NPY2 RECEPTOR AGONISTS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to PYY analogues that are neuropeptide Y2 (NPY2) receptor agonists, and to their medical use in the treatment and/or prevention of a variety of diseases, conditions or disorders, such as treatment and/or prevention of excess food intake, excess body weight, obesity, metabolic diseases, and other conditions or disorders related to excess body weight or obesity, e.g. diabetes and cardiovascular diseases.

BACKGROUND INFORMATION

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. In this regard, overweight and obesity are major risk factors for a number of chronic diseases, including type 2 diabetes, cardiovascular diseases and cancer. According to the WHO overweight and obesity are no longer considered a problem limited to high income countries but are now dramatically on the rise in low- and middle-income countries. WHO´s Global Health Observatory indicate that, in 2016, 39% of women or men aged 18 and over were overweight and 11% of men and 15% of women were obese.

Despite long-standing efforts, the number of overweight and obese patients is still growing. First line therapy for overweight and obese patients comprise diet and exercise but often are not sufficiently efficacious. Second line treatment options are bariatric surgery and pharmacotherapy. Available pharmacological treatments seem to lack in efficacy and/or safety, and only a limited number of approved therapies are available in the US and in Europe.

Therefore, there is still a high medical need for more efficacious and safe treatment options.

NPY (Neuropeptide Y; SEQ ID No:1 – human sequence), PYY (Peptide YY; SEQ ID No: 2 – human sequence), and PP (Pancreatic Polypeptide; SEQ ID No:3 – human sequence) are naturally secreted homologous, 36 amino acid, C-terminally amidated peptides and belong to the PP-fold family of peptides.

Sequence of hPYY (3-36):
Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr (SEQ ID No:4)

PYY is cleaved to PYY(3-36) by dipeptidyl peptidase IV (DPP IV). PYY(3-36) displays increased selectivity for the neuropeptide Y2 receptor over neuropeptide Y1, Y4 and Y5 receptors as compared to PYY(1-36), albeit some Y1 and Y5 affinity is retained. It is believed that PYY or PYY(3-36) exhibits the feeding suppressive action via activation of the neuropeptide Y2 receptor (*Inhibition of Food Intake in Obese Subjects by Peptide YY_{3-36}*, N Engl J Med 2003;349;941-8).

However, PYY and also PYY(3-36) have a short half-life in the body and show undesirable chemical or physical properties, e.g. low stability. Further, the pharmacologic effect, e.g. its efficacy as body weight lowering agent, seems limited.

WO2014/178018 discloses PYY analogues and their ability to reduce food intake in mice. WO2011/033068 and WO2011/058165 disclose long acting Y2 receptor agonists. WO2015/071355, WO2016/198682 and WO2020/092191 relate to PYY compounds, which are selective Y2 receptor agonists. PYY compounds are disclosed comprising a covalently attached substituent or modifying group also referred therein as a protracting moiety.

There is a need in the art for further (long acting) PYY analogues selectively acting on the NPY2 receptor. For example, it would be desirable to increase further the solubility of PYY analogues, preferably to increase the solubility around pH 7 and/or around pH 6. This would increase the formulation options for a ready-to-use application and potentially allow combinations with other (peptide) therapeutics to improve their efficacy.

It has been found that the PYY analogues of the present invention generally are soluble around pH 6 and pH 7.

SUMMARY

The disclosure provides a PYY analogue, wherein the analogue comprises

i) alanine at the position corresponding to position 4 of hPYY(3-36)

ii) lysine at the position corresponding to position 7 of hPYY(3-36)

iii) the sequence QRY at its C-terminal end,

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21, 22 or 30, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at positions 14 or 30.

In some examples, the PYY analogue comprises

i) alanine at the position corresponding to position 4 of hPYY(3-36)

ii) lysine at the position corresponding to position 7 of hPYY(3-36)

iii) glutamate at the position corresponding to position 9 of hPYY(3-36)

iv) tyrosine at the position corresponding to position 20 of hPYY(3-36)

v) tryptophan at the position corresponding to position 30 of hPYY(3-36)

vi) leucine at the position corresponding to position 31 of hPYY(3-36)

vii) the sequence RQRY at its C-terminal end,

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21 or 22, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at position 14.

In some examples, the PYY analogue comprises

i) alanine at the position corresponding to position 4 of hPYY(3-36)

ii) lysine at the position corresponding to position 7 of hPYY(3-36)

iii) glutamate at the position corresponding to position 9 of hPYY(3-36)

iv) tyrosine at the position corresponding to position 20 of hPYY(3-36)

v) the sequence WLTRQRY at its C-terminal end,

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21, or 22, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at positions 14 .

In some examples, the PYY analogue is in accordance to one of the above examples and bears a half-life extending group, which is attached to the epsilon amino group of the lysine at position 7.

In some examples of the present disclosure, the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is an amino acid sequence of formula III:

Ala-Pro-X6-Lys-Pro-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-Val-X23-Leu-Arg-His-Tyr-Tyr-Asn-Trp-Leu-Thr-Arg-Gln-Arg-Tyr (III)

wherein

X6 is selected from the group consisting of Ala and Glu;

X9 is selected from the group consisting of Glu and Gly;

X10 is selected from the group consisting of Ala and Glu;

X11 is selected from the group consisting of Ala, Asp, Glu and Pro;

X12 is selected from the group consisting of Ala and Ser;

X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;

X14 is selected from the group consisting of Ala, Glu and Pro;

X15 is selected from the group consisting of Ala and Glu,

X16 is selected from the group consisting of Ala and Glu;

X17 is selected from the group consisting of Ile, Leu, Thr and Val;

X18 is selected from the group consisting of Glu and Gln;

X19 is selected from the group consisting of Ala, Glu, Arg, Lys, and Gln;

X21 is selected from the group consisting of Glu and Tyr;

X23 is selected from the group consisting of Ala, Glu, Ser and Thr;

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7.

In some examples, the half-life extending group consists of a lipophilic substituent X and a linker U, wherein the linker U is attached to the amino acid side chain and X is attached to U, and the linker U consists of one, two or three sub-moieties (U1, U2, U3).

Thus, in one aspect, the present disclosure provides a PYY analogue comprising an amino acid sequence corresponding to the amino acid sequence of hPYY(3-36), wherein the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is a peptide comprising an amino acid sequence of formula II:

Ala-X5-X6-Lys-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-X22-X23-X24-Arg-X26-Tyr-X28-X29-X30-X31-X32-Arg-Gln-Arg-Tyr (II)

wherein

X5 is selected from the group consisting of Pro and Hyp;

X6 is selected from the group consisting of Ala and Glu;

X8 is selected from the group consisting of Ala, Pro, and Hyp;

X9 is selected from the group consisting of Glu, Gly, Gln and Pro;

X10 is selected from the group consisting of Ala and Glu;

X11 is selected from the group consisting of Ala, Asp, Glu and Pro;

X12 is selected from the group consisting of Ala, Gly, and Ser;

X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;

X14 is selected from the group consisting of Ala, Glu, Pro and Hyp;

X15 is selected from the group consisting of Ala, Glu and Ser;
X16 is selected from the group consisting of Glu and Ser;
X17 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;
X18 is selected from the group consisting of Glu and Gln;
X19 is selected from the group consisting of Ala, Glu, Arg, Lys and Gln
X21 is selected from the group consisting of Ala, Glu, Gln and Tyr;
X22 is selected from the group consisting of Ile, Ser, Thr and Val;
X23 is selected from the group consisting of Ala, Glu, Ser and Thr;
X24 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;
X26 is selected from the group consisting of Ala, His and Lys;
X28 is selected from the group consisting of Trp and Tyr;
X29 is selected from the group consisting of Asn, and Tyr;
X30 is selected from the group consisting of His, Trp, and Tyr;
X31 is selected from the group consisting of Ala, Ile, and Leu;
X32 is selected from the group consisting of Gln, and Thr;

and wherein only one of X6 , X10 and X15 is Ala, and
at least two amino acids from X6, X10, X11, X13 and X23 are selected from the group consisting of Asp and Glu, and
wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7, the half-life extending group consists of a lipophilic substituent X and a linker U, wherein the linker U is attached to the epsilon amino group of lysine and X is attached to U, wherein X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl, and U consists of one, two or three sub-moieties independently selected from the group consisting of γ-Glu, and OEG.

In some examples, the lipophilic substituent X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl, and the linker U consists of one, two or three sub-moieties independently selected from the group consisting of Gly, Glu, γ-Glu, ε-Lys, Ser, and OEG, or independently selected from the group consisting of γ-Glu, and OEG.

In some embodiment the PYY analogue is selected from the compounds 1 to 244 described herein.

In some examples the PYY analogue is in the form of a salt, preferably in the form of a pharmaceutically acceptable salt.

The disclosure further provides a composition comprising a PYY analogue as described herein. In another aspect, the present disclosure provides a pharmaceutical composition comprising at least one PYY analogue as described herein and one or more pharmaceutically acceptable excipient.

The present disclosure further provides a PYY analogue for use in a method of medical treatment, e.g. for use in the treatment of obesity and various obesity-related conditions, diseases, or disorders such as type 2 diabetes, NAFLD or NASH.

The disclosure provides a PYY analogue of the disclosure for use in a method of treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing excess body weight. In another aspect, the present disclosure provides a PYY analogue as described herein for use in the treatment of obesity or obesity-related conditions, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea and cardiovascular disease. In another aspect, the present disclosure provides use of a PYY analogue as described herein in the manufacture of a medicament for the treatment of obesity or obesity-related conditions, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

DETAILED DESCRIPTION OF THE INVENTION

TERMS, DEFINITIONS AND CONVENTIONS

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the following terms have the meaning indicated and the following conventions are adhered to.

Throughout this specification, amino acid positions of the PYY analogues are numbered according to the corresponding position in native human PYY having the sequence shown above.

PYY analogues

A PYY analogue is a peptide comprising an amino acid sequence corresponding to the amino acid sequence of hPYY(3-36). In other words a PYY analogue is a peptide, whose structure is related to PYY, in which one or more amino acid residues have been modified when compared to hPYY(3-36). Possible modifications are substitutions, insertions, or deletions of amino acids at specific positions. A PYY analogue of the invention relates to a peptide that has retained a certain binding affinity (Ki) towards the hNPY2 receptor.

The term “PYY analogue” comprises the peptide itself, as well as the peptide in ionized state (e.g. when one or more side chains of its amino acids are ionized, i.e. (de)protonated).

The term “PYY analogue” may also refer to peptides, in which a half-life extending group is attached to one or more amino acids of the peptide. In such cases, a side chain of an amino acid bears a covalently attached half-life extending group.

As used herein, the term “pharmaceutically acceptable salt” is intended to indicate a salt which is not harmful to a patient or subject to which the salt in question is administered. It may suitably be a salt chosen, e.g., among acid addition salts and basic salts. As used herein, "pharmaceutically acceptable salt" refer to derivatives of the disclosed analogues or compounds wherein the parent analogue or compound is modified by making acid or base salts thereof. Examples of acid addition salts include chloride salts, citrate salts and acetate

salts. Examples of basic salts include salts, where the cation is selected among alkali metal cations, such as sodium or potassium ions, alkaline earth metal cations, such as calcium or magnesium ions, as well as substituted ammonium ions, such as ions of the type $N(R^1)(R^2)(R^3)(R^4)^+$, where R^1, R^2, R^3 and R^4 independently will typically designate hydrogen or optionally substituted C_{1-6}-alkyl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985 (and more recent editions thereof), in the "Encyclopaedia of Pharmaceutical Technology", 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA (Inc.), NY, USA, 2007, and in *J. Pharm. Sci.* 66: 2 (1977).

The term "agonist" as employed in the context of the invention refers to a substance that activates the receptor type in question, typically by binding to it (i.e. as a ligand).

Each embodiment of the invention described herein may be taken alone or in combination with one or more other embodiments of the invention.

Throughout the present specification, unless naturally occurring amino acids are referred to by their full name (e.g. alanine, arginine, etc.), they are designated by their conventional three-letter or single-letter abbreviations (e.g. Ala or A for alanine, Arg or R for arginine, etc.).

Unless otherwise indicated, reference is made to the L-isomeric forms of the amino acids in question.

Additional abbreviations include the following:

Hyp:	4-hydroxyproline, e.g. (2S,4R)-4-hydroxyproline [also denoted (4R)-4-hydroxy-L-proline]
γ-Glu:	γ-glutamic acid [also denoted gGlu]

The term "C_{1-n} alkyl", wherein n is an integer selected from 2, 3, 4, 5 or 6, either alone or in combination with another radical, denotes an acyclic, saturated, branched or linear hydrocarbon radical with 1 to n C atoms. For example the term C_{1-5}-alkyl embraces the

radicals H_3C-, H_3C-CH_2-, H_3C-CH_2-CH_2-, H_3C-$CH(CH_3)$-, H_3C-CH_2-CH_2-CH_2-, H_3C-CH_2-$CH(CH_3)$-, H_3C-$CH(CH_3)$-CH_2-, H_3C-$C(CH_3)_2$-, H_3C-CH_2-CH_2-CH2-CH2-, H_3C-CH_2-CH_2-$CH(CH_3)$-, H_3C-CH_2-$CH(CH_3)$-CH_2-, H_3C-$CH(CH_3)$-CH_2-CH_2-, H_3C-CH_2-$C(CH_3)_2$-, H_3C-$C(CH_3)_2$-CH_2-, H_3C-$CH(CH_3)$-$CH(CH_3)$- and H_3C-CH_2-$CH(CH_2CH_3)$-.

The term "C_{3-n}-cycloalkyl", wherein n is an integer from 4 to n, either alone or in combination with another radical denotes a cyclic, saturated, unbranched hydrocarbon radical with 3 to n C atoms. For example, the term C_{3-6}-cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

"C(O)" or "C(=O)" refers to a carbonyl group.

Nomenclature of compounds:

As an example,

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PGEDASPEELQRYYVSLRHYYNALTRQRY-NH_2,

wherein iVal represents 3-methylbutanoyl (-$C(O)CH_2CH(CH_3)_2$),

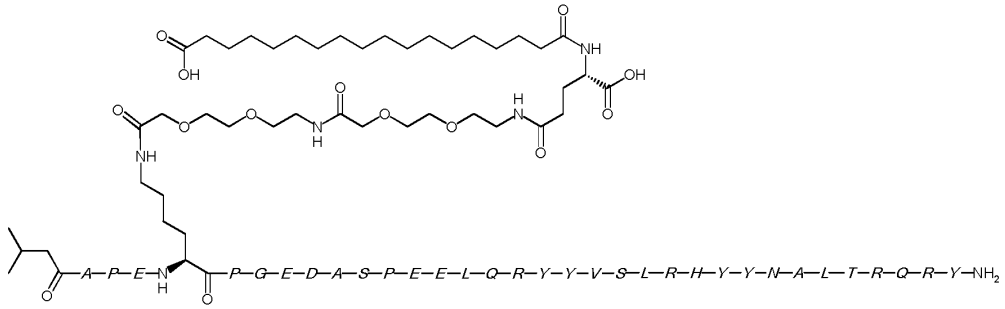
C18DA represents 17-carboxyheptadecanoyl,

gGlu represents L-γ-glutamyl, connected via its amino-group to C18DA and via its γ-carboxy-group to OEG1, and

OEG1 represents 2-[2-(2-aminoethoxy)ethoxy]acetoyl, connected via its amino-group to gGlu and via its carboxy-group to OEG2, and

OEG2 represents 2-[2-(2-aminoethoxy)ethoxy]acetoyl, connected via its amino-group to OEG1 and via its carboxy-group to ε-amino-group of lysine (K),

completely defines the PYY analogue of the following structure:



Alternatively, the same compound can be defined in the following way:

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18Q,22V,28Y,30A,31L]hPYY(4-36).

The terms "treatment" and grammatical variants thereof (e.g. “treated”, “treating”, “treat”) as employed in the present context refer to an approach for obtaining beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization (i.e. not worsening) of state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival relative to expected survival time if not receiving treatment. A subject (e.g. a human) in need of treatment may thus be a subject already afflicted with the disease or disorder in question. The term “treatment” includes inhibition or reduction of an increase in severity of a pathological state or symptoms (e.g. weight gain or hyperglycemia) relative to the absence of treatment and is not necessarily meant to imply complete cessation of the relevant disease, disorder or condition.

The terms "prevention" and grammatical variants thereof (e.g., “prevented”, “preventing”, “prevent”) as employed in the present context refer to an approach for hindering or preventing the development of, or altering the pathology of, a condition, disease or disorder. Accordingly, "prevention" may refer to prophylactic or preventive measures. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, prevention or slowing of symptoms, progression or development of a disease, whether detectable or undetectable. A subject (e.g. a human) in need of “prevention” may thus be a subject not yet afflicted with the disease or disorder in question. The term “prevention” thus includes inhibiting or slowing the onset of disease relative to the absence of treatment and is not necessarily meant to imply permanent prevention of the relevant disease, disorder or condition.

Half-life extending group

As described herein, a half-life extending group is covalently attached to a functional group of a side chain of an amino acid of the PYY analogue. The half-life extending group comprises or consists of a lipophilic substituent (X) and optionally a linker (U), wherein one end of the linker U (if present) is attached to an amino acid of the PYY analogue and the other end is connected to the lipophilic substituent (-U-X).

Without wishing to be bound by any particular theory, it is thought that such lipophilic substituents (and other classes of half-life extending moieties) bind albumin and other plasma components in the blood stream, thereby shielding the compound of the invention from renal filtration as well as enzymatic degradation and thus possibly enhancing the half-life of the compound *in vivo*. The lipophilic substituent may also modulate the potency of the compound as an agonist to the NPY2 receptor or other receptors of the NPY receptor family.

The lipophilic substituent X is attached to the linker U via an ester, ether, a sulfonyl ester, a thioester, an amide, an amine, triazole or a sulfonamide. Accordingly, it will be understood that preferably the lipophilic substituent X includes an acyl group, a sulfonyl group, an alkyne, an azide, an N atom, an O atom or an S atom, which forms part of the ester, sulfonyl ester, thioester, triazole, amide, amine or sulfonamide. Preferably, an acyl group, or an O or N atom in the lipophilic substituent X forms part of an amide or ester with the linker U.

The half-life extending group (the linker U thereof, if present) is attached to an amino acid residue of the PYY analogue via an ester, a sulfonyl ester, a thioester, an amide, an amine or a sulfonamide. Accordingly, it will be understood that preferably the half-life extending group (the linker U thereof, if present) includes an acyl group, a sulfonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulfonyl ester, thioester, amide, amine or sulfonamide. Preferably, an acyl group, or an O or N atom in the linker U forms part of an amide or ester with the amino acid residue.

The lipophilic substituent X may comprise a hydrocarbon chain having from 10 to 24 C atoms, e.g. from 14 to 22 C atoms, e.g. from 16 to 20 C atoms. Preferably, it has at least 14 C atoms, and preferably has 20 C atoms or fewer. For example, the hydrocarbon chain may contain 14, 15, 16, 17, 18, 19 or 20 carbon atoms. The hydrocarbon chain may be linear or branched and may be saturated or unsaturated. Furthermore, it can include a

functional group at the end of the hydrocarbon chain, e.g. a carboxylic acid group, a sulphonic acid group, or a tetrazole group. From the discussion above it will also be understood that the hydrocarbon chain is preferably substituted with a moiety, which forms part of the attachment to an amino acid residue of the PYY analogue or to the linker U, for example an acyl group, a sulfonyl group, an N atom, an O atom or an S atom.

Most preferably, the hydrocarbon chain is substituted with an acyl group (for the attachment to the linker U), and accordingly the hydrocarbon chain may be part of an alkanoyl group, for example a dodecanoyl, 2-butyloctanoyl, tetradecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl or eicosanoyl group. These hydrocarbon chains substituted with an acyl group at one end may further be functionalized with a carboxylic acid group at the other end of the chain. Examples of functionalized hydrocarbon chains (e.g. lipophilic substituents X) are 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl and 19-carboxy-nonadecanoyl.

In certain embodiments, the linker moiety U may itself comprise one, two, three or more linked sub-moieties (U^1, U^2, U^3, etc). In some of these embodiments the linker may comprise one or more (e.g. one, two or three) linked amino acid residues, which may each independently be a residue of any naturally occurring or non-naturally occurring amino acid. For example, the linker may comprise one, two or three linked amino acid residues, each of which may independently be a residue of Gly, Pro, Ala, Val, Leu, Ile, Cys, Phe, Tyr, His, Lys, Arg, Gln, Asn, α-Glu, γ-Glu, ε-Lys, Asp, β-Asp, Ser, Thr, Aib, AEA (2-(2-aminoethoxy)acetic acid), AEEEA (2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetic acid), H_2N-dPEG(4)-COOH (15-amino-4,7,10,13-tetraoxa-pentadecanoic acid), H_2N-dPEG(6)-COOH (1-amino-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid) , H_2N-dPEG(12)-COOH (1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid), OEG-OEG (2-[2-(2-{2-[2-(2-aminoethoxy)ethoxy]acetamido}ethoxy)ethoxy]acetic acid), H-Ebes (3-({2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamoyl)propanoic acid), H-DOOA-DIG-OH (2-[({2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamoyl)methoxy]acetic acid), H-TTD-DIG-OH (2-{[(3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propyl)carbamoyl]methoxy} acetic acid), H-TTDS-OH (3-[(3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propyl)-carbamoyl]propanoic acid), or 8Ado (i.e. 8-amino-3,6-dioxaoctanoyl also denoted OEG herein).

References to γ-Glu, ε-Lys, and β-Asp indicate residues of amino acids which participate in bonds via their side chain carboxyl or amine functional groups. Thus γ-Glu, and β-Asp participate in bonds via their alpha amino and side chain carboxyl groups, while ε-Lys participates via its carboxyl and side chain amino groups. In the context of the present invention, γ-Glu, gGlu and isoGlu are used interchangeably.

In certain embodiments, the linker U comprises or consists of one, two or three independently selected sub-moieties (U^1, U^2, U^3) selected from the group consisting of Ala, Glu, γ-Glu, Gly, ε-Lys, Ser, OEG and OEG-OEG.

Linkers consisting of γ-Glu, γ-Glu-γ-Glu, γ-Glu-OEG, OEG-OEG, γ-Glu-γ-Glu-OEG, γ-Glu-OEG-OEG may be preferred.

Synthesis of PYY analogues

The invention provides a method of synthesis of a PYY analogue of the invention. The PYY analogues may be manufactured by standard synthetic methods, including standard solid-phase or liquid-phase methodology. Peptides are assembled either stepwise or by merging fragments, and optionally isolated and purified yielding the final peptide product. Synthesis examples are described in numerous publications, including Fields, G.B. et al., "Principles and Practice of Solid-Phase Peptide Synthesis" in Synthetic Peptides, Grant G.A. (ed.), Oxford University Press (2^{nd} edition, 2002).

EMBODIMENTS

In a first aspect, the invention provides a PYY analogue, wherein the analogue comprises

i) alanine at the position corresponding to position 4 of hPYY(3-36)

ii) lysine at the position corresponding to position 7 of hPYY(3-36)

iii) the sequence QRY at its C-terminal end,

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21, 22 or 30, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at positions 14 or 30.

In some embodiments, the PYY analogue comprises glutamate at the position corresponding to position 9 of hPYY(3-36).

In some embodiments, the PYY analogue comprises tyrosine at the position corresponding to position 20 of hPYY(3-36).

In some embodiment, the PYY analogue comprises tryptophan at the position corresponding to position 30 of hPYY(3-36).

In some embodiments, the PYY analogue comprises leucine at the position corresponding to position 31 of hPYY(3-36).

In some embodiments, the PYY analogue comprises arginine or lysine at the position corresponding to position 33 of hPYY(3-36).

In some embodiments, the PYY analogue bears a half-life extending group, which is attached to the epsilon amino group of the lysine at position 7.

In some embodiments of the present invention, the half-life extending group consists of a lipophilic substituent X and a linker U, wherein the linker U is attached to the amino acid side chain, and X is attached to U.
In some embodiments, the lipophilic substituent X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl.
In some embodiments the linker U comprises one or more sub-moieties, wherein at least one of the sub-moieties is OEG.
In some embodiments the linker U consists of one, two or three sub-moieties (U1, U2, U3), wherein the sub-moiety is independently selected from the group consisting of Gly, Glu, γ-Glu, ε-Lys, Ser and OEG.

In some embodiments, the PYY analogue comprises

i) alanine at the position corresponding to position 4 of hPYY(3-36)
ii) lysine at the position corresponding to position 7 of hPYY(3-36)
iii) glutamate at the position corresponding to position 9 of hPYY(3-36)
iv) tyrosine at the position corresponding to position 20 of hPYY(3-36)
v) tryptophan at the position corresponding to position 30 of hPYY(3-36)
vi) leucine at the position corresponding to position 31 of hPYY(3-36)
vii) the sequence RQRY at its C-terminal end,
and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21 or 22, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at position 14.

In some embodiments, the PYY analogue comprises threonine at the position corresponding to position 32 of hPYY(3-36).

In some embodiments, the PYY analogue comprises the sequence WLTRQRY at its C-terminal end.

In some embodiments, the PYY analogue bears a half-life extending group, which is attached to the epsilon amino group of the lysine at position 7.

In some embodiments, the PYY analogue comprises
i) alanine at the position corresponding to position 4 of hPYY(3-36)
ii) lysine at the position corresponding to position 7 of hPYY(3-36)
iii) glutamate at the position corresponding to position 9 of hPYY(3-36)
iv) tyrosine at the position corresponding to position 20 of hPYY(3-36)
v) the sequence WLTRQRY at its C-terminal end,
and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21, or 22, or to the carboxylic acid group of the side chain of a aspartate or a glutamate at positions 14 .

In some embodiments, the PYY analogue comprises arginine at the position corresponding to position 25 of hPYY(3-36).

In some embodiments, the PYY analogue bears a half-life extending group, which is attached to the epsilon amino group of the lysine at position 7.

In some embodiments, exactly one half-life extending group is attached to the PYY analogue, said half-life extending group being attached to the epsilon amino group of the lysine at position 7.

In some embodiments of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is a peptide comprising an amino acid sequence of formula I:

Ala-X5-X6-Lys-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-X22-X23-X24-Arg-X26-X27-X28-X29-X30-X31-X32-Arg-Gln-Arg-Tyr (I)

wherein

X5 is selected from the group consisting of Pro and Hyp;

X6 is selected from the group consisting of Ala and Glu;

X8 is selected from the group consisting of Ala, Ile, Pro, Thr, Val and Hyp;

X9 is selected from the group consisting of Glu, Gly, Gln and Pro;

X10 is selected from the group consisting of Ala and Glu;

X11 is selected from the group consisting of Ala, Asp, Glu and Pro;

X12 is selected from the group consisting of Ala, Gly, Ser, Thr and Val;

X13 is selected from the group consisting of Ala, Glu, Ser, Gln, Thr and Pro;

X14 is selected from the group consisting of Ala, Glu, Gly, Pro and Hyp;

X15 is selected from the group consisting of Ala, Glu and Ser;

X16 is selected from the group consisting of Ala, Glu and Ser;

X17 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;

X18 is selected from the group consisting of Glu and Gln;
X19 is selected from the group consisting of Ala, Glu, Arg, Lys and Gln;
X21 is selected from the group consisting of Ala, Glu, Gln and Tyr;
X22 is selected from the group consisting of Ile, Ser, Thr and Val;
X23 is selected from the group consisting of Ala, Glu, Ser and Thr;
X24 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;
X26 is selected from the group consisting of Ala, His and Lys;
X27 is selected from the group consisting of Gln and Tyr;
X28 is selected from the group consisting of His, Trp and Tyr;
X29 is selected from the group consisting of Asn, Trp and Tyr;
X30 is selected from the group consisting of Ala, His, Trp, and Tyr;
X31 is selected from the group consisting of Ala, Ile, Leu and Thr;
X32 is selected from the group consisting of Gln, Leu and Thr;

wherein one to three amino acids of X5, X6, X8-X19, X21-X24 and X26-X32 may be absent,

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7.

In a further embodiment the PYY analogue is according to the previous embodiment, wherein none of X5, X6, X8-X19, X21-X24 and X26-X32 is absent.

In a further embodiment R^1 is -C(O)C_{1-4} alkyl, -C(O)C_{3-5} cycloalkyl, -C(O)C_{1-3} alkyl-C_{3-4} cycloalkyl, C_{1-4} alkyl or C_{1-3} alkyl-C_{3-4} cycloalkyl.

In a further embodiment R^1 is -C(O)$CH_2CH(CH_3)_2$, -C(O)CH_2-cyclobutyl, -C(O)CH_2-cyclopropyl.

It has been found that the PYY analogues of the present invention – bearing alanine at position 4 and lysine at position 7 - generally are soluble around pH 6 and pH 7.

Peptide therapeutics are usually provided as pharmaceutical liquid formulation in a pre-filled ready-to-use injection device. These peptide formulations for subcutaneous administration have limited application volumes. Therefore, good solubility of the peptides is a requirement for the application in a ready-to-use injection device.

A further important aspect is the long-term stability and solubility of the peptides in the liquid formulation. A property of fundamental importance for physical stability is the intrinsic solubility (at a given pH value).

A broad pH range, within which a peptide therapeutic is reasonably soluble (solubility window), is also desirable as it allows more flexibility for pharmaceutical formulation development. This flexibility might be desirable as other factors, such as chemical stability, are also pH dependent. In general, a peptide formulation around pH 6.0 is believed to show reduced rates of oxidation (e.g. Cys oxidation, disulphide crosslinking, and oxidation of Trp residues), deamination and aspartate isomerization as compared to a formulation at pH 7. For instance, Bak et al. (A. Bak, D. Leung, S. E. Barrett, S. Forster, E. D. Minnihan, A. W. Leithead, J. Cunningham, N. Toussaint, L. S. Crocker, The AAPS Journal, Vol. 17, No. 1, 2015, p. 144 - 155) states that oxidation propensity generally lessens at lower pH and suggests maintenance at pH <7 as a strategy for mitigation risks related to oxidation. Therefore, it might be desirable to have the option to formulate around pH 6 in case chemical stability of peptides containing asparagine, aspartate or glutamine, tryptophan, cysteine or methionine is an issue.

Efficacy of obesity therapeutics is limited. However, efficacy might be improved by combining different therapeutic principles. NPY2 receptor agonists seem attractive partners for combination with other weight reducing therapeutics. For example, NPY2 receptor agonists show enhanced weight loss efficacy with GLP-1 receptor agonists (e.g. WO2005/077072, WO2014/178018, WO2018/081370) or amylin (e.g. WO2006/066024, WO2009/064298). Native amylin and many amylin (or calcitonin) analogues comprise a disulphide bridge. Therefore, a fix-dose combination of a PYY analogue with an amylin analogue might benefit from the opportunity to formulate at a lower pH where the disulphide bridge of the amylin analogue potentially shows improved stability (e.g. due to reduced intramolecular disulphide bond crosslinking reactions). This illustrates that the advantage to be able to formulate at a pH below 7 may not (only) be due to a higher stability

of the NPY analogue but may lie therein that the combination partner shows improved stability (or solubility) at a lower pH.
Therefore, to enable co-formulation development with a diverse range of partners, it is highly desirable to identify NPY2 receptor agonists with a wide solubility window.

Increased solubility, however, should not come at the cost of reduced activity or potency. It was surprisingly found that the alanine at position 4 increases solubility around pH 6 in the PYY analogues of the invention with no or only small negative effects on activity or potency or other important properties (e.g. chemical or physical stability).

Long in-vivo half-life is also a beneficial property for agents to reduce food intake in over-weight or obese patients. Compounds with a long-acting profile (as compared to the very short in-vivo half-life of native (human) PYY) reducing the frequency of administration are desirable.

In one aspect, the invention relates to PYY analogues being NPY2 receptor agonists.
In one aspect, the invention relates to PYY analogues showing selectivity towards the NPY receptor subtype Y2 as compared to Y receptor subtypes Y1, Y4 and Y5.
In one aspect, the invention relates to PYY analogues with extended half-live, e.g. with longer half-life than the half-life of hPYY(3-36). For example, the PYY analogues of the invention are suitable for once weekly administration.
Additionally or alternatively, the invention relates to PYY analogues having high chemical and/or physical stability, e.g. around pH 6 or pH 7.

Further, more specific embodiments are defined below:

In a further embodiment of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,
wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:
R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is an amino acid sequence of formula II:

Ala-X5-X6-Lys-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-X22-X23-X24-Arg-X26-Tyr-X28-X29-X30-X31-X32-Arg-Gln-Arg-Tyr (II)

wherein
X5 is selected from the group consisting of Pro and Hyp;
X6 is selected from the group consisting of Ala and Glu;
X8 is selected from the group consisting of Ala, Pro and Hyp;
X9 is selected from the group consisting of Gln, Gly, Glu and Pro;
X10 is selected from the group consisting of Ala and Glu;
X11 is selected from the group consisting of Ala, Asp, Glu and Pro;
X12 is selected from the group consisting of Ala, Gly and Ser;
X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;
X14 is selected from the group consisting of Ala, Glu, Pro and Hyp;
X15 is selected from the group consisting of Ala, Glu and Ser;
X16 is selected from the group consisting of Ala, Glu and Ser;
X17 is selected from the group consisting of Ile, Leu, Thr and Val;
X18 is selected from the group consisting of Gln and Glu;
X19 is selected from the group consisting of Ala, Glu, Arg, Lys and Gln
X21 is selected from the group consisting of Ala, Glu, Gln and Tyr;
X22 is selected from the group consisting of Ile, Ser, Thr and Val;
X23 is selected from the group consisting of Ala, Glu, Ser and Thr;
X24 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;
X26 is selected from the group consisting of Ala, His and Lys;
X28 is selected from the group consisting of Trp and Tyr;
X29 is selected from the group consisting of Asn and Tyr;
X30 is selected from the group consisting of His, Trp, and Tyr;
X31 is selected from the group consisting of Ala, Ile and Leu;
X32 is selected from the group consisting of Gln and Thr;

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7.

In some embodiments in line with the embodiment described immediately above, the half-life extending group consists of a lipophilic substituent X and a linker U, wherein the linker U is attached to the amino acid side chain, and X is attached to U, and wherein the lipophilic substituent X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl, and the linker U consists of one, two or three sub-moieties (U1, U2, U3), wherein the sub-moiety is independently selected from the group consisting of Gly, Glu, γ-Glu, ε-Lys, Ser and OEG.

In some embodiments of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is an amino acid sequence of formula III:

Ala-Pro-X6-Lys-Pro-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-Val-X23-Leu-Arg-His-Tyr-Tyr-Asn-Trp-Leu-Thr-Arg-Gln-Arg-Tyr (III)

wherein

X6 is selected from the group consisting of Ala and Glu;

X9 is selected from the group consisting of Glu and Gly;

X10 is selected from the group consisting of Ala and Glu;

X11 is selected from the group consisting of Ala, Asp, Glu and Pro;

X12 is selected from the group consisting of Ala and Ser;

X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;

X14 is selected from the group consisting of Ala, Glu and Pro;

X15 is selected from the group consisting of Ala and Glu,

X16 is selected from the group consisting of Ala and Glu;

X17 is selected from the group consisting of Ile, Leu, Thr and Val;

X18 is selected from the group consisting of Glu and Gln;

X19 is selected from the group consisting of Ala, Glu, Arg, Lys, and Gln;
X21 is selected from the group consisting of Glu and Tyr;
X23 is selected from the group consisting of Ala, Glu, Ser and Thr;

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7.

In further embodiments, the PYY analogue is a compound according to formula I to III, wherein at least two amino acids from X6, X10, X11, X13 and X23 are selected from the group consisting of Asp and Glu.

In further embodiments, the PYY analogue is a compound according to any of the previous embodiments, wherein only one of X6, X10 and X15 is Ala.

In a further embodiment of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,
wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:
R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and
Z is an amino acid sequence of formula IV:

Ala-Pro-X6-Lys-Pro-Glu-X10-X11-Ala-X13-X14-Glu-Glu-X17-Gln-X19-Tyr-Tyr-Val-X23-Leu-Arg-His-Tyr-Tyr-Asn-Trp-Leu-Thr-Arg-Gln-Arg-Tyr (IV)

wherein
X6 is selected from the group consisting of Ala and Glu;
X10 is selected from the group consisting of Ala and Glu;
X11 is selected from the group consisting of Ala, Asp and Glu;
X13 is selected from the group consisting of Ala, Glu, Ser, and Thr;
X14 is selected from the group consisting of Ala, Pro and Hyp;
X17 is selected from the group consisting of Ile and Leu;

X19 is selected from the group consisting of Arg, Lys, and Gln;
X23 is selected from the group consisting of Ala, Glu, and Ser;

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7.

In a further embodiment, the PYY analogue is a compound according to formula IV, wherein at least 2 amino acid from X6, X10, X11, X13 and X23 are selected from Asp or Glu.

In further embodiments, the PYY analogue is a compound according to formula IV, wherein only one of X6, and X10 is Ala.

In further embodiments, the PYY analogue is a compound according to any of the previous embodiments, wherein X6 is Ala.

In further embodiments, R^1 is hydrogen, $-C(O)C_{1-6}$ alkyl, $-C(O)C_{3-6}$ cycloalkyl, or C_{1-6} alkyl.
In further embodiments, R^1 is hydrogen or $-C(O)C_{1-6}$ alkyl.
In further embodiments, R^1 is hydrogen or $-C(O)C_{1-4}$ alkyl.
In more specific embodiments, R^1 is $-C(O)CH_2CH(CH_3)_2$.

In more specific embodiments, R^2 is NH_2.

In some embodiments of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,
wherein R^1 and R^2 are defined as in any of the definitions above;and
Z is an amino acid sequence selected from Table 1:

cf. compound	Sequence	SEQ ID NO
1	APEKPEEDAQPEELQEYYVSLRHYYNWLTRQRY	5
2	AHypEKPEEDASPEELQRYYVSLRHYYNWLTRQRY	6
3	APEKPEEEASPAELQRYYVSLRHYYNWLTRQRY	7
4	APEKPEEDASPEELQRYYVSLRHYYNWLQRQRY	8
5	APEKPEEEVSPEELQRYYVSLRHYYNWLTRQRY	9
6	APEKPEEEASPEELQRYYVSARHYYNWLTRQRY	10
7	APEKPEEDASPEELQAYYVSLRHYYNWLTRQRY	11
8	APEKPEEDASEEELQQYYVSLRHYYNWLTRQRY	12
9	APEKPEEDASPEEIQRYYVELRHYYNWLTRQRY	13
10	APEKPEADASPEETQRYYVSLRHYYNWLTRQRY	14
11	APEKPEEDASPEEIQQYYVSLRHYYNWLTRQRY	15
12	APEKPEEDAPGEELQRYYVSLRHYYNWLTRQRY	16
13	APEKPEEDAAPEELQQYYVSLRHYYNWLTRQRY	17
14	APEKPEADATPEELQRYYVSLRHYYNWLTRQRY	18
15	APEKPEEDASPEEIQRYEVSLRHYYNWLTRQRY	19
16	APEKPEEDASPEETQRYYVTLRHYYNWLTRQRY	20
17	APEKPEEDASPAELQRYYVSLRHYYNWLTRQRY	21
18	APEKPEEDAEPEELQQYYVSLRHYYNWLTRQRY	22
19	APEKPEADASPEELQQYYVSLRHYYNWLTRQRY	23
20	APEKPEEDAAPEELQRYYVELRHYYNWLTRQRY	24
21	APEKPEEDASPEELQKYYVSLRHYYNWLTRQRY	25
22	APEKPEEDASPEELQRYYTSLRHYYNWLTRQRY	26
23	APAKPEEDASPEEIQRYYVSLRHYYNWLTRQRY	27
24	APEKPEEDASPEELQRYYVSLRKYYNWLTRQRY	28
25	APEKPEEEASAEELQRYYVSLRHYYNWLTRQRY	29
26	APEKPPEDATPEELQRYYVSLRHYYNWLTRQRY	30
27	APEKPPEDAEPEELQRYYVSLRHYYNWLTRQRY	31
28	APEKPEEDTSPEELQRYYVELRHYYNWLTRQRY	32
29	APEKPEAPASPEELQRYYVELRHYYNWLTRQRY	33
30	APEKPEADASEEELQRYYVSLRHYYNWLTRQRY	34

31	APEKPEEDASPEETQRYYVALRHYYNWLTRQRY	35
32	APEKPEEDTSPEELQRYEVSLRHYYNWLTRQRY	36
33	APEKPEEPASPEELQRYYVELRHYYNWLTRQRY	37
34	APEKPEEDASPEELQRYYVSTRHYYNWLTRQRY	38
35	APEKPEEDAAPEETQRYYVSLRHYYNWLTRQRY	39
36	APEKAEEDAEPEELQRYYVSLRHYYNWLTRQRY	40
37	APEKPEEDASPEETQRYEVSLRHYYNWLTRQRY	41
38	APEKPPEDASPEELQRYYVALRHYYNWLTRQRY	42
39	APEKPPADAEPEELQRYYVSLRHYYNWLTRQRY	43
40	APEKPEEDATPEETQRYYVSLRHYYNWLTRQRY	44
41	APEKPEEEATPEELQRYYVSLRHYYNWLTRQRY	45
42	APEKPEEAASPEELQRYYVSTRHYYNWLTRQRY	46
43	APEKPEAPASPEELQRYYVSLRHYYNWLTRQRY	47
44	APEKPEEEASPEELQRYYVSLRHYYNWITRQRY	48
45	APEKPEEEASPESLQRYYVSLRHYYNWLTRQRY	49
46	APEKPEEEASPEELQRYYVSLRHYYNWLTRQRY	50
47	APEKPPADASPEELQRYYVSLRHYYNWLTRQRY	51
48	APEKPEEDTEPEELQRYYVSLRHYYNWLTRQRY	52
49	APEKPEEDASPEELQQYYVELRHYYNWLTRQRY	53
50	APEKPEEDASAEELQRYYVSLRHYYNWITRQRY	54
51	APEKAEEEASPEELQRYYVSLRHYYNWLTRQRY	55
52	APEKAEEDASEEELQRYYVSLRHYYNWLTRQRY	56
53	APEKPEADASPEEIQRYYVSLRHYYNWLTRQRY	57
54	APEKPEEDASPEELQRYYVSLRHYYNWLTRQRY	58
55	APEKPEEDASPEEAQRYYVSLRHYYNWLTRQRY	59
56	APEKPEADAEPEELQRYYVSLRHYYNWLTRQRY	60
57	APEKPEEDASAEEIQRYYVSLRHYYNWLTRQRY	61
58	APEKPEEDASPEELQQYYVSLRHYYNWLTRQRY	62
59	APEKPEEDASPEETEQYYVSLRHYYNWLTRQRY	63
60	APEKPEEAASPEELEQYYVSLRHYYNWLTRQRY	64
61	APEKPPEDASPEEIQRYYVSLRHYYNWLTRQRY	65
62	APEKPGEDASPEELERYYVSLRHYYNWLTRQRY	66

63	APEKPEEDASPEELQRYYVSLRAYYNWLTRQRY	67
64	APEKPGEDASPEELQRYYVSLRHYYNWLTRQRY	68
65	APEKPEEDASPEELQRYYVEIRHYYNWLTRQRY	69
66	APEKPEEDASAEETQRYYVSLRHYYNWLTRQRY	70
67	APEKPEEDSAPEELQRYYVSLRHYYNWLTRQRY	71
68	APEKPEEEASPEELQAYYVSLRHYYNWLTRQRY	72
69	APEKPEEDSSAEELQRYYVSLRHYYNWLTRQRY	73
70	APEKPEEAASPEELQRYEVSLRHYYNWLTRQRY	74
71	APEKPEEEASPSELQRYYVSLRHYYNWLTRQRY	75
72	APEKPEEEGSPEELQRYYVSLRHYYNWLTRQRY	76
73	APEKPEEEASPEELQRYYVSLRAYYNWLTRQRY	77
74	APEKPEEDASAEELQRYYVALRHYYNWLTRQRY	78
75	APEKPEEDASAEELQRYYVTLRHYYNWLTRQRY	79
76	APEKPGEDASPEELQRYYVSLRHYYNALTRQRY	80
77	APEKPEEDASEEELQRYYVELRHYYNWLTRQRY	81
78	APEKPEEDAEPEELQQYYVTLRHYYNWLTRQRY	82
79	APEKPEEEASEEELQRYYVSLRHYYNWLTRQRY	83
80	APEKPEEEASPEETQRYYVSLRHYYNWLTRQRY	84
81	APEKPEEDASPEELQRYYVSIRHYYNWLTRQRY	85
82	APEKPEEDASPEALQRYYVALRHYYNWLTRQRY	86
83	APEKPEADASPAELQRYYVSLRHYYNWLTRQRY	87
84	APEKPEEDASPEEIQRYYVSLRHYYNWLTRQRY	88
85	APEKPEEDASPEELQRYAVSLRHYYNWLTRQRY	89
86	APEKPEADASPEELQRYEVSLRHYYNWLTRQRY	90
87	APEKPEEDSSPAELQRYYVSLRHYYNWLTRQRY	91
88	APEKPPEDASPEELQRYYVELRHYYNWLTRQRY	92
89	APEKPEEEAEPEELQRYYVSLRHYYNWLTRQRY	93
90	APEKPEEDASPEELQRYEVSLRHYYNWLTRQRY	94
91	APEKPEEDAPAEELQRYYVELRHYYNWLTRQRY	95
92	APEKPEEDTSPEELQRYYVSLRHYYNWLTRQRY	96
93	APEKPEEDASAEELQKYYVSLRHYYNWLTRQRY	97
94	APEKPEEDASPEETQRYYVELRHYYNWLTRQRY	98

95	APEKPEEDASPAEIQRYYVSLRHYYNWLTRQRY	99
96	APEKPEADASPEELQRYYVSLRHYYNWLTRQRY	100
97	APEKPEEDASPEELQRYYVALRHYYNWLTRQRY	101
98	APEKPEEDASPEELQRYYSALRHYYNWLTRQRY	102
99	APEKPEEDAEAEELQRYYVSLRHYYNWLTRQRY	103
100	APAKPPEDASPEELQRYYVSLRHYYNWLTRQRY	104
101	APEKPEEDATPEELQRYYVELRHYYNWLTRQRY	105
102	APEKPEEDASPEELQRYYVTLRHYYNWLTRQRY	106
103	APAKPEEDASEEELQRYYVSLRHYYNWLTRQRY	107
104	APEKPEEDASAEELQRYYVSLRHYYNWLTRQRY	108
105	APEKPEEDAEPEELQEYYVSLRHYYNWLTRQRY	109
106	APAKPEEDATPEELQRYYVSLRHYYNWLTRQRY	110
107	APEKPEEDASPEELQKYYVALRHYYNWLTRQRY	111
108	APEKPEEDASPEELQRYYVELRHYYNWLTRQRY	112
109	APEKPEEAASPEELQRYYVSLRHYYNWLTRQRY	113
110	APEKPEEDAEPEEIQRYYVSLRHYYNWLTRQRY	114
111	APEKPEEDAEPEELQRYYVELRHYYNWLTRQRY	115
112	APEKPEEDATPEELQRYYVSLRHYYNWLTRQRY	116
113	APEKAEEDATPEELQRYYVSLRHYYNWLTRQRY	117
114	APEKPEEDASPEELQRYYVSLRHYHWYLTRQRY	118
115	APEKPEEEASPEALQRYYVSLRHYYNWLTRQRY	119
116	APEKPEEDAAAEELQRYYVSLRHYYNWLTRQRY	120
117	APAKPEEDASPEELQRYYVALRHYYNWLTRQRY	121
118	APEKPEEDASAAELQRYYVSLRHYYNWLTRQRY	122
119	APEKPEEEASPEELQRYYVTLRHYYNWLTRQRY	123
120	APEKPGEDAEEEELQEYYVSLRHYYNWLTRQRY	124
121	APAKPEADASPEELQRYYVSLRHYYNWLTRQRY	125
122	APEKPEEDAAPEELQRYYVSLRHYYNWLTRQRY	126
123	APEKPEEDASPEELQRYYVSLRHYWYHLTRQRY	127
124	APEKPEEDAAPEEIQRYYVSLRHYYNWLTRQRY	128
125	APEKPGEDASPEELQRYYVSLRHYYNWATRQRY	129
126	APAKPEEDSSPEELQRYYVSLRHYYNWLTRQRY	130

127	APAKPEEDASAEELQRYYVSLRHYYNWLTRQRY	131
128	APEKPEEDAPAEEIQRYYVSLRHYYNWLTRQRY	132
129	APEKPEEDASPEETQRYYTALRHYYNWLTRQRY	133
130	APEKPEEDTSAEELQRYYVSLRHYYNWLTRQRY	134
131	APEKAEEDASPEELQRYYVELRHYYNWLTRQRY	135
132	APEKPEEDASPEELQKYYVTLRHYYNWLTRQRY	136
133	APEKPEEDASPEEVQRYYVELRHYYNWLTRQRY	137
134	APEKPEEDASPEEIQRYYTELRHYYNWLTRQRY	138
135	APEKPEADASPEELQRYYVELRHYYNWLTRQRY	139
136	APEKPEADSSPEELQRYYVSLRHYYNWLTRQRY	140
137	APEKPEEDASPEELQRYYVSLRHYYNWITRQRY	141
138	APAKPEEDASPEELQQYYVSLRHYYNWLTRQRY	142
139	APEKPEEESSPEELQRYYVSLRHYYNWLTRQRY	143
140	APEKPGEDASPEELEQYYVSLRHYYNWLTRQRY	144
141	APEKPEEAASPEELQRYYVSARHYYNWLTRQRY	145
142	APEKPPEEASPEELQRYYVSLRHYYNWLTRQRY	146
143	APEKPEEDATPEELQRYYVALRHYYNWLTRQRY	147
144	APEKAEEDASPEELQQYYVSLRHYYNWLTRQRY	148
145	APEKPEEDSSPEEIQRYYVSLRHYYNWLTRQRY	149
146	APEKPEEEASPEELQRYYVSTRHYYNWLTRQRY	150
147	APEKPEEDASPEETQKYYVSLRHYYNWLTRQRY	151
148	APEKPEEDAPAEELQRYYVSLRHYYNWLTRQRY	152
149	APEKPEEAASPEELQRYYVELRHYYNWLTRQRY	153
150	APEKPEEDAEPEELQKYYVSLRHYYNWLTRQRY	154
151	APEKPEAEASPEELQRYYVSLRHYYNWLTRQRY	155
152	APEKAEEDASPEEIQRYYVSLRHYYNWLTRQRY	156
153	APEKVEEEASPEELQRYYVSLRHYYNWLTRQRY	157
154	APEKPEEEASPEELQRYEVSLRHYYNWLTRQRY	158
155	APEKPEEDASPEETQRYYVSLRHYYNWLTRQRY	159
156	APEKPEEDASEEELEQYYVSLRHYYNWLTRQRY	160
157	APAKPEEDASPEETQRYYVSLRHYYNWLTRQRY	161
158	APEKPEEDASPEELQRYYVSLRHYWNYLTRQRY	162

159	APEKPEADASPEELEQYYVSLRHYYNWLTRQRY	163
160	APEKPEADAEPEELQRYYTSLRHYYNWLTRQRY	164
161	APEKPEEDATPEELQQYYVSLRHYYNWLTRQRY	165
162	APEKPEEDASPEELQRYEVSLRAYYNWLTRQRY	166
163	APEKPEEAASPEELQRYYVALRHYYNWLTRQRY	167
164	APEKPEEDASEEELQEYYVSLRHYYNWLTRQRY	168
165	APEKPEEDASPEELQRYQVSLRHYYNWLTRQRY	169
166	APEKPPADASPEELQRYYVELRHYYNWLTRQRY	170
167	APEKPEEDASPEELQQYEVSLRHYYNWLTRQRY	171
168	APEKPEEDAEPEELQRYYVSLRHYYNWITRQRY	172
169	APEKPEEEASPEELQRYYVALRHYYNWLTRQRY	173
170	APEKPEEEASPEELEQYYVSLRHYYNWLTRQRY	174
171	APEKPEEDATPEEIQRYYVSLRHYYNWLTRQRY	175
172	APEKPEEDAEPEELQRYYVALRHYYNWLTRQRY	176
173	APAKPEEDASPEELQRYYVELRHYYNWLTRQRY	177
174	APAKPEEDAEPEELQRYYVSLRHYYNWLTRQRY	178
175	APEKPEEDAAPEELQRYYVALRHYYNWLTRQRY	179
176	APEKPEEDASEEELQRYYVSLRHYYNWLTRQRY	180
177	APEKPEEDSSPEELQRYYVELRHYYNWLTRQRY	181
178	APEKPEEDATPEETERYYVSLRHYYNWLTRQRY	182
179	APAKPEEDASPAELQRYYVSLRHYYNWLTRQRY	183
180	APEKPEEDASPEETQQYYVSLRHYYNWLTRQRY	184
181	APEKPEADASPEELQRYYVALRHYYNWLTRQRY	185
182	APEKPEEDGSPEELQRYYVELRHYYNWLTRQRY	186
183	APEKPPEDASPEELQRYYVSLRHYYNWLTRQRY	187
184	APEKPEEAASPEELQRYYVTLRHYYNWLTRQRY	188
185	APEKPEEEASPEELQRYYVELRHYYNWLTRQRY	189
186	APEKPEEDASAEELQRYYVELRHYYNWLTRQRY	190
187	APEKPEEDASPEELQRYYVSLRHQYNWLTRQRY	191
188	APEKPEEDAEPEELQRYYVSLRHYYNWLTRQRY	192
189	APEKPEEDASEAELQRYYVSLRHYYNWLTRQRY	193
190	APEKPEEEAPPEELQRYYVSLRHYYNWLTRQRY	194

191	APEKPEEDASPEELQRYYISLRHYYNWLTRQRY	195
192	APEKPEEDAAPAELQRYYVSLRHYYNWLTRQRY	196
193	APEKPEEDASEEEIQRYYVSLRHYYNWLTRQRY	197
194	AHypEKPEEDASPEELQRYYVSLRHYYNWTLRQRY	198
195	APEKPEEDASPEALQRYYVELRHYYNWLTRQRY	199
196	APAKPPEDAEPEELQRYYVSLRHYYNWLTRQRY	200
197	APAKPEADASPEELQRYYVELRHYYNWLTRQRY	201
198	APEKPEEDAEPEALQRYYVSLRHYYNWLTRQRY	202
199	APEKPEEDAEPEETQRYYVSLRHYYNWLTRQRY	203
200	APEKHypEEDASPEELQRYYVSLRHYYNWLTRQRY	204
201	APEKPQEDAEPEELQEYYVSLRHYYNWLTRQRY	205
202	APEKPEEDAAPEELQKYYVSLRHYYNWLTRQRY	206
203	APAKPEEDASPEELQRYYVSLRHYYNWLTRQRY	207
204	APEKPEEEASPEEVQRYYVSLRHYYNWLTRQRY	208
205	APEKPEEEASPEELQKYYVSLRHYYNWLTRQRY	209
206	APEKPEEDASPEELEQYEVSLRHYYNWLTRQRY	210
207	APAKPEADAEPEELQRYYVSLRHYYNWLTRQRY	211
208	APEKPEEDASEEETQRYYVSLRHYYNWLTRQRY	212
209	APEKPEEDSEPEELQRYYVSLRHYYNWLTRQRY	213
210	APEKIEEEASPEELQRYYVSLRHYYNWLTRQRY	214
211	APEKPEEDATAEELQRYYVSLRHYYNWLTRQRY	215
212	APEKPEEDTAPEELQRYYVSLRHYYNWLTRQRY	216
213	APEKPEEETSPEELQRYYVSLRHYYNWLTRQRY	217
214	APEKPEEDASEEELQRYYVALRHYYNWLTRQRY	218
215	APEKPEEDASPAELQRYYVELRHYYNWLTRQRY	219
216	APAKPEEDASPEELEQYYVSLRHYYNWLTRQRY	220
217	APEKPEEDASPAELQRYYVALRHYYNWLTRQRY	221
218	APEKPEEDSSPEELQRYYVALRHYYNWLTRQRY	222
219	APEKPEEEAAPEELQRYYVSLRHYYNWLTRQRY	223
220	APEKPEEAASAEELQRYYVSLRHYYNWLTRQRY	224
221	APEKPEEDAPEEELQRYYVSLRHYYNWLTRQRY	225
222	APEKPEEDASAEELQQYYVSLRHYYNWLTRQRY	226

223	APEKPEEAAEPEELQRYYVSLRHYYNWLTRQRY	227
224	APEKPGEEASPEELEQYYVSLRHYYNWLTRQRY	228
225	APEKPGEDAEPEELEQYYVSLRHYYNWLTRQRY	229
226	APEKPEEAASPEETQRYYVSLRHYYNWLTRQRY	230
227	APEKAEEDASPEELEQYYVSLRHYYNWLTRQRY	231
228	APEKPEEDASPEELQQYYVSLRAYYNWLTRQRY	232
229	APEKPEEDASPEELQRYYVSARHYYNWLTRQRY	233
230	APEKPEEDASPEEIQRYYVALRHYYNWLTRQRY	234
231	APEKPEEDATPEELQKYYVSLRHYYNWLTRQRY	235
232	APAKPPEDASPEELQRYYVELRHYYNWLTRQRY	236
233	APEKPEEAASPEELQQYYVSLRHYYNWLTRQRY	237
234	APAKPEEEASPEELQRYYVSLRHYYNWLTRQRY	238
235	APEKPEEDASHypEELQRYYVSLRHYYNWLTRQRY	239
236	APEKPEEEAPAEELQRYYVSLRHYYNWLTRQRY	240
237	APEKPEEDASPEELQRYYVEVRHYYNWLTRQRY	241
238	APEKTEEEASPEELQRYYVSLRHYYNWLTRQRY	242
239	APEKPEEDASEEELQRYEVSLRHYYNWLTRQRY	243
240	APEKPEEDASPEALQRYYVSLRHYYNWLTRQRY	244
241	APEKPEEEASPEEIQRYYVSLRHYYNWLTRQRY	245
242	APEKPEADASAEELQRYYVSLRHYYNWLTRQRY	246
243	APEKPEEEASPEELQQYYVSLRHYYNWLTRQRY	247
244	APEKPEEDAEPAELQRYYVSLRHYYNWLTRQRY	248

Table 1

and wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 or of a lysine at positions 6, 10, 11, 14, 17, 21 or 22, or to the carboxylic acid group of the side chain of an aspartate or a glutamate at position 14.

In an embodiment of the present invention, the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 and R^2 are as defined above;

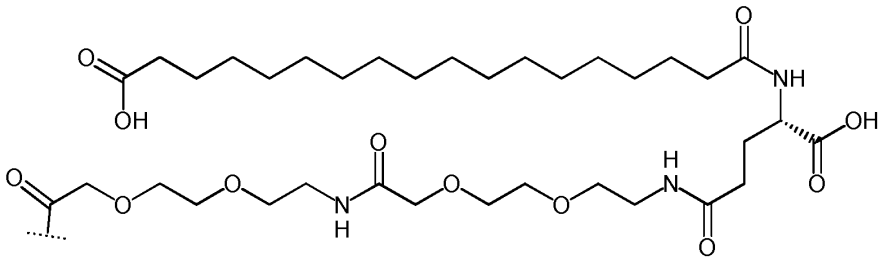
Z is an amino acid sequence of selected from Table 1; and
wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7 and consists of a lipophilic substituent X and a linker U,
wherein the linker U is attached to the amino acid side chain and X is attached to U, and
the linker U consists of one, two or three sub-moieties (U1, U2, U3).

In some embodiments, the lipophilic substituent, X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl.

In some embodiments the linker U consists of one, two or three sub-moieties (U^1, U^2, U^3) independently selected from the group consisting of Gly, Glu, γ-Glu, ε-Lys, Ser and OEG, or independently selected from the group consisting of γ-Glu and OEG.

In certain embodiments, the linker U is selected from the group consisting of γ-Glu, γ-Glu-γ-Glu, γ-Glu-OEG, OEG-OEG, γ-Glu-γ-Glu-OEG, γ-Glu-OEG-OEG.

In specific embodiments, the half-life extending group is C18DA-γ-Glu-OEG-OEG-, i.e.



In some embodiments of the present invention, the PYY analogue is a compound selected from the group consisting of compound 1 to compound 244 as defined below.

In some embodiments, the PYY analogue has a maximum of 15 amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has a maximum of 14 amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has a maximum of 13 amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has a maximum of 12 amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has a maximum of 11 amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 7 and 15 (i.e. 7, 8, 9, 10, 11, 12, 13, 14 or 15) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 8 and 14 (i.e. 8, 9, 10, 11, 12, 13 or 14) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 5 and 13 (i.e. 5, 6, 7, 8, 9, 10, 11, 12 or 13) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 7 and 13 (i.e. 7, 8, 9, 10, 11, 12 or 13) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 8 and 13 (i.e. 8, 9, 10, 11, 12 or 13) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 9 and 13 (i.e. 9, 10, 11, 12 or 13) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 10 and 13 (i.e. 10, 11, 12 or 13) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 8 and 12 (i.e. 8, 9, 10, 11 or 12) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 9 and 12 (i.e. 9, 10, 11 or 12) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 10 and 12 (i.e. 10, 11 or 12) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 9 and 11 (i.e. 9, 10 or 11) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue has between 11 and 12 (i.e. 11 or 12) amino acid modifications as compared to hPYY(3-36).

In some embodiments, the PYY analogue or compound of the above-mentioned embodiments is in the form of a salt, preferably in the form of a pharmaceutically acceptable salt.

The PYY analogues of the invention are able to bind to the human NPY2 receptor (hNPY2-R).

Binding to biological receptors can be measured by appropriate assays known in the art. For instance, binding of PYY analogues to the NPY2 receptor can be evaluated by radio-ligand binding competition assays, e.g. as described in Example 1, below.

In some embodiments of compounds of the present invention, the binding affinity (Ki) towards hNPY2 receptor is below 100 nM (e.g. 0.01 to 100 nM).
In some embodiments of compounds of the present invention, the binding affinity (Ki) towards hNPY2 receptor is below 50 nM (e.g. 0.01 to 50 nM).
In some embodiments of compounds of the present invention, the binding affinity (Ki) towards hNPY2 receptor is below 10 nM (e.g. 0.01 to 10 nM).

In some embodiments of compounds of the present invention, the binding affinity (Ki) towards hNPY2 receptor is below 5 nM (e.g. 0.01 to 5 nM).
In some embodiments of compounds of the present invention, the binding affinity (Ki) towards hNPY2 receptor is below 2 nM (e.g. 0.01 to 2 nM).

The PYY analogues of the invention activate the human NPY2 receptor, i.e. they are NPY2 agonists.

In general, it is preferred to use a biological assay which measures intracellular signalling caused by binding of the compound to the relevant receptor. Activation of the NPY2 receptor by compounds of the invention (which behave as agonists of the receptor) reduces cAMP concentrations effecting intracellular signalling pathways. Thus, reduction of cAMP or any other suitable parameter in cells expressing the receptor can be used to monitor agonist activity towards the receptor. The skilled person will be aware of suitable assay formats, and examples are provided below.

EC_{50} values may be used as a numerical measure of agonist potency at a given receptor. An EC_{50} value is a measure of the concentration of a compound required to achieve half of that compound's maximal activity in a particular assay, e.g. in the assay as described in Example 2, below.

In some embodiments of compounds of the present invention, the EC_{50} towards hNPY2 receptor is below 100 nM (e.g. 0.001 to 100 nM).
In some embodiments of compounds of the present invention, the EC_{50} towards hNPY2 receptor is below 50 nM (e.g. 0.001 to 50 nM).
In some embodiments of compounds of the present invention, the EC_{50} towards hNPY2 receptor is below 10 nM (e.g. 0.001 to 10 nM).
In some embodiments of compounds of the present invention, the EC_{50} towards hNPY2 receptor is below 5 nM (e.g. 0.001 to 5 nM).
In some embodiments of compounds of the present invention, the EC_{50} towards hNPY2 receptor is below 2 nM (e.g. 0.001 to 2 nM).

As mentioned above the PYY analogues or compounds of the present invention are generally soluble around pH 7 and 6. There are several techniques known to the skilled person in the art how to determine solubility. One such experiment is described below under Example 3. If specific solubility in mg/ml is provided herein, it is referred to the solubility determination as in Example 3.

In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 1.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 3.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 5.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is equal to or greater than 7.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is equal to or greater than 8.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2).

In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 1.0 mg/ml around pH 7 (e.g. at pH 6.8 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 5.0 mg/ml around pH 7 (e.g. at pH 6.8 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 7.0 mg/ml around pH 7 (e.g. at pH 6.8 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is equal to or greater than 8.0 mg/ml around pH 7 (e.g. at pH 6.8 ± 0.2).

In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 1.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2) and around pH 7 (e.g. at pH 6.8 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 3.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2) and around pH 7 (e.g. at pH 6.8 ± 0.2).

In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 5.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2) and around pH 7 (e.g. at pH 6.8 ± 0.2).
In some embodiments, the solubility of the PYY analogues or compounds of the invention is greater than 6.0 mg/ml around pH 6 (e.g. at pH 6.1 ± 0.2) and around pH 7 (e.g. at pH 6.8 ± 0.2).

In some embodiments the PYY analogues or compounds of the invention have favourable pharmacokinetic properties. In this regard, in some embodiments of the invention, the in-vivo half-life of the PYY analogues or compounds is at least 3 hours in the mouse (NMRI mice, see measurement described in Example 5). In some embodiments, the in-vivo half-life is at least 5 hours in the mouse. In some embodiments, the in-vivo half-life is at least 7 hours in the mouse. In some embodiments, the in-vivo half-life is at least 10 hours in the mouse.

The invention further provides a composition comprising a PYY analogue as described above. The composition may be a pharmaceutical composition, and may comprise a pharmaceutically acceptable carrier, excipient or vehicle.

The invention further provides a method for the synthesis of a PYY analogue as described above. The method may comprise the steps of synthesising the peptide by solid-phase or liquid-phase methodology, and optionally isolating and/or purifying the final product.

METHOD OF TREATMENT

The present invention is directed to PYY analogues or a compound according to the above-mentioned embodiments, which are useful in the prevention and/or treatment of a disease and/or condition associated with or modulated by NPY2 receptor activity, including but not limited to the treatment and/or prevention of obesity and various obesity-related conditions, diseases, or co-morbidities, such as type 2 diabetes and NASH (non-alcoholic steatohepatitis).

The compounds described herein find use, inter alia, in preventing weight gain or promoting weight loss. By "preventing" is meant inhibiting or reducing when compared to the

absence of treatment, and is not necessarily meant to imply complete cessation of weight gain. The peptides may cause a decrease in food intake and/or increased energy expenditure, and may have a beneficial effect on glucose control and/or on circulating cholesterol levels, being capable of lowering circulating LDL levels and increasing HDL/LDL ratio. Thus, the compounds of the invention can be used for direct or indirect therapy of any condition caused or characterised by excess body weight, such as the treatment and/or prevention of obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, and obesity related sleep apnea. They may also be used for the prevention of conditions or treatment of obesity associated co-comorbidities caused or characterised by inadequate glucose control or dyslipidaemia (e.g. elevated LDL levels or reduced HDL/LDL ratio), Type 2 diabetes, metabolic syndrome, hypertension, atherogenic dyslipidemia, and cardiovascular diseases such as atherosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease, and cancer. Their effects in these conditions may be as a result of or associated with their effect on body weight, or may be independent thereof.

As mentioned above the PYY analogues or compounds according to the above mentioned embodiments are useful in the reduction of food intake, promotion of weight loss, and inhibition or reduction of weight gain. As a result, they may be used for treatment of a variety of conditions, diseases, or disorders in a subject, including, but not limited to, obesity and various obesity-related conditions, diseases, or co-morbidities, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea, cardiovascular disease, hepatic steatosis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and cancer. The subject may be affected by obesity accompanied by at least one weight-related co-morbid condition, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea, cardiovascular disease, hepatic steatosis, NAFLD and NASH. It will be understood that the PYY analogues may thus be administered to subjects affected by conditions characterised by inadequate control of appetite or otherwise over-feeding, such as binge-eating disorder and Prader-Willi syndrome. It will be clear that the analogues can be used for treatment of combinations of the conditions described.

Thus, the invention provides a PYY analogue for use in a method of medical treatment, e.g. for treating, inhibiting or reducing weight gain, promoting weight loss and/or reducing

excess body weight. Treatment may be achieved, for example, by control of appetite, feeding, food intake, caloric intake and/or energy expenditure.

The invention also provides a PYY analogue of the invention for use in a method of treating obesity as well as associated diseases, disorders and health conditions, including, but not limited to, morbid obesity, obesity prior to surgery, obesity-linked inflammation, obesity-linked gallbladder disease and obesity related sleep apnea and respiratory problems, degeneration of cartilage, osteoarthritis, and reproductive health complications of obesity or overweight such as infertility. The subject may be affected by obesity accompanied by at least one weight-related co-morbidity, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea, cardiovascular disease, cancer, hepatic steatosis, NAFLD and NASH.

The invention also provides a PYY analogue of the invention for use in a method of prevention or treatment of conditions mentioned above.

Accordingly, the present invention relates to a PYY analogue or a compound according to the above-mentioned embodiments for use as a medicament.

Furthermore, the present invention relates to the use of a PYY analogue or a compound according to the above-mentioned embodiments for the treatment and/or prevention of a disease and/or condition associated with or modulated by NPY2 receptor activation.

Furthermore, the present invention relates to the use of a PYY analogue or a compound according to the above mentioned embodiments for the treatment and/or prevention of obesity and various obesity-related conditions, diseases, or co-morbidities, such as type 2 diabetes and NASH (non-alcoholic steatohepatitis) and others as mentioned above.

In a further aspect the present invention relates to the use of a PYY analogue or a compound according to the above mentioned embodiments for the preparation of a medicament for the treatment and/or prevention of above-mentioned diseases and conditions.

In a further aspect the present invention relates to methods for the treatment or prevention of above mentioned diseases and conditions, which method comprises the administration

of an effective amount of a PYY analogue or a compound according to the above-mentioned embodiments to a human being.

The dose range of the compounds of general formula 1 applicable per week is usually from 0.01 to 100 mg for humans (subcutaneous administration).

The actual pharmaceutically effective amount or therapeutic dosage will usually depend on factors known by those skilled in the art such as age and weight of the patient, route of administration and severity of disease. In any case, the compounds will be administered at dosages and in a manner, which allows a pharmaceutically effective amount to be delivered based upon patient's unique condition.

COMBINATION THERAPY

A PYY analogue of the invention may be administered as part of a combination therapy together with another active agent for the treatment of the disease or disorder in question, e.g. an anti-obesity agent, an anti-diabetic agent, an agent for treatment of metabolic syndrome, an anti-dyslipidemia agent, an anti-hypertensive agent, a proton pump inhibitor, or an anti-inflammatory agent. In such cases, the two active agents may be given together or separately, e.g. as constituents in the same pharmaceutical composition or formulation, or as separate formulations.

Thus, a peptide of the invention may be used in combination with an anti-obesity agent of known type. The anti-obesity agent may be a GIP or GLP-1 receptor agonist (including GLP-1 or a GLP-1 analogue, exendin-4 or an exendin-4 analogue, any other GLP-1 receptor agonist including Liraglutide (Saxenda™), Semaglutide, Dulaglutide, Albiglutide, MK-8521, or a glucagon-GLP-1 dual agonist (e.g. HM-12525, SAR-425899, MEDI-0382, NN-9277 or as described in WO2008/101017, WO2008/152403, WO2010/070252, WO2010/070253, WO2010/070255, WO2010/070251, WO2011/006497, WO2011/160630, WO2011/160633, WO2013/092703, WO2014/041195, WO2015/055801, WO2015/055802, WO2016/166289), oxyntomodulin or an oxyntomodulin analog (e.g. TT-401) or a GLP-1/GIP dual agonist (e.g. Tirzepatide or as described in WO2013/164483), or a GLP-1/GIP/glucagon triple agonists (e.g. NN-9423 or

as described in WO2015/067716, WO2016/198624, WO2017/116204, WO2017/116205, WO2018/100134, WO2018/100135).

The anti-obesity agent may be amylin or an amylin analogue, e.g. pramlintide, NN-9838, or an amylin (or calcitonin) analogue disclosed in WO2012/168430, WO2012/168431, WO2012/168432, WO2015/040182, WO2015/071229, WO2016/146739, WO2018/046719, or WO2018/172390.

Alternatively, the anti-obesity agent may be Orlistat™, Sibutramine™, phentermine, a melanin concentrating hormone receptor 1 antagonist, CCK, leptin analogue, a GOAT inhibitor, a ghrelin-receptor antagonist, a further neuropeptide Y (NPY) analogue, a NPY4 receptor agonist, a NPY5 receptor antagonist, a cannabinoid receptor 1 antagonist, a beta-3 agonist, a lipase inhibitor, Human proIslet Peptide (HIP), a melanocortin receptor 4 agonist, as well as analogues thereof.

Moreover, a peptide of the invention may have some benefit if administered in combination with an anti-diabetic agent of known type, including, but not limited to, metformin, a sulfonylurea, a glinide, a DPP-IV inhibitor, a glitazone, a GLP-1 receptor agonist (including GLP-1 or a GLP-1 analogue, an exendin-4 or an exendin-4 analogue, any other GLP-1 receptor agonist including Liraglutide (Victoza™), Semaglutide, Dulaglutide, Albiglutide, MK-8521, or a glucagon-GLP-1 dual agonist (e.g. HM-12525, SAR-425899, MEDI-0382, NN-9277 or as described in WO2008/101017, WO2008/152403, WO2010/070252, WO2010/070253, WO2010/070255, WO2010/070251, WO2011/006497, WO2011/160630, WO2011/160633, WO2013/092703, WO2014/041195, WO2015/055801, WO2015/055802, WO2016/166289), oxyntomodulin, or an oxyntomodulin analog (e.g. TT-401), or a SGLT2 inhibitor (i.e. an inhibitor of sodium-glucose transport, e.g. a gliflozin such as empagliflozin, canagliflozin, dapagliflozin or ipragliflozin), a GPR40 agonist (FFAR1/FFA1 agonist, e.g. fasiglifam), or an insulin or an insulin analogue. Examples of appropriate insulin analogues include, but are not limited to, Lantus™, Novorapid™, Humalog™, Novomix™, Actraphane™ HM, Levemir™ Degludec™ and Apidra™. Other relevant anti-diabetic agents in this connection include GLP-1 receptor agonists, such as exenatide (Byetta™ and Bydureon™ exendin-4) and Byetta LAR™, and lixisenatide (Lyxumia™).

According to more specific embodiments, the PYY analogue of the present invention is administered as part of a combination therapy together with

- a GLP-1 receptor agonist selected from the group consisting of Liraglutide, Semaglutide, Dulaglutide and Albiglutide or a glucagon-GLP-1 dual agonist described in WO2011/006497, WO2014/041195, WO2015/055801, WO2015/055802, WO2016/166289 or
- an amylin receptor agonist selected from the group consisting of pramlintide or an amylin analogue disclosed in WO2012/168430, WO2012/168431, WO2012/168432, WO2015/040182, WO2016/146739, or WO2018/046719.

A peptide of the invention may further be used in combination with medications targeting cardiovascular diseases treating hypertension, dyslipidemia, inflammation and platelet function. The medication treating hypertension can be selected from the group including, but not limited to, an angiotensin-converting enzyme inhibitor, an angiotensin II receptor blocker, a diuretic, a beta-blocker or a calcium channel blocker.

A peptide of the invention may still further be used in combination with an anti-dyslipidemia agent of known type, including, but not limited to, a statin, a fibrate, a niacin, a PSCK9 (Proprotein convertase subtilisin/kexin type 9) inhibitor, or a cholesterol absorption inhibitor.

A peptide of the invention may also be used in combination with a proton pump inhibitor (i.e. a pharmaceutical agent possessing pharmacological activity as an inhibitor of H^+/K^+-ATPase) of known type, including, but not limited to, an agent of the benzimidazole derivative type or of the imidazopyridine derivative type, such as Omeprazole™, Lansoprazole™, Dexlansoprazole™, Esomeprazole™, Pantoprazole™, Rabeprazole™, Zolpidem™, Alpidem™, Saripidem™ or Necopidem™.

In addition, with regard to anti-inflammatory treatment, a peptide of the invention may be beneficial if administered in combination with an anti-inflammatory agent of known type, including, but not limited to:

steroids and corticosteroids, such as beclomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and hydrocortisone;

non-steroidal anti-inflammatory agents (NSAIDs), such as propionic acid derivatives (e.g. alminoprofen, benoxaprofen, bucloxic acid, carprofen, fenbufen, fenoprofen, fluprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, miroprofen, naproxen, oxaprozin,

pirprofen, pranoprofen, suprofen, tiaprofenic acid and tioxaprofen); acetic acid derivatives (e.g. indomethacin, acemetacin, alclofenac, clidanac, diclofenac, fenclofenac, fenclozic acid, fentiazac, furofenac, ibufenac, isoxepac, oxpinac, sulindac, tiopinac, tolmetin, zidometacin and zomepirac); fenamic acid derivatives (e.g. flufenamic acid, meclofenamic acid, mefenamic acid, niflumic acid and tolfenamic acid); biphenylcarboxylic acid derivatives (e.g. diflunisal and flufenisal); oxicams (e.g. isoxicam, piroxicam, sudoxicam and tenoxicam); salicylates (e.g. acetylsalicylic acid and sulfasalazine); and pyrazolones (e.g. apazone, bezpiperylon, feprazone, mofebutazone, oxyphenbutazone and phenylbutazone);

COX II inhibitors, such as rofecoxib and celecoxib; preparations of interferon beta (e.g. interferon beta-1a or interferon beta-1b);

and certain other compounds, such as 5-aminosalicylic acid and prodrugs and pharmaceutically acceptable salts thereof.

PREPARATION

General procedure for solid phase synthesis of peptides

All peptides were synthesized by standard Fmoc-based solid phase peptide chemistry on a Tentagel S RAM resin (loading 0.23-0.25 mmol/g, bead size 90 µm) supplied by Iris Biotech GmbH or Rapp Polymere GmbH.

The following protected amino acids were used: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu-OtBu, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Dde)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH. The L-form of the amino acid building blocks was utilized if not specified otherwise. The modular half-life extending group was built up by solid-phase peptide synthesis (SPPS) using protected building blocks such as, but not limited to, 18-(tert-butoxy)-18-oxooctadecanoic acid (C18DA(tBu)), 2-[2-[2-[[2-[2-[2-(9H-fluoren-9-ylmethoxycarbonyl-amino)ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (Fmoc-OEG-OEG-OH), 2-

[2-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethoxy]ethoxy] acetic acid (Fmoc-OEG-OH) and Fmoc-Glu-OtBu.

The amino acids, Fmoc-Glu-OtBu, Oxyma and DIC, were purchased from standard suppliers, e.g. Bachem, Novabiochem, ABCR, Iris Biotech GmbH, Sigma-Aldrich. 18-(Tert-butoxy)-18-oxooctadecanoic acid (C18DA(tBu)) was supplied by Cool Pharm Ltd. or AstraTech, 2-[2-[2-[[2-[2-[2-(9H-Fluoren-9-ylmethoxycarbonyl-amino)ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (Fmoc-OEG-OEG-OH) was supplied by ABCR GmbH & CO. KG or Iris Biotech GmbH, 2-[2-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethoxy]ethoxy] acetic acid (Fmoc-OEG-OH) was supplied by Combi Blocks Inc., Iris Biotech GmbH or Hangzhou APIChem Technology Co., Ltd. 3-Methylbutanoic acid was supplied by Sigma-Aldrich GmbH.

Assembly of peptides started from the C-terminus by stepwise chain elongation towards the N-terminus according to the respective sequences until the N-terminal capping group was reached. Deprotection of the side chain of the branching amino acid, e.g. Lys(Dde), was followed by assembly of the half-life extending group.

The PYY analogues were obtained as TFA salts from the cleavage/deprotection or from the HPLC purification. The trifluoroacetate can be exchanged by common procedures, such as resin-ion exchange procedures, e.g. as disclosed in *Roux, St. et al. J. Pept. Sci. 2008; 14: 354-359.*

Synthesis Method 1 (S01)

Peptides were synthesized by microwave-assisted solid-phase peptide synthesis (SPPS) on a CEM Liberty Blue Peptide Synthesizer at 0.25 mmol scale on Tentagel S RAM resin using the Fmoc strategy.

Standard coupling of amino acids was performed with 4 eq of suitably protected amino acid in DMF (0.2 mol/l, 5 ml), 4 eq of Oxyma in DMF (1 mol/l, 1 ml) and 8 eq DIC in DMF (1 mol/l, 2 ml) at 90 °C for 4 min. Fmoc-Arg(Pbf)-OH was coupled 2 times at 90 °C for 4 min, Fmoc-His(Trt)-OH was coupled 2 times at 50 °C for 12 min and Fmoc-Glu-OtBu was coupled 4 times at 50 °C for 12 min. Fmoc-OEG-OH, Fmoc-OEG-OEG-OH and C18DA(tBu) were coupled 2 times for 4 min at 90 °C. Capping of the N-terminus was achieved by coupling 3-methylbutanoic acid 3 times at 90 °C for 4 min.

N^{α} Fmoc deprotection was performed with 20% piperidine/DMF (10 ml) for 1 min at 90 °C. Deprotection of the Lys(Dde)-group was carried out 2 times with 5% hydrazine hydrate in DMF (10 ml) for 3 min at 90 °C.

Raw products were washed on resin with DCM and dried prior to cleavage. Cleavage from resin and deprotection was performed with a mixture of 95% TFA/water (10 ml) and triisopropylsilane (250 µl) for 40 min at 42 °C. Crude peptides were precipitated with cold diethyl ether, dissolved in 50% acetonitrile/water and purified by preparative HPLC (P01).

Synthesis method 2 (S02)

Peptides were synthesized by SPPS on a MultiSynTech SYRO II at 0.2 mmol scale.

Standard coupling of amino acids was achieved by using 4 eq of suitably protected amino acids dissolved in 0.5 mol/l Oxyma-DMF solution (0.5 mol/l, 1.6 ml) and 4.5 eq DIC (0.5 mol/l, 1.8 ml) in DMF. Fmoc-Phe-OH was dissolved in 0.5 mol/l Oxyma-NMP and 4 eq were used for coupling (0.5 mol/l, 1.6 ml).

Coupling time of the first 15 amino acids starting from the C-terminus was 2 h at RT. Subsequent couplings were realized by double coupling (2 × 2h at RT). Capping of the N-terminus was achieved by coupling with 3-methylbutanoic acid (2 × 2h at RT). Deprotection of Lys(Dde)-group was carried out selectively using 5 % hydrazine in DMF (5 × 5 min at RT, 4 ml) followed by coupling of 4eq Fmoc-OEG-OEG-OH (3 × 2h at RT), 4 eq Fmoc-Glu-OtBu and 4eq C18DA(tBu) (2 × 2 h at RT).

N^{α} Fmoc deprotections were performed using 40% piperidine in NMP (4 ml) for 3 min followed by 20% piperidine in NMP (4 ml) for 15 min at RT.

Peptides were cleaved from resin and side-chains deprotected by adding 15 ml 95:2:1:2 TFA:DODT:TES:water for 4 h at RT or 45 min at 45 °C. The peptides were precipitated with cold diethyl ether, dissolved in acetonitrile/water and purified by preparative HPLC(P02).

Synthesis method 3 (S03)

Peptides were synthesized using microwave-assisted SPPS on a Biotage Alstra Synthesizer at 0.2 mmol scale.

Standard coupling of amino acids was achieved by using 5 eq of suitably protected amino acid dissolved in 0.5 mol/l Oxyma-DMF solution (0.5 mol/l, 2 ml) and 5 eq DIC in DMF

(0.5 mol/l, 2 ml). Coupling of the first 15 amino acids starting from the C-terminus was achieved by heating for 5 min to 75 °C. Subsequent couplings were realized by double coupling (2 x 5 min at 75 °C).

N^{α} Fmoc deprotections were carried out using 20% piperidine in DMF for 30 sec followed by 20% piperidine for 3 min at 75 °C.

Special conditions were used for: coupling of Fmoc-His(Trt)-OH (2 × 12 min at 50 °C) and subsequent Fmoc-deprotection at RT (20% piperidine in DMF for 3 min followed by 20% piperidine in DMF for 10 min); coupling of Fmoc-Glu-OtBu (2 × 6 min at 75 °C) and Fmoc-Arg(Pbf)-OH (2 × 5 min at 75 °C); coupling of Fmoc-Asp(tBu)-OH and subsequent Fmoc-deprotection at RT (20% piperidine in DMF for 3 min followed by 20% piperidine in DMF for 10 min); Dde deprotection with 5 % hydrazine in DMF (5 × 5 min at RT); coupling of 4 eq Fmoc-OEG-OEG-OH (3 × 10 min at 75 °C) and 2.5 eq C18DA(tBu) (2 × 10 min at 75 °C).

Cleavage from resin and side-chain deprotection was performed by adding 8 ml 95:2:1:2 TFA:DODT:TES:water for 4 hours at RT or 45 min at 45 °C. Crude peptides were precipitated with cold diethyl ether, dissolved in 50% acetonitrile/water and purified by preparative HPLC (P02).

Purification Method 1 (P01)

Crude peptides were purified by reversed phase chromatography using an Agilent preparative HPLC-MS System with preparative pumps G1361A, a diode array detector G1315B, a mass-spectrometer G1956B and a fraction collector CTC PAL IFC. A Waters XSelect CSH Prep C18 column (130 Å, 5 µm, OBD, 30 mm x 150 mm) served as stationary phase. The mobile phase was run with a gradient of buffer A (0.1% TFA in H_2O) and buffer B (0.1% TFA in ACN, gradient: 20-42% over 44 min) at a flow rate of 50 ml/min at 40 °C. The relevant fractions were pooled and lyophilized. The final product was characterized by analytical HPLC-MS (A01).

Purification Method 2 (P02)

Crude peptides were purified by reversed phase HPLC using a Waters preparative HPLC with C8 column (Reprosil Gold 200 Å, 5µm, 40 mm × 250 mm), preparative pumps (waters 2545), UV/VIS detector (Waters 2489) and a Waters fraction collector III. The

mobile phase was run with a gradient of buffer A (0.1% TFA in H_2O) and buffer B (0.1% TFA in ACN, gradient: 35-45% B over 10 min) at a flow rate of 50 ml/min at RT. Relevant fractions were analysed, pooled and lyophilized. The final product was characterized by analytical UPLC-MS (A02).

Analytical Method 1 (A01)

Peptide purity and mass were determined by analytical HPLC-MS on a Kinetex C8 column (Phenomenex, 100 Å, 2.6 µm, 4.6 mm x 150 mm) using a Waters Acquity HPLC System equipped with 3100 Mass Detector. Analysis was performed by gradient elution with buffer A (0.3% TFA in H_2O) and buffer B (0.24% TFA in ACN) at a temperature of 40 °C. Details of the gradient and flow rates are summarized in the table below. Retention times and masses were recorded.

Method Name:	A01	Gradient/ Solvent Time [min]	% Sol [Water 0.3% TFA (v/v)]	% Sol [ACN 0.24% TFA (v/v)]	Flow [ml/min]	Temp [°C]
Device description:	Waters Acquity with 3100 MS	0.0	65.0	35.0	0.5	40.0
Column:	Kinetex C8_4.6 x 150 mm_2.6 µm	15.0	45.0	55.0	0.5	40.0
Column producer:	Phenomenex	16.0	10.0	90.0	1.0	40.0
		17.01	65.0	35.0	1.0	40.0
		18.0	65.0	35.0	1.0	40.0

Analytical Method 2 (A02)

Analytical UPLC-MS was performed on a Waters Acquity class H using a Waters Acquity UPLC C18 column (peptide CSH™ 130Å, 1.7 µm, 2.1 mm × 100 mm) and a gradient flow of buffer C (0.3% TFA in H_2O) and buffer D (10% H_2O + 0.3 % TFA in ACN; 38-48% D over 14 min) connected to a SQ Detector 2 (ESI, Waters).

List of abbreviations

ACN: acetonitrile

Boc: tert-butyloxycarