or agonize the GLP-1 receptor, *in vitro* potency assays on Baby Hamster Kidney (BHK) cells expressing the human GLP-1 receptor (hGLP-1R) were performed as described below. To assess how the activation of the receptors is potentially influenced by the presence of human serum albumin (HSA), the *in vitro* assays were performed in the absence of HSA and presence of 1% (w/v) HSA. Unless stated otherwise the reference to the "GLP-1 receptor assay as described in Example 4" throughout the specification shall refer to the described herein assay procedure method A (hGLP-1R assay) in the absence of HSA.

15 Assay principle

Activation of the human GLP-1 receptor leads to increased intra-cellular concentrations of cyclic AMP (cAMP) and the consequent transcription activation from promoters containing multiple copies of the cAMP response element (CRE). It is thus possible to measure GLP-1

receptor activity using a CRE-luciferase reporter gene introduced into Baby Hamster Kidney (BHK) cells co-expressing the human GLP-1 receptor.

Cells and assay reagents

5 Cell stocks were prepared by culturing of a cell line stably expressing the human GLP-1 receptor and the CRE responsive luciferase (CRE-Luc) reporter gene (BHK 467-12A KZ-10 prepared according to methods known to the person skilled in the art) in growth medium consisting of DMEM (Gibco, 61965-026) supplemented with 10% FBS (Gibco, 16140-071 or 10100-147), 1% Penicillin-Streptomycin (Gibco, 15140-122), 1 mM Na-Pyruvate (Gibco, 11360-039), 1 mg/mL G418 (Gibco, 10131-027) and 240 nM Methotrexate (Pfizer, 15936). Cells at approximately 80-90% confluence were washed once in PBS (Gibco 14190-094) and loosened from the cell flasks with Versene (Gibco, 15040-033). After centrifugation, the cell pellet was resuspended and diluted to approximately 1.5×10^6 ($1.5E+6$) cells/mL in Recovery™ Cell Culture Freezing Medium (Gibco, 12648-010) or in medium 15 consisting of DMEM (Gibco, 61965-026) supplemented with 20% FBS (Gibco, 16140-071 or 10100-147), 1% Penicillin-Streptomycin (Gibco, 15140-122), 1mM Na-Pyruvate (Gibco, 11360-039), 1 mg/mL G418 (Gibco, 10131-027), 240 nM Methotrexate (Pfizer, 15936) and 10% DMSO (Sigma, D2650). Cells were aliquoted and stored at -180°C until use.

The assay buffer consisted of DMEM without phenol red (Gibco, 11880-028) 20 supplemented with 1X GlutaMAX (Gibco, 35050-038), 10 mM HEPES (Gibco, 15630-056), 1% (w/v) ovalbumin (Sigma, A5503) and 0.1% (v/v) Pluronic F-68 (Gibco, 24040-032) either with HSA or without HSA (Sigma, A9511).

Procedure – Method A (hGLP-1R assay)

25 To perform the assay, serial dilutions (10-fold dilutions, 8 concentrations per compound) of reference compounds and GLP-1-/GIP-/amylin-receptor tri-agonists were performed in assay buffer without HSA in a 96-well plate. Frozen stocks of hGLP-1R BHK CRE-Luc cells were thawed in a 37°C water bath, washed once in PBS (Gibco 14190-094) and diluted to 1.5×10^6 ($1.5E+6$) cells/mL in assay buffer with or without 2% (w/v) HSA 30 (Sigma, A9511). For each dilution, 50 μL aliquots of reference compounds or GLP-1-/GIP-/amylin-receptor tri-agonists were transferred to two 96-well assay plates (ThermoFisher, 237105) to which 50 μL of the cell suspension with or without 2% (w/v) HSA was added (5.0×10^3 ($5.0E+3$) cells/well). The assay plates were incubated for 3 hours at 37°C in 5% CO_2 , left at room temperature for 5 minutes after which 100 μL steadylite plus™ 35 (PerkinElmer/Revvity, 6066759) was added to each well. Plates were sealed and incubated

at room temperature with gentle shaking for 30 minutes while protected from light.

Luminescence was detected on a luminescence plate reader e.g., a Synergy 2 (BioTek). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = 1) using GraphPad Prism (GraphPad Software, Boston, MA, USA) or by means of TIBCO Enterprise Runtime for R (TIBCO Software, Palo Alto, CA, USA).

Procedure – Method B (hGLP-1R assay)

To perform the assay, serial dilutions (7-fold dilutions, 7 concentrations per compound and one well containing only assay buffer) of reference compounds and GLP-1-/GIP-/amylin-receptor tri-agonists were performed in assay buffer in a 96-well plate. Serial dilutions were transferred to a 384-well assay plate (PerkinElmer/Revvity, 6007688) and mixed with an equal volume (10 µL) of assay buffer without or with 3% (w/v) HSA (Sigma, A-9511). Frozen stocks of hGLP-1R BHK Cre-Luc cells were thawed in a 37 °C water bath, washed once in PBS (Gibco 14190-094), diluted to 1.5 x10⁵ (1.5E+5) cells/mL in assay buffer (without HSA) and added (10 µL) to each well of the 384-well assay plate. After a short centrifugation, the assay plates were incubated for 3 hours at 37 °C in 5% CO₂ and let to equilibrated at room temperature for 10 minutes before the addition of 30 µL steadylite plusTM PerkinElmer/Revvity, 6066759) per well. Plates were sealed and incubated at room temperature with gentle shaking for 30 minutes while protected from light. Luminescence was detected on a luminescence plate reader e.g. a Synergy 2 (BioTek). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = 1) using GraphPad Prism (GraphPad Software, Boston, MA, USA) or by means of TIBCO Enterprise Runtime for R (TIBCO Software, Palo Alto, CA, USA).

25 GIP receptor assay

To determine the ability of compounds to activate or agonize the GIP receptor, *in vitro* potency assays on Baby Hamster Kidney (BHK) cells expressing the human GIP receptor (hGIPR) were performed as described below. To assess how the activation of the receptors is potentially influenced by the presence of human serum albumin (HSA), the *in vitro* assays were performed in the absence of HSA and presence of 1% (w/v) HSA. Unless stated otherwise the reference to the “GIP receptor assay as described in Example 4” throughout the specification shall refer to the described herein assay procedure method A (hGIPR assay) in the absence of HSA.

Assay principle

Activation of the human GIP receptor leads to increased intra-cellular concentrations of cyclic AMP (cAMP) and the consequent transcription activation from promoters containing multiple copies of the cAMP response element (CRE). It is thus possible to measure GIP receptor activity using a CRE-luciferase reporter gene introduced into Baby Hamster Kidney (BHK) cells co-expressing the human GIP receptor.

Cells and assay reagents

- Cell stocks were prepared by culturing of a cell line stably expressing the human GIP receptor and containing the CRE responsive luciferase (CRE-Luc) reporter gene (hGIPR BHK Cre-Luc2p clone#5 prepared according to methods known to the person skilled in the art) at 5% CO₂ and 37 °C in growth medium consisting of in DMEM (Gibco, 61965-026) supplemented with 10% fetal calf serum (Gibco, 16140-071 or 10100-147), 0.5 mg/ml G418 (Gibco, 10131-027), 1% Penicillin-Streptomycin (Gibco, 15140-122) and 0.3 mg/ml Hygromycin B (Invitrogen, 10687010). Cells at about 80-90% confluency were washed once with PBS (Gibco 14190-094) and detached from the cell flasks with Versene (Gibco, 15040-066). After centrifugation, the cells were counted, resuspended and diluted to approximately 1.5-3.0 x10⁶ (1.5E+6 to 3.0E+6) cells/mL in Recovery Cell Culture Freezing Medium (Gibco, 12648-010) and stored at -180 °C in suitable aliquots until use.
- The assay buffer consisted of DMEM without phenol red (Gibco, 11880-028) supplemented with 1X GlutaMAX (Gibco, 35050-038), 10 mM HEPES (Gibco, 15630-056), 1% (w/v) ovalbumin (Sigma, A5503) and 0.1% (v/v) Pluronic F-68 (Gibco, 24040-032) either with HSA or without HSA (Sigma, A9511).

Procedure – Method A (hGIPR assay)

- On the day before the assay hGIPR BHK Cre-Luc cells were thawed and plated out in 96 well Culture Plates (PerkinElmer/Revvity, 6005680) at 5.0 x10³ (5.0E+3) cells/well in growth medium as described above. The plates were then incubated for 21-23 hours at 37 °C with 5% CO₂. On the day of the assay GLP-1-/GIP-/amylin-receptor tri-agonists or reference compounds were diluted in assay buffer using a 7-point, 10-fold titration plus a blank (absence of compound). Each compound was diluted and tested in duplicates in each experiment. In the plates containing the cells the growth medium was removed, and cells were washed twice with 100 µL PBS (Gibco 14190-094). 50 µL of the test compound dilutions were added to the plates containing cells preceded by 50 µL assay buffer with or without 2% (w/v) HSA (Sigma, A9511). The cell plates were incubated for 3 hours in a 5%

CO₂ incubator at 37 °C. The plates were then transferred to room temperature followed by addition of 100 µL steadylite plus™ (PerkinElmer/Revvity, 6066759). The plates were sealed and shaken for 30 minutes at room temperature while protected from light. Finally, luminescence (as an indicator of receptor activation) was measured on a Mithras reader (Berthold Technologies, DE). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = "shared value for all datasets") using GraphPad Prism (GraphPad Software, Boston, MA, USA).

Procedure – Method B (hGIPR assay)

- 10 To perform the assay, serial dilutions (7-fold dilutions, 7 concentrations per compound and one well containing only assay buffer) of reference compounds and GLP-1-/GIP-/amylin-receptor tri-agonists were prepared in assay buffer in a 96-well plate. Serial dilutions were transferred to a 384-well assay plate (PerkinElmer/Revvity, 6007688) and mixed with an equal volume (10 µL) of assay buffer without or with 3% HSA (Sigma, A-9511).
- 15 Frozen stocks of hGIPR BHK Cre-Luc cells were thawed in a 37 °C water bath, washed once in PBS (Gibco 14190-094), diluted to 1.5×10^5 ($1.5E+5$) cells/mL in assay buffer (without HSA) and added (10 µL) to each well of the 384-well assay plate. After a short centrifugation, the assay plates were incubated for 3 hours at 37 °C in 5% CO₂ and let to equilibrated at room temperature for 10 minutes before the addition of 30 µL steadylite plus™
- 20 (PerkinElmer/Revvity, 6066759) per well. Plates were sealed and incubated at room temperature with gentle shaking for 30 minutes while protected from light. Luminescence was detected on a luminescence plate reader e.g. a Synergy 2 (BioTek). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = 1) using GraphPad Prism (GraphPad Software, Boston, MA, USA) or by means of
- 25 TIBCO Enterprise Runtime for R (TIBCO Software, Palo Alto, CA, USA).

Amylin receptor assay

- To determine the ability of compounds to activate or agonize the amylin receptor, *in vitro* potency assays on Baby Hamster Kidney (BHK) cells expressing the human amylin receptor (hAMYR3) were performed as described below. To assess how the activation of the receptors is potentially influenced by the presence of human serum albumin (HSA), the *in vitro* assays were performed in the absence of HSA and presence of 1% (w/v) HSA. Unless stated otherwise the reference to the "amylin receptor assay as described in Example 4" throughout the specification shall refer to the described herein assay procedure method A
- 35 (hAMYR3 assay) in the absence of HSA.

Assay principle

Activation of the human amylin 3 receptor leads to increased intra-cellular concentrations of cAMP and the consequent transcription activation from promoters containing multiple copies of the cAMP response element (CRE). It is thus possible to measure hAMYR3 activity using a CRE-luciferase reporter gene introduced into Baby Hamster Kidney (BHK) cells co-expressing the hAMYR3.

Cells and assay reagents

A BHK cell line was transfected to stably express the human calcitonin receptor_(a) and a CRE-responsive luciferase (CRE-Luc) reporter gene according to methods known to the person skilled in the art (Holle-1 cell line, obtained from Zymogenetics described in US patent 5,622,839). The cell line was further transfected with human receptor modifying protein 3 (RAMP3) using standard methods. This turns the human calcitonin receptor into a human amylin-3(a) receptor (hAMYR3).

Cells stocks were prepared by culturing of the hAMYR3 BHK Cre-Luc cell line in growth medium consisting of DMEM (Gibco, 31966-021) supplemented with 10% FBS (Gibco, 16140-071 or 10100-147), 1% Penicillin-Streptomycin (Gibco, 15140-122), 0.5 mg/mL Geneticin (Gibco, 10131-027), 0.4 mg/mL Hygromycin (Invitrogen, 10687010) and 250 nM Methotrexate (Sigma, A6770). Cells at approximately 80-90% confluence were washed once with PBS (Gibco 14190-094) and loosened from the cell flasks with Versene (Gibco, 15040-033) or TrypLE™ (Gibco, 12605-010). After centrifugation, the cell pellet was resuspended and diluted to approximately $2.5-4.0 \times 10^6$ ($2.5E+6$ to $4.0E+6$) cells/mL in Recovery™ Cell Culture Freezing Medium (Gibco, 12648-010). Cells were aliquoted and stored at -180°C until use.

The assay buffer consisted of DMEM without phenol red (Gibco, 11880-028) supplemented with 1X GlutaMAX (Gibco, 35050-038), 10 mM HEPES (Gibco, 15630-056) and 1% (w/v) ovalbumin (Sigma, A5503) either with or without 0.1% (v/v) Pluronic F-68 (Gibco, 24040-032) and either with or without HSA (Sigma, A9511).

Procedure - Method A (hAMYR3 assay)

To perform the assay, BHK hAMYR3/CRE-Luc cells were thawed, washed once in PBS (Gibco 14190-094), and seeded in 40 μL growth medium in a white 384-well culture plate (PerkinElmer/Revvity, 6007688) at a cell density of 4.0×10^3 ($4.0E+3$) cells/well on the day before the experiment. The plate was incubated over night at 37°C in 5% CO_2 . On the day of the assay, cells were washed once in assay PBS (Gibco 14190-094). Serial dilutions

(7-fold dilutions, 7 concentrations pr. compound and one well containing only assay buffer) of reference compounds and GLP-1-/GIP-/amylin-receptor tri-agonists were performed in assay buffer with or without 1% (w/v) HSA (Sigma, A9511) in 96-well plates and 30 µL of each concentration added to the 384-well assay plate with cells. The assay plate was incubated for 3 hours at 37 °C in 5% CO₂ after which 30 µL steadylite plus™ (PerkinElmer/Revvity, 6066759) was added to each well. The assay plate was sealed, incubated at room temperature with gentle shaking for 5 minutes followed by 30 minutes incubation without shaking while protected from light. Luminescence was detected on a luminescence plate reader e.g., a Synergy 2 (BioTek). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = 1.5, shared bottom response within each plate) using GraphPad Prism (GraphPad Software, Boston, MA, USA) or by means of TIBCO Enterprise Runtime for R (TIBCO Software, Palo Alto, CA, USA).

Procedure – Method B (hAMYR3)

To perform the assay, hAMYR3 BHK Cre-Luc cells were thawed, washed once in PBS (Gibco 14190-094) and seeded in 40 µL growth medium in a white 384-well culture plate (PerkinElmer/Revvity, 6007688) at a cell density of 4.0×10^3 (4.0E+3) cells/well the day before the experiment. The plate was incubated over night at 37 °C in 5% CO₂. On the day of the assay, serial dilutions (7-fold dilutions, 7 concentrations per compound and one well containing only assay buffer) of reference compounds and GLP-1-/GIP-/amylin-receptor tri-agonists were prepared in assay buffer in 96-well plates. Serial dilutions were then mixed in a new 96-well plate with equal volume (1:1:1 ratio) of assay buffer and either assay buffer without or with 3% HSA (Sigma, A-9511). Twenty microliters of the solution mix were transferred to the cells which previously were washed once with PBS (Gibco 14190-094). After a short centrifugation, the assay plates were incubated for 3 hours at 37 °C in 5% CO₂ and let to equilibrated at room temperature for 10 minutes before the addition of 30 µL steadylite plus™ PerkinElmer/Revvity, 6066759) per well. Plates were sealed and incubated at room temperature with gentle shaking for 30 minutes while protected from light. Luminescence was detected on a luminescence plate reader e.g., a Synergy 2 (BioTek). The EC₅₀-values [pM] were calculated by non-linear curve fitting applying a four-parameter logistic model (Hill slope = 1.5, shared bottom response within each plate) using GraphPad Prism (GraphPad Software, Boston, MA, USA) or by means of TIBCO Enterprise Runtime for R (TIBCO Software, Palo Alto, CA, USA).

Table 7a: In vitro activity data for reference compounds on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods A) in the absence of HSA

Reference compound number	hGLP-1R Potency, no HSA, EC ₅₀ [pM]	hGIPR Potency no HSA, EC ₅₀ [pM]	hAMYR3 Potency, no HSA, EC ₅₀ [pM]
1	1516.05	29.63	5.29
2	586.71	3.16	20.27
3	267.92	2.31	4.49
4	nd	1.01	nd
5	43.0	0.91	nd
6	nd	nd	1.47
7	2.67	nd	nd

5 Table 7b: In vitro activity data for reference compounds on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods B) in the absence of HSA

Reference compound number	hGLP-1R Potency, no HSA, EC ₅₀ [pM]	hGIPR Potency no HSA, EC ₅₀ [pM]	hAMYR3 Potency, no HSA, EC ₅₀ [pM]
4	nd	2.41	nd
6	nd	nd	2.47
7	4.97	nd	nd

The results in Table 7a and Table 7b show that the reference compounds 4 to 7 are agonists or co-agonists on one or two of the GLP-1 receptor, GIP receptor, and amylin receptor (hAMYR3).

10 According to Table 7a, the activity data for reference compound 2 show that linking the C-terminus of a potent GLP-1/GIP co-agonist (tirzepatide), via a peptide linker, to the N-terminus of a potent amylin receptor agonist (cagrilintide) does not result in a compound that is equally potent on these three receptors and that can necessarily function as a GLP-1-/GIP-
15 the reference compound 2 with the reference compound 5 (tirzepatide) and reference compound 6 (cagrilintide) illustrates this point. The reference compound 2 significantly loses potency on the GLP-1 receptor and show further some loss of potency on the amylin receptor, when compared to the original compounds, reference compounds 5 (tirzepatide) and 6 (cagrilintide).

The reference compounds 1 to 3 show a functional activation of all three receptors but have all an impaired potency on the GLP-1 receptor, and therefore are unbalanced tri-agonists having a potency ratio (A/B) between 116 and 287.

- 5 *Table 8a: In vitro activity data for the synthesized compound of the invention on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods A) in the absence of HSA*

Compound number	hGLP-1R Potency, no HSA, EC ₅₀ [pM]	hGIPR Potency, no HSA, EC ₅₀ [pM]	hAMYR3 Potency, no HSA, EC ₅₀ [pM]
10	13.83	2.11	6.8
11	10.43	1.69	12.79
12	0.8	2.62	6.23
13	37.92	2.4	2.26
14	2.05	5.84	2.21
15	1.41	3.2	5.01
16	2.74	4.27	5.7
17	2.72	5.04	5.13
18	52.83	7.25	3.93
19	57.91	9.44	4.24
20	40.64	4.82	3.65
21	41.18	8.63	11.98
22	11.33	35.77	10.92
23	15.35	1.2	5.17
24	51.49	2.34	4.24
25	2.87	4.27	3.22
26	20.71	1.75	11.8
27	26.24	2.11	3.82
28	95.98	3.5	2.28
29	27.08	2.62	3.09
30	6.37	6.8	2.28
31	47.78	4.73	2.69
32	27.55	8.09	10.09
33	32.13	3.11	10.26
34	21.71	1.7	6.81
35	23.26	1.97	4.21

36	3.19	1.96	3.84
37	3.94	2.34	2.98
38	11.97	1.37	3.63
39	23.74	1.62	5.75
40	23.28	1.63	5.49
41	40.79	5.02	6.72
42	61.34	8.01	3.42
43	4.31	4.38	3.13
44	3.68	6.3	3.8
45	4.78	2.63	4.69
46	4.59	5.42	4.41
47	3.15	2.02	2.57
48	64.76	7.02	3.2
49	33.26	4.36	1.85
50	16.18	2.36	3.2
51	7.68	8.37	6.19
52	11.03	1.83	2.85
53	23.34	3.25	3.22
54	28.83	5.56	3.02
55	23.02	3.0	2.22
56	18.22	2.65	2.36
57	12.75	1.98	3.0
58	12.73	1.88	2.91
59	50.63	7.72	3.85
60	94.38	6.06	3.85
61	2.79	1.3	2.97
62	4.13	2.26	3.35
63	4.77	1.6	2.86
64	3.71	2.03	1.91
65	4.86	1.89	2.84
66	5.25	2.39	2.32
67	15.4	4.28	3.66
68	12.08	1.83	2.57
69	3.45	1.9	3.43

70	3.48	5.89	4.53
71	3.34	2.77	8.66
72	13.05	2.12	6.57
73	4.55	4.98	1.99
74	29.76	2.51	2.73
75	3.66	2.65	2.49
76	19.9	2.82	3.11
77	19.69	2.53	3.13
78	4.03	3.49	1.83
79	38.68	4.7	3.53
80	24.76	4.01	3.23
81	16.0	3.31	4.07
82	3.45	3.7	3.26
83	2.3	2.92	2.9
84	3.31	3.0	4.41
85	3.52	2.71	4.4
86	6.69	5.11	3.31
87	8.49	7.26	4.99
88	4.12	5.68	3.7
89	3.86	6.47	3.12
90	4.16	5.4	5.45
91	3.79	3.21	2.26
92	2.55	3.12	2.64
93	4.11	2.54	2.23
94	16.08	2.9	3.91
95	17.85	3.13	3.76
96	16.83	3.62	3.73
97	16.06	2.3	2.81
98	13.26	2.58	2.3
99	16.73	2.09	2.82
100	5.85	3.95	3.05
101	3.24	1.87	3.08
102	10.97	2.24	3.26
103	5.24	3.14	2.42

104	4.16	3.57	1.49
105	12.57	3.08	2.88
106	3.33	4.8	2.07
107	4.1	3.87	3.07
108	4.57	4.01	2.48
109	68.94	7.42	3.65
110	107.07	8.01	3.27
111	15.88	3.66	3.33
112	4.37	2.42	3.65
113	14.43	2.02	2.53
114	43.29	4.29	2.27

- 5 *Table 8b: In vitro activity data for the synthesized compound of the invention on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods B) in the absence of HSA*

Compound number	hGLP-1R Potency, no HSA, EC ₅₀ [pM]	hGIPR Potency, no HSA, EC ₅₀ [pM]	hAMYR3 Potency, no HSA, EC ₅₀ [pM]
115	19.4	10.3	7.13
120	25.3	2.9	6.31
121	9.5	2.95	7.63
122	21.0	3.73	7.68
123	19.9	3.69	7.89
124	21.3	3.4	15.2
125	23.8	3.06	6.26
126	21.7	3.38	11.
127	5.28	3.15	12.4
128	43.9	6.31	7.5
129	25.	3.66	5.46
130	21.2	4.05	7.24
131	29.6	4.24	5.44
132	29.8	3.69	5.29
133	21.4	3.67	8.78
134	27.9	3.88	7.33

135	33.6	4.86	8.3
136	34.3	4.64	10.2
137	28.3	3.71	5.1
138	19.7	2.3	5.5
139	19.0	2.1	7.1
140	28.0	3.3	10.2
141	24.2	2.53	7.64
142	26.9	3.9	6.43
143	28.8	3.89	6.16
144	20.8	2.72	8.07
145	25.0	4.15	10.2
146	34.0	2.93	6.83
147	33.6	3.06	7.12
148	25.7	2.35	6.56
149	30.8	3.14	8.86
150	26.2	2.99	7.61
151	27.7	3.99	9.67
152	22.4	3.83	8.5
153	21.2	2.25	7.54
154	33.8	3.42	8.11
155	10.0	4.56	11.1
156	9.03	6.59	11.8
157	8.48	5.6	9.6
158	11.2	3.6	11.8
159	26.2	3.16	7.42
160	17.6	2.2	8.2
161	19.3	2.6	9.7
162	15.1	1.79	10.4
163	15.7	1.63	20.4
164	14.4	1.93	10.5
165	25.1	2.39	9.4
166	15.0	1.76	9.12
167	23.0	2.0	9.89
168	25.8	2.31	6.59

169	13.1	1.9	15.0
170	15.2	1.8	6.9
171	10.7	2.1	11.0
172	12.8	2.1	20.8
173	11.8	1.8	22.2
174	14.0	2.1	72.0
175	14.3	2.3	34.3
176	29.9	3.66	11.2
177	35.9	4.13	8.37
178	27.0	3.59	12.9
179	23.3	4.36	10.0
180	13.3	2.2	9.93
181	28.8	3.25	8.46
182	21.0	3.2	17.2
183	14.7	2.0	6.2
184	13.3	1.72	7.57
185	15.2	2.2	11.2
186	14.1	2.14	16.5
187	31.5	4.73	17.2
188	20.2	3.52	31.9
189	26.9	3.18	12.7
190	6.0	2.1	10.6
191	7.9	2.7	16.0
192	35.0	3.68	8.17
193	49.0	3.74	8.67
194	303.0	2.89	11.0
195	244.0	3.58	14.0
196	42.5	4.56	12.8
197	18.3	3.92	8.16
210	16.4	9.57	7.28
211	15.2	1.83	3.8
212	12.7	1.92	3.6
213	11.1	1.9	3.6
214	12.2	1.64	3.8

215	11.6	1.7	3.8
216	9.9	1.7	4.5
217	9.0	1.7	5.9
218	8.8	1.6	5.4
219	2.7	0.5	5.0
220	12.5	2.22	7.05
221	17.0	2.39	6.76

The results in Table 8a/b show that the compounds of the present invention display potent functional activation of all three receptors, namely the human GLP-1 receptor, human GIP receptor, and human amylin receptor (hAMYR3).

5 Most of the GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention agonize the different receptors with EC₅₀ values comparable to the GIP, GLP-1 and amylin receptor agonists and GLP-1/GIP co-agonists disclosed herein as reference compounds 4 to 7. Furthermore, the GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention, as shown in Table 8a/b, display a potent and balanced functional activation of all three
10 receptors, and are, in contrast to the reference compounds 1, 2 and 3, balanced GLP-1-/GIP-/amylin-receptor tri-agonists.

15 *Table 9a: In vitro activity data for reference compounds on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods A) in the presence of 1% HSA*

Reference compound number	hGLP-1R Potency, 1% HSA, EC ₅₀ [pM]	hGIPR Potency, 1% HSA, EC ₅₀ [pM]	hAMYR3 Potency, 1% HSA, EC ₅₀ [pM]
4	nd	78.27	nd
6	nd	nd	51.8
7	138.18	nd	nd

Table 9b: In vitro activity data for reference compounds on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods B) in the presence of 1% HSA

Reference compound number	hGLP-1R Potency, 1% HSA, EC ₅₀ [pM]	hGIPR Potency, 1% HSA, EC ₅₀ [pM]	hAMYR3 Potency, 1% HSA, EC ₅₀ [pM]
4	nd	80.21	nd
6	nd	nd	84.41
7	138.82	nd	nd

- 5 Table 10a In vitro activity data for the synthesized compounds of the invention on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods A) in the presence of 1% HSA

Compound number	hGLP-1R Potency, 1% HSA, EC ₅₀ [pM]	hGIPR Potency, 1% HSA, EC ₅₀ [pM]	hAMYR3 Potency, 1% HSA, EC ₅₀ [pM]
10	245.54	42.37	174.93
11	204.33	58.3	211.21
12	10.22	59.11	55.04
14	75.92	254.17	33.38
15	102.87	356.21	82.21
16	238.41	485.28	165.52
17	13.14	36.93	33.58
26	1675.65	557.59	349.42
36	207.7	226.46	136.95
37	188.46	132.8	75.7
38	2347.55	335.89	171.67
39	1925.86	352.39	140.43
43	41.47	186.48	56.46
45	111.93	131.5	62.4
47	67.45	162.38	55.79
48	105.34	54.01	78.51
49	110.45	16.72	106.32
50	66.29	15.09	185.14
51	32.25	61.85	57.15
52	26.88	6.79	118.58
53	79.17	14.55	75.87

55	45.28	10.29	34.01
56	58.42	8.48	44.89
57	29.55	5.85	84.99
58	25.6	7.58	134.14
59	39.75	27.18	24.17
60	172.53	28.33	191.08
61	14.69	15.96	30.75
62	11.51	47.16	28.78
63	100.94	63.21	70.25
64	67.85	100.01	108.96
65	100.45	118.01	127.16
67	105.24	37.02	190.49
68	51.87	11.91	229.55
69	20.35	8.05	78.88
70	18.41	19.14	59.36
71	18.43	9.28	274.0
72	41.74	9.79	589.32
73	54.08	96.54	61.51
74	140.97	18.71	134.37
75	25.19	11.87	109.5
76	63.59	11.1	429.53
77	51.35	13.36	381.81
78	17.79	10.4	17.14
79	168.07	24.71	53.44
80	111.24	20.25	62.37
81	51.37	15.81	187.55
82	24.2	19.05	37.97
83	9.89	13.6	45.74
84	25.9	11.64	175.37
85	30.32	14.64	249.74
86	129.61	122.39	58.35
87	39.32	38.57	36.55
88	48.76	40.38	101.5
89	29.46	30.61	39.66

90	38.6	32.54	182.87
91	17.44	9.31	28.77
92	13.37	8.53	75.11
93	16.44	8.58	66.39
94	130.12	29.54	377.38
95	146.48	22.83	233.45
96	162.02	29.95	594.92
97	108.91	8.38	131.5
98	43.14	11.18	107.95
99	36.16	7.41	128.85
100	306.66	217.28	210.38
101	179.06	128.91	95.53
102	46.99	12.19	134.0
103	33.14	13.31	73.6
104	19.09	17.29	293.01
105	48.9	12.5	113.18
106	11.07	10.27	31.24
107	13.92	8.76	93.1
108	15.0	11.45	59.95
109	94.68	17.44	31.96
110	169.38	29.31	93.98
111	34.79	12.22	66.36
112	335.79	321.43	268.55
113	57.52	8.96	136.32
114	126.11	17.18	45.2

Table 10b: In vitro activity data for the synthesized compounds of the invention on hGLP-1R, hGIPR, and hAMYR3 measured (according to methods B) in the presence of 1% HSA

Compound number	hGLP-1R Potency, 1% HSA, EC ₅₀ [pM]	hGIPR Potency, 1% HSA, EC ₅₀ [pM]	hAMYR3 Potency, 1% HSA, EC ₅₀ [pM]
115	92.9	38.92	551.2
120	128.7	20.0	774.1
121	47.41	15.98	472.3

122	1120	116.0	147.0
123	1190	89.5	90.3
124	1120	152.0	228.5
125	89.3	12.62	555.0
126	865.0	102.0	129.7
127	276.7	98.45	88.0
128	165.0	17.0	429.0
129	998.0	72.6	62.6
130	1140	99.1	220.0
131	225.0	27.2	949.0
132	64.9	12.7	568.0
133	105.0	12.7	917.0
134	220.0	34.7	1200
135	123.0	18.6	568.0
136	120.8	20.53	629.0
137	123.0	13.0	704.0
138	56.2	8.0	555.0
139	59.7	6.0	489.0
140	200.0	19.6	878.0
141	119.1	11.69	771.3
142	102.0	9.99	453.0
143	93.5	12.7	483.0
144	76.07	8.05	778.8
145	111.0	15.2	6130
146	92.5	12.9	842.0
147	83.0	11.0	837.3
148	72.71	7.979	727.8
149	88.4	16.3	1060
150	65.3	10.7	961.0
151	72.6	13.9	949.5
152	67.4	11.4	913.8
153	66.49	10.48	912.9
154	80.6	14.2	1145
155	55.6	14.0	652.0

156	91.8	26.5	876.0
157	77.4	16.3	636.0
158	112.0	19.6	638.0
159	103.0	14.1	716.0
160	55.9	8.7	715.0
161	54.2	7.7	1150
162	61.2	6.00	988.0
163	68.12	7.43	2230
164	60.27	7.59	1180
165	88.79	7.64	537.0
166	54.78	6.19	1296
167	66.49	5.85	1341
168	63.0	6.42	914.9
169	517.0	74.1	184.0
170	685.0	36.8	127.0
171	468.0	39.0	110.0
172	578.0	85.2	353.0
173	750.0	63.8	290.0
174	602.0	93.2	1650
175	731.0	82.3	407.0
176	178.0	23.8	798.0
177	181.0	18.7	779.0
178	173.0	17.0	1480
179	170.0	25.5	1350
180	80.49	11.9	1103
181	134.5	20.1	1300
182	139.4	18.64	2743
183	744.4	71.41	108.1
184	718.1	61.57	122.6
185	954.2	72.34	192.0
186	829.7	85.46	208.0
187	1110	123.0	476.0
188	1154	153.8	1036
189	81.9	9.18	1210

190	21.8	3.9	386.0
191	26.0	6.4	815.0
192	55.7	8.22	291.0
193	110.0	12.0	275.0
194	763.0	15.7	1660
195	528.0	9.52	1690
196	201.0	21.6	641.0
197	148.0	18.4	882.0

Example 5: Experimental protocol for efficacy testing on appetite using an ad libitum fed rat model

- Male Sprague Dawley (SD) rats from Taconic, Denmark were used for the acute food intake experiments, wherein the principles of laboratory animal care were followed.
- The rats were of normal weight of 250-350 g at start of experiment. The rats arrived at least 10-14 days before the start of the experiment to allow acclimatization to experimental settings. During this period, the animals were handled (fixation by restrain in neck skin) at least 2 times. Immediately upon arrival, rats were changed to a reversed light cycle (dark from 11am – 11pm) and were transferred to an automated food intake measuring system (HM2 system, MBRose; Faaborg, Denmark). Rats had ad libitum access to chow (Altromin cat. No. 1324, Brogaarden, Lyngø, Denmark) and water, and were housed at room temperature (~22°C). To enable recording of individual food intake, rats were ID chipped. Three rats were housed in each cage. During the acclimatization period, in which the rats get used to the new light cycle and diet (LF 10% (D12450B), from Research Diets Inc.), the animals had free access to food and water. Since rats are normally active and consume most of their daily calories during the dark period, rats were dosed in the morning right before lights were turned off. Such a set-up results in the lowest data variation and highest test sensitivity. Each dose of tri-agonist was tested in a group of 5-8 rats. A vehicle group of 6-8 rats was included in each set of testing. In each cage there were animals from three different treatment groups (to eliminate potential cage effects (e.g. cage malfunction) on primary readout: food intake). The rats were dosed once subcutaneously (s.c.) with the peptide of interest according to body weight (10 or 30 nmol/kg) in vehicle (0.5 ml/kg) using a NovoPen® (Novo Nordisk, Bagsværd, Denmark).
- The compounds of the invention were formulated (20 or 60 nmol/ml) in the following vehicle: 8 mM phosphate; 250 mM glycerol; 0.007 % polysorbate 20, pH 7.4.

After dosing, the rats were returned to their home cages, where they continued to have ad libitum access to chow and water. The food consumption was recorded individually and continuously by the HM2 system from 0-72 h after test compound administration. Data obtained by the HM-2 system were stored in a HMBase SQL database (Firebird® relational database management system) and were processed by HM2Lab software (MBRose; Faaborg, Denmark) installed in an embedded computer. The feeding system is a highly sensitive system with a load resolution of 0.001 g. In addition to recording food intake, the system records number of feeding events, which is defined as a 0.001 g reduction of food within 5s (Detailed information's of the system can be found in Rathod, Y.D., and Di Fulvio, M. (2021). The feeding microstructure of male and female mice. PLoS One 16, e0246569.)

At the end of the experimental session, the animals were euthanized.

Table 11 shows acute food intake in normal weight (lean) rats based on above-described protocol for efficacy testing on appetite. The results allowed assessment of *in vivo* effect on food intake and provided an indication of the compounds' duration of action. Data are expressed as average percent inhibition relative to average food intake in vehicle group at each study day (day 1 [0-24 hours], day 2 [24-48 hours] and day 3 [48-72 hours]) and food intake was performed in rats up to 72 h. Food intake at each study day (e.g. day 1) means cumulative food intake during, i.e. over the course of this day (i.e. the 24 hours period).

Table 11: Results for acute food intake in normal weight rats after a single dose of tri-agonist

Compound number	Food intake (relative to vehicle in [%])					
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	10 nmol/kg			30 nmol/kg		
10	-20*	-4	-4			
11				-46	-45	-27
12	-70	-39	-8			
14	-78	-85	-32			
15	-30	-42	-9			
16	-17	-11	-10	-39	-22	-17
17	-84	-78	-11			
26				-11	-18	0
35				-42	-18	-16
36	-44	-45	-3			
37	-66	-80	-24			
38	-3	-19	1			
50	-54	-60	-21			
52	-80	-93	-35			
53	-75	-90	-33			
55	-85	-78	-27			

56	-69	-77	-10			
57	-75	-88	-14			
58	-71	-90	-18			
61	-86	-92	-34			
62	-74	-42	13			
63	-79	-80	-17			
68	-65	-72	-35			
69	-81	-68	-6			
77	-54	-89	-54			
78	-96	-57	-3			
79	-70	-54	-5			
80	-84	-78	-23			
82	-94	-73	-27			
87	-91	-86	-24			
89	-93	-78	-28			
91	-96	-84	-33			
93	-94	-61	-25			
97	-65	-82	-42			
99	-69	-84	-46			
100	-42	-64	-40			
101	-76	-92	-63			
109	-85	-34	-28			
110	-75	-54	-23			
115	-44.5	-76.5	-38			
120	-42	-57	-13			
121	-51	-85	-52			
122	-44	-44	-11			
124	-24	-35	4			
125	-53	-58	-10			
126	-23	-37	6			
129	-68	-67	-32			
130	-61	-58	1			
131	-27	-56	-8			
132	-36	-67	-16			
133	-27	-35	-8			
134	-10	-25	0			
136	-12	-19	27			
137	-24	-49	4			
144	-38	-6	-11			
145	-31	-52	-14			
146	-55	-66	-23			
147	-32	-62	-2			
148	-34	-38	-5			
152	-26	-30	10			
176	-12	2	7			

* "-20" means that food intake is reduced by 20 % when compared to vehicle. In general, "-X" means that food intake is reduced by "X %" when compared to vehicle.

Following dosing of the GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention to rats, it was observed that many of them induced profound food intake inhibition, compared to vehicle treatment, as can be deduced from the data presented in Table 11. In general, the compounds in Table 11 showed similar or improved reduction in food intake compared to reference compound 1 as disclosed in WO 2023/288313 at a much lower dose than the disclosed dosage level of 50 nmol/kg of reference compound 1.

Example 6: Pharmacokinetic study in minipigs

The purpose of this study is to determine the half-life ($t_{1/2}$) *in vivo* of the inventive GLP-1-/GIP-/amylin-receptor tri-agonists after intravenous (i.v.) administration to minipigs, i.e., the residence time in the body and thereby their time of action. This is done in a pharmacokinetic (PK) study, where the terminal half-life ($t_{1/2}$) of the compound or derivative in question is determined. By terminal half-life is meant the time it takes to halve a certain plasma concentration in the terminal elimination phase.

Study

Female Göttingen minipigs, obtained from Ellegaard Göttingen Minipigs (Dalmose, Denmark), approximately 8-12 months of age and weighing approximately 20-30 kg were used in the studies. The minipigs were housed individually (pigs with permanent catheters) in pens with straw as bedding and fed restrictedly once daily with Altromin 9023 minipig diet (Altromin Spezialfutter GmbH & Co. KG).

After three weeks of acclimatisation two permanent central venous catheters were implanted in *vena cava caudalis* in each animal. The animals were allowed at least 10 days recovery after the surgery and were then used for repeated pharmacokinetic studies with a suitable wash-out period between successive dosing.

The compounds of the invention were formulated (40 nmol/ml) in the following vehicle: 8 mM phosphate; 250 mM glycerol; 0.007 % polysorbate 20, pH 7.4.

Intravenous injections (the volume corresponding to 0.05 ml/kg and dose of 2 nmol/kg/derivative) of the derivatives were given through one catheter, and blood was sampled at predefined time points for up till 14 days post dosing (preferably from the other catheter).

Blood samples (for example 1.3 ml) were collected in EDTA (1.3 ml tube containing K₃EDTA to yield 1.6 mg K₃EDTA/ml blood) coated tubes and then centrifuged at 4 °C and 2000 x g for 10 minutes.

Sampling and analysis

Plasma was within 30 min after centrifugation pipetted into Micronic tubes stored on dry ice and were afterwards kept at -20 °C until analysed for plasma concentration of the compounds using LCMS.

5 Plasma concentrations of the peptides of the invention were assayed by plasma protein precipitation and analysed by liquid chromatography mass spectrometry (LC-MS). Calibrators were prepared by spiking blank plasma from minipigs with the compounds in the typical range from 0.05 to 200 nM. LLoQ was typically in the range of 0.2-2 nM. Calibrators, plasma blanks or study samples were prepared for LC-MS by protein precipitation by adding
10 4 volumes of ethanol containing 20 nM of internal standard (structurally similar analogue with different mass) to one volume of sample followed by centrifugation at 6200 rpm at 4 °C for 10 minutes. The supernatant was diluted with 1 volumes of Milli-Q water containing 1% Formic acid before injection on the LC-MS system. Individual plasma concentration-time profiles were analysed by a non-compartmental pharmacokinetic method (NCA) in Phoenix v. 6.4
15 (Pharsight Inc., Mountain View, CA, USA), and the resulting terminal half-lives (harmonic mean) determined. The LC-MS analysis was carried out using a TurboFlow HPLC system from Thermo Fisher Scientific (Bremen, Germany) coupled to either a Q Exactive Orbitrap or Altis Triple Quadrupole (TQ) Mass Spectrometer. The LC mobile phases consisted of A: MQ water with 5% organic solvent (50% methanol / 50% acetonitrile) and 1% formic acid and B:
20 MQ water with 95% organic solvent (50% methanol / 50% acetonitrile) and 1% formic acid. A TurboFlow Cyclone 0,5 x 100 mm column from Thermo Fischer Scientific (Bremen, Germany) was used for extraction, before analytical elution on a XBridge Peptide BEH C18 300Å, 3.5 µm, 2.1x50 mm column for the analysis both operated at 60 °C. Typically 40-45% B and 75-80% B were used for loading and elution on the TurboFlow column, respectively,
25 followed by a linear gradient elution typically from around 45% B to 85% B over 2.33 minutes on the analytical column. The Orbitrap mass spectrometer were operating in positive ionization mode with a spray voltage of 4.0 kV using Parallel Reaction Monitoring scan mode using 5 *m/z* isolation windows on the most abundant charge state of the compounds with a resolution of 35K on the Orbitrap MS. The TQ mass spectrometer were operating in positive
30 ionization mode with a spray voltage of 4.0 kV using Single Reaction Monitoring scan mode using Q1 and Q3 resolutions of 1.2 (FWHM).

For all compounds, individual optimal fragmentation collision energies were found and used. The data was processed using the Quan Browser in the Xcalibur software from Thermo Fisher Scientific (Bremen, Germany) by fitted the data to linear calibration curves
35 (weighed $1/x^2$), used for calculating the concentration in the plasma samples. Quality control

samples were included. The deviation between nominal and calculated concentration in the calibrators and quality control samples were below 15%.

Results:

5 Table 12: Terminal half-life as measured after i.v. administration to minipigs

Terminal half-life as measured after i.v. administration to minipigs	
Compound number	t _{1/2} [hours]
11	119
12	81
15	122
16	138
37	118
52	120
55	88
57	90
58	97
61	61
64	117
68	125
77	140
91	43
93	69
101	122
115	115
120	95
125	100

As shown in Table 12, the tested GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention have very long half-lives (t_{1/2}) of at least 43 hours in minipigs. It is contemplated that, based on these half-lives in minipigs, half-lives in humans will be
10 sufficient for at least once-weekly administration via liquid subcutaneous injection or at least once daily administration via oral tablet.

Example 7: Chemical stability assessment in formulation

The assay was performed to investigate the extend of chemical degradation *in vitro* over time upon incubation at 37 °C for a period of 2 weeks.
15 Peptide solutions were prepared by dissolving freeze-dried powder in 8 mM phosphate buffer pH 7.4 to a target of 1 mg/mL. The pH of peptide solutions was adjusted to 7.4 with 0.02 M HCl or 0.02 M NaOH. Samples were filled in Agilent HPLC vials with fixed

insert. Vials were capped to prevent evaporation. The HPLC vials were incubated at 37 °C and samples were withdrawn at different time points over a period of 2 weeks, flash frozen at -80 °C, and stored at -20 °C until analysis.

- Sample analysis was carried out using UPLC coupled to UV detection at 215 nm and MS (UPLC-UV-MS). One μL of sample was injected into a Waters Acquity UPLC with a flow-through-needle injection system and on to a Waters Acquity CSH C18 column (1*150 mm), with a particle size of 1.7 μm and held at 55 °C. A flow-rate of 100 $\mu\text{L}/\text{min}$ was delivered with a Binary solvent manager pump having 0.1 % formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B. Gradient elution was carried out using 20 % B from 0 to 2 min followed by 20 to 50% B from 2 to 20 min in a total run time of 30 min. The identity of the peptide was confirmed by MS and the peak purity, area%, from the UV signal at 215 nm was plotted against time and the slope from linear regression was used to calculate the purity loss per week (Table 13).

- 15 *Table 13: Chemical stability assessment of peptides in 8 mM phosphate buffer pH 7.4 (1 mg/mL) at 37 °C. Data is given as purity loss in % per week.*

Compound number	Purity loss per week at 37°C [in %]
12	5.6
15	3.8
17	4.2
35	0.3
37	2.3
47	2.6
50	0.7
52	0.9
53	0.8
55	0.9
56	0.6
57	0.2
58	0.3
61	2.0
62	3.0
63	3.7
64	3.3
65	2.1
73	2.8
77	1.2
78	3.5

79	1.3
80	0.8
83	2.3
84	2.9
85	2.0
91	2.8
92	2.8
93	2.8
115	1.1
120	1.2
121	1.5
122	1.3
123	1.0
124	1.5
125	0.5
126	1.2
127	1.7
129	1.7
130	1.6
132	1.3
133	1.4
134	1.4
146	1.4
147	1.0
148	1.4
149	1.1
150	1.6
151	1.3
152	1.3
153	1.4
154	1.4
160	1.2
161	0.9
162	1.0
163	1.4
164	1.2
165	1.0
171	1.0
172	0.9
180	0.7
183	1.3
184	1.0
185	1.7

186	1.0
-----	-----

All GLP-1-/GIP-/amylin-receptor tri-agonists tested in this assay show an acceptable chemical stability with an acceptable rate of degradation (less than 6 % of purity loss per week) in an aqueous buffer (at 37 °C). Most of the tested GLP-1-/GIP-/amylin-receptor tri-agonists show a good chemical stability having less than 3.0 % purity loss per week, or even more an excellent chemical stability (less than 1.5 % purity loss per week). Accordingly, the GLP-1-/GIP-/amylin-receptor tri-agonists of the present invention are considered to be chemically stable in solution.

Example 8: Sub-chronic treatment in diet-induced obese (DIO) rats

The purpose of this example is to assess the *in vivo* effect of selected tri-agonists on pharmacodynamic parameters such as body weight and food intake in diet-induced obese (DIO) rats. The animals were treated once daily via subcutaneous injection with a liquid formulation of the triple agonist to be tested to assess effects on body weight and food intake (daily and cumulative).

Diet-induced obese male rats (Sprague Dawley) were purchased from Charles River (Écully, France). Rats were housed in pairs of two at room temperature with ad libitum access to 45% high fat diet (D12451, Research Diets, Inc. NJ) initially following a 12h:12h light:dark cycle with lights on at 0600h. Two weeks before start of study, rats were switched to reverse light:dark cycle (12:12h) with lights on at 21.00-09.00h. Five days prior to start of treatment, animals were weighted and MRI scanned (EchoMRITM, TX, USA) to obtain body composition data. Rats were divided into seven groups (n=8) matched on body weight and fat mass (P>0.91 between groups for both body weight and fat mass as tested by Oneway ANOVA followed by Tukey Multiple comparison test), ensuring that no cage had two rats from the same group. Group details are shown in Table 14 below. A group of age matched normal weight controls, who had been on standard chow (Altromin 1324, Altromin International) for the entirety of their lives was included as reference. At start of study, average weight of DIO rats was 958±16.4 g (mean±SEM). Average weight of normal weight controls was 796±21.4 g (mean±SEM). Body weight and daily food intake was collected by manual weighing (0-1h before onset of dark during four days prior to start of treatment (pre-treatment/baseline phase). In continuation to this, rats were mock handed to accustom them to the dosing procedure during treatment (restrain in neck skin). During baseline and treatment phases, food was replaced daily. Treatment phase lasted four weeks (28 days) with daily subcutaneous injection (QD, s.c.) at a volume of 0.5 ml/kg.

The compounds of the invention were formulated (0.6 nmol/ml, 2 nmol/ml, 6 nmol/ml, 12 nmol/ml, and 20 nmol/ml, depending on administered dose) in the following vehicle: 8 mM phosphate; 250 mM glycerol; 0.007 % polysorbate 20, pH 7.4. Rats were dosed with a NovoPen® (Novo Nordisk, Bagsværd, Denmark) 0-1h before start of dark phase immediately after weighing. The two test compounds were titrated in small increments towards maintenance doses of either 3 nmol/kg or 10 nmol/kg as shown in Table 14.

Table 14: Dose titration schedule for DIO rat study described in Table 15 (Dosing in nmol/kg body weight)

Compound	n	Day 1+2	Day 3+4	Day 5+6	Day 7+8	Day 9-28
Vehicle	8	0	0	0	0	0
Compound 52, 3 nmol/kg	8	0.3	1	3	3	3
Compound 52, 10 nmol/kg	8	0.3	1	3	6	10
Compound 77, 3 nmol/kg	8	0.3	1	3	3	3
Compound 77 10 nmol/kg	8	0.3	1	3	6	10

The results of this study are shown in Fig. 1, Fig. 2, and Fig. 3, and in Table 15. Table 15 shows the effects on cumulative food intake, absolute body weight and relative body weight of DIO rats treated daily for up to 28 days with vehicle and compound 52 or 77 of the present invention (at 3 nmol/kg and 10 nmol/kg, respectively) following the dose titration schedule shown in Table 14.

Table 15: Effects on food intake and body weight in DIO rats treated daily for up to 28 days with vehicle, compound 52 or compound 77 following the dose titration schedule shown in Table 14

Compound	Cumulative food intake in [kcal]	Absolute body weight in [g]		body weight in [%] at day 28 compared to day 0
		Day 0	Day 28	Day 28
Vehicle, DIO	2688 ± 109.4	968 ± 61.6	969 ± 56.9	100 ± 1.04
77 (3 nmol/kg)	1795 ± 116.9	962 ± 45.5	831 ± 47.8	86.2 ± 1.27
77 (10 nmol/kg)	1193 ± 75.60	954 ± 37.1	765 ± 37.9	80.0 ± 1.35
52 (3 nmol/kg)	1696 ± 92.90	949 ± 32.7	836 ± 32.3	87.8 ± 0.61
52 (10 nmol/kg)	1028 ± 99.45	940 ± 32.6	742 ± 29.1	78.9 ± 0.97

Results are expressed as means ± SEM, n=6-8.

The second column in Table 15 shows cumulative food intake from treatment day 0-28 (in kcal). The DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14. Data are shown as means $\pm$ SEM, n = 6-8. These results are also shown in figure 3. It can be seen that treatment with the GLP-1-/GIP-/amylin-receptor triple agonist compounds 52 and 77 induced a reduction in food intake.

The third column in Table 15 shows absolute body weight (in g) of the rats, dosed with vehicle, compound 52 or compound 77 (at 3 nmol/kg and 10 nmol/kg, respectively) at day 0 and day 28 of the treatment period. The DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14. Data are shown as means $\pm$ SEM, n = 6-8. The last column in Table 15 shows body weight relative to day 0 in percent (%body weight) in DIO rats during the treatment period of 28 days with compounds 52 and 77 (at 3 nmol/kg and 10 nmol/kg, respectively). For example, a relative body weight of 80 % at day 28 means that the rat possesses only 80% of its body weight at day 0, or in other words, the rat has lost 20 % of its initial body weight (at day 0). The DIO rats received a subcutaneous dose once daily following the titration schedule described in Table 14. Data are shown as means $\pm$ SEM, n = 6-8. These results are also shown in figure 1. It can be seen that treatment with the GLP-1-/GIP-/amylin-receptor triple agonist compounds 52 and 77 induced a reduction in body weight in absolute and relative manner.

From Table 15 it is seen that treatment with the GLP-1-/GIP-/amylin-receptor triple agonist compounds 52 and 77 induced at all concentrations a reduction in food intake that resulted in body weight loss. The treatment with the GLP-1-/GIP-/amylin-receptor triple agonist compound 52 and compound 77 with same concentration (3 nmol/kg or 10 nmol/kg) induced a comparable reduction in food intake that resulted in a comparable body weight loss. The effects of reduction in food intake and body weight loss were concentration dependent, with a lower reduction in food intake and body weight loss at 3 nmol/kg and a higher reduction in food intake and body weight loss at 10 nmol/kg.

This invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary without departing from the scope of the present disclosure. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the above, the elements of the present invention were described. These elements were listed with specific embodiments. However, it should be understood that said embodiments may be combined in any manner and in any number to create additional embodiments, which all fall within the scope of the present disclosure. The various described
5 examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered
10 disclosed by the description of the present application unless the context indicates otherwise.

Documents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such
15 disclosure by virtue of prior invention.

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 claims are intended to cover all such modifications and changes as fall within the true spirit of the
20 invention.

CLAIMS

1. A GLP-1-/GIP-/amylin-receptor tri-agonist comprising a peptide according to Formula I:

5 Z1—L1—Z2 (I),

comprising one lysine (Lys, K) residue; wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

10 YX₂EGTFTSDYSILLEEQAAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,
and

Z1 comprises an amino acid sequence according to Formula X (SEQ ID NO: 161):

15 X₂₁X₂₂X₂₃GTFTSDYSX₂₄LLEEX₂₅AAX₂₆EFIX₂₇WLX₂₈X₂₉GGPSX₃₀X₃₁ (X),

wherein

X₂₁ represents His (H) or Tyr (Y),

X₂₂ represents Aib,

X₂₃ represents Glu (E) or His (H),

X₂₄ represents Ile (I) or Lys (K),

20 X₂₅ represents Gln (Q) or Ile (I),

X₂₆ represents Arg (R) or Gln (Q),

X₂₇ represents Ala (A), Glu (E) or Gln (Q),

X₂₈ represents Leu (L) or I (Ile),

X₂₉ represents Ala (A) or Gln (Q),

25 X₃₀ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₃₁ represents Gly (G), Glu (E) or Lys (K);

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

30 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula XII (SEQ ID NO:164):

35 AX₃₂X₃₃LSTAX₃₄X₃₅X₃₆RLSAX₃₇LHX₃₈LX₃₉X₄₀X₄₁PX₄₂TETGSGX₄₃P (XII),

wherein

- X₃₂ represents Gly (G) or Ser (S),
 X₃₃ represents Gln (Q), Glu (E), His (H) or Lys (K),
 X₃₄ represents Ala (A) or Gln (Q),
 X₃₅ represents Gln (Q), Leu (L) or Thr (T),
 5 X₃₆ represents Ala (A), Gly (G) or Gln (Q),
 X₃₇ represents Glu (E) or Lys (K),
 X₃₈ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),
 X₃₉ represents Ala (A) or Lys (K),
 10 X₄₀ represents Asp (D) or Thr (T),
 X₄₁ represents Leu (L) or Glu (E),
 X₄₂ represents Arg (R) or Lys (K),
 X₄₃ represents Ala (A) or Ser (S).

15 2. The GLP-1-/GIP-/amylin-receptor tri-agonist according to claim 1, wherein:

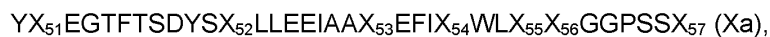
- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):



wherein the amino acid at position X₂ represents Aib,

20 and

Z1 comprises an amino acid sequence according to Formula Xa (SEQ ID NO: 162):



wherein

- 25 X₅₁ represents Aib,
 X₅₂ represents Ile (I) or Lys (K),
 X₅₃ represents Arg (R) or Gln (Q),
 X₅₄ represents Ala (A), Glu (E) or Gln (Q),
 X₅₅ represents Leu (L) or I (Ile),
 30 X₅₆ represents Ala (A) or Gln (Q),
 X₅₇ represents Gly (G) or Glu (E);

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula XIIa (SEQ ID NO: 165):

5 AX₅₈X₅₉LSTAX₆₀X₆₁X₆₂RLSAELHX₆₃LATX₆₄PRTETGSGSP (XIIa),

wherein

X₅₈ represents Gly (G) or Ser (S),

X₅₉ represents Gln (Q), Glu (E), or His (H),

X₆₀ represents Ala (A) or Gln (Q),

10 X₆₁ represents Leu (L) or Thr (T),

X₆₂ represents Ala (A), Gly (G) or Gln (Q),

X₆₃ represents Gln (Q), Glu (E), or Lys (K),

X₆₄ represents Leu (L) or Glu (E).

15 3. The GLP-1-/GIP-/amylin-receptor tri-agonist according to claim 1, wherein:

- Z1 is a peptide having a maximum of 4 amino acid substitutions relative to Formula II (SEQ ID NO: 1):

YX₂EGTFTSDYSILLEEQAAAREFIEWLLAGGPSKG (II),

wherein the amino acid at position X₂ represents Aib,

20 and

Z1 comprises an amino acid sequence according to Formula IIa (SEQ ID NO: 3):

X₁X₂X₃GTFTSDYSILLEEQAAAREFIEWLLAGGPSX₄X₅ (IIa),

wherein

X₁ represents His (H) or Tyr (Y),

25 X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G), Lys (K) or Ser (S),

X₅ represents Gly (G) or Lys (K);

- L1 is a peptide linker; and
- Z2 is a peptide comprising a C-terminal amide, and having a maximum of 10 amino acid substitutions relative to Formula III (SEQ ID NO: 2):

30 ASELSTAALGRLSAELHELATLPRTETGSGSP (III),

and

Z2 comprises an amino acid sequence according to Formula IIIa (SEQ ID NO: 4):

35

ASX₆LSTAX₇X₈X₉RLSAX₁₀LHX₁₁LX₁₂X₁₃LPX₁₄TETGSGX₁₅P (IIIa),

wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

X₇ represents Ala (A) or Gln (Q),

5 X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

10 X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

X₁₅ represents Ala (A) or Ser (S).

15 4. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 1 to 3, wherein the peptide linker L1 comprises 1 to 14, 1 to 10, 4 to 10 or 9 to 10 amino acid residues.

20 5. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 1 to 4, wherein the peptide linker L1 comprises the amino acid sequence according to Formula IV:

X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄ (IV),

wherein

X₁ represents Ala (A), Glu (E), Gly (G),

25 X₂ represents Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P) or is absent,

X₃ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or absent,

X₄ represents Ala (A), Gln (Q), Glu (E), Gly (G), Pro (P) or absent,

X₅ represents Glu (E), Gly (G), Pro (P), Ser (S), Thr (T) or is absent,

X₆ represents Glu (E), Gly (G), Leu (L), Gln (Q) or is absent,

30 X₇ represents Ala (A), Gln (Q), Glu (E), Gly (G), Phe (F) or is absent,

X₈ represents Ala (A), Gln (Q), Glu (E), Gly (G), Thr (T), Pro (P), Val (V) or is absent,

X₉ represents Glu (E), Asn (N), Pro (P), Thr (T) or is absent,

X₁₀ represents Ala (A), Gln (Q), Glu (E), Gly (G), Leu (L), Pro (P), Ser (S), Val (V) or is absent,

35 X₁₁ represents Ala (A) or is absent,

X₁₂ represents Gln (Q) or is absent,

X₁₃ represents Thr (T) or is absent,

X₁₄ represents Leu (L) or is absent.

- 5 6. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 3 to 5,
wherein the peptide Z1 comprises the amino acid sequence according to Formula IIa
(SEQ ID NO: 3):



wherein

10 X₁ represents His (H) or Tyr (Y),

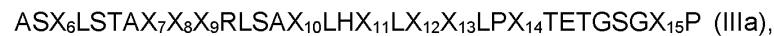
X₂ represents Aib,

X₃ represents Glu (E) or His (H),

X₄ represents Arg (R), Gly (G) or Ser (S),

X₅ represents Gly (G); and

- 15 wherein the peptide Z2 comprises the amino acid sequence according to Formula IIIa
(SEQ ID NO: 4):



wherein

X₆ represents Gln (Q), Glu (E), His (H) or Lys (K),

20 X₇ represents Ala (A) or Gln (Q),

X₈ represents Gln (Q), Leu (L) or Thr (T),

X₉ represents Ala (A), Gly (G) or Gln (Q),

X₁₀ represents Glu (E) or Lys (K),

X₁₁ represents Arg (R), Gln (Q), Glu (E), Gly (G), His (H), Lys (K), Thr (T) or Tyr (Y),

25 X₁₂ represents Ala (A) or Lys (K),

X₁₃ represents Asp (D) or Thr (T),

X₁₄ represents Arg (R) or Lys (K),

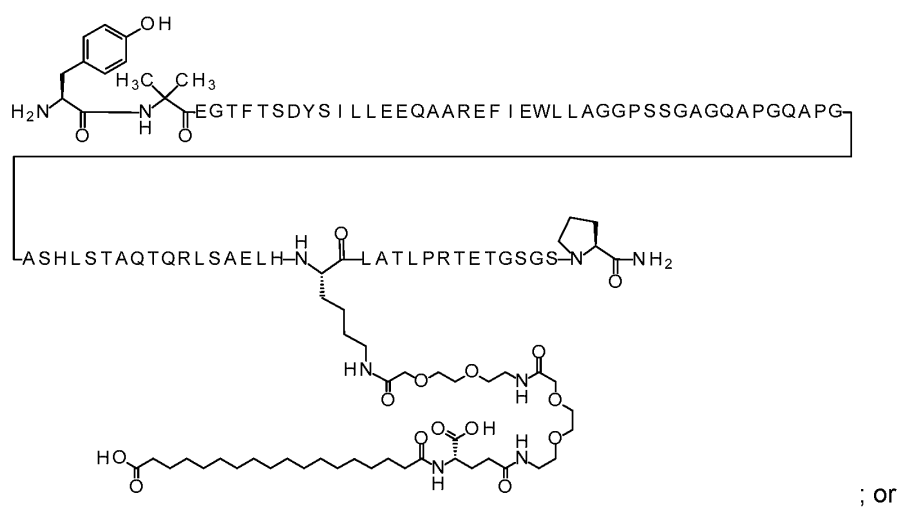
X₁₅ represents Ala (A) or Ser (S).

- 30 7. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 1 to 6,
wherein the peptide comprises an amino acid sequence selected from the group
consisting of SEQ ID NOs 20 to 124 and 170 to 242, and wherein X represents Aib.

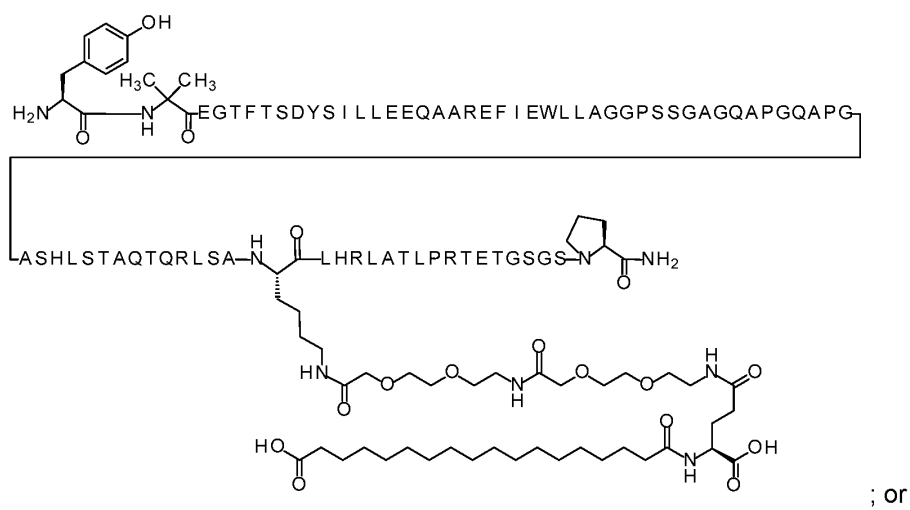
- 35 8. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 3 to 7,
wherein the amino acid sequence of the peptide comprises

YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
 SAELHKLATLPRTETGSGSP (SEQ ID NO: 62), or
 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRL
 SAKLHRLATLPRTETGSGSP (SEQ ID NO: 65), or
 5 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRLS
 AELHKLATLPRTETGSGSP (SEQ ID NO: 68), or
 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRLS
 RTETGSGSP (SEQ ID NO: 78), or
 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPLASHLSTAQTQRLS
 10 AELHKLATLPRTETGSGSP (SEQ ID NO: 87), or
 HXHGFTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTARLSA
 ELHQLATLPRTETGSGSP (SEQ ID NO: 111); or
 YXEGTFTSDYSILLEEQAAREFIEWLLAGGPSSGAGQAPGQAPGASHLSTAQTQRLS
 AELHKLATLPRTETGSGSP (SEQ ID NO: 170);
 15 wherein in each amino acid sequence X represents Aib.

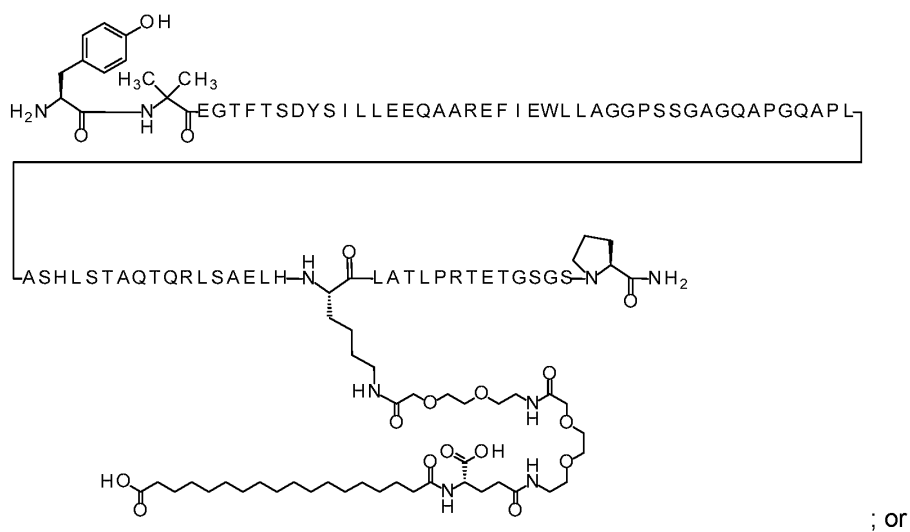
9. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 1 to 8,
 wherein the peptide is a peptide derivative comprising a protraction moiety.
- 20 10. The GLP-1-/GIP-/amylin-receptor tri-agonist according to claim 9, wherein the peptide
 is a peptide derivative comprising a protraction moiety, and wherein said protraction
 moiety comprises a protractor P being a C₁₂-C₂₀ diacid.
- 25 11. The GLP-1-/GIP-/amylin-receptor tri-agonist according to any one of claims 1 to 10,
 wherein the peptide is a peptide derivative comprising a protraction moiety, wherein
 said protraction moiety further comprises a linker L_P.
12. A GLP-1-/GIP-/amylin-receptor tri-agonist, which is
 compound 52



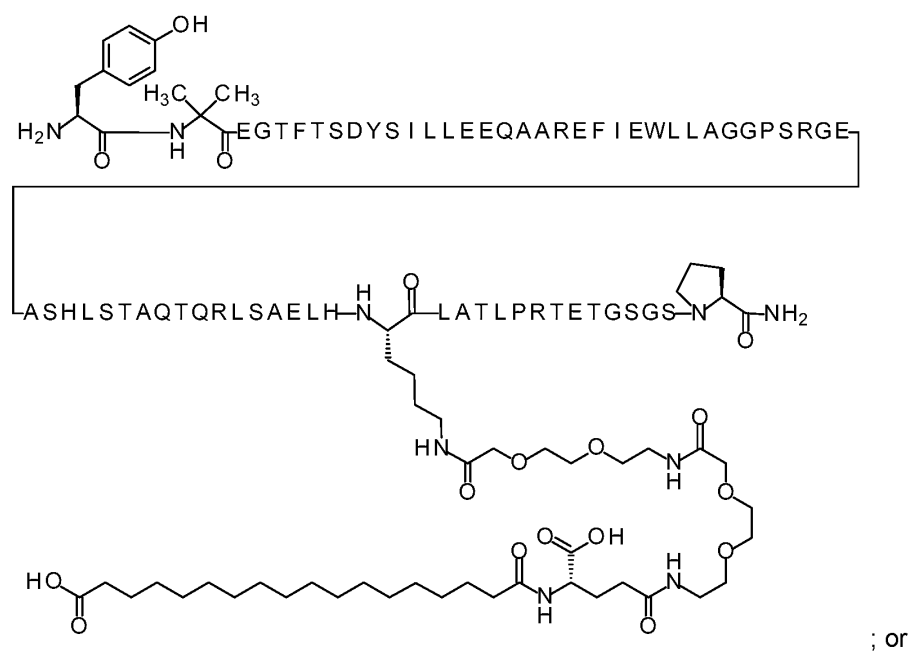
compound 55



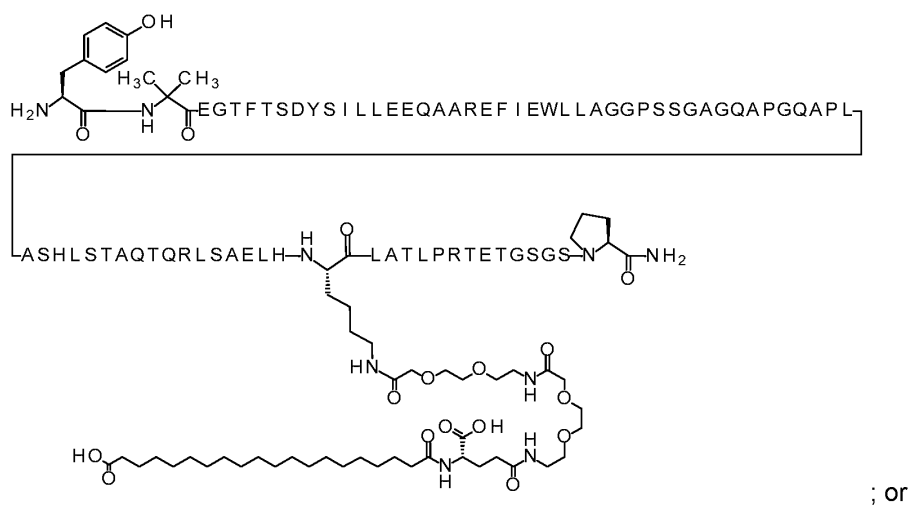
compound 58



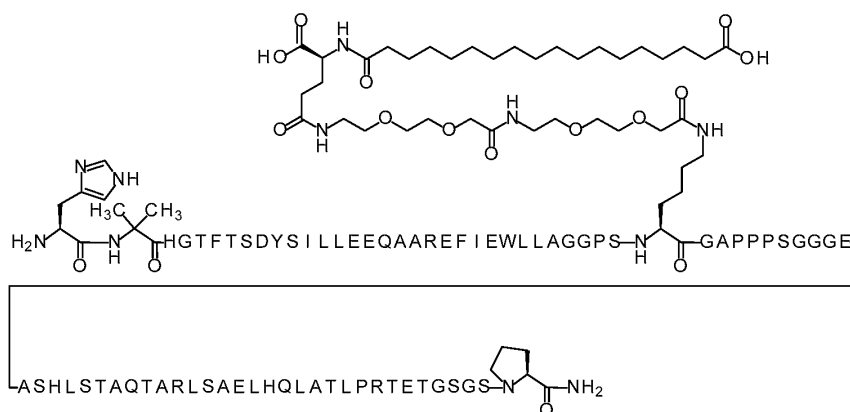
compound 68



compound 77



compound 101



5

13. A pharmaceutical composition comprising a GLP-1-/GIP-/amylin-receptor triple agonist according to any one of claims 1 to 12, and one or more pharmaceutically acceptable excipients.
14. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13 for use as a medicament.
15. The GLP-1-/GIP-/amylin-receptor triple agonist according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 13, for use in the treatment of a subject with an initial body mass index (BMI) of 25 or more, 27 or more, or 28 or more.

more, or 30 or more; optionally in the presence of at least one weight-related co-morbidity.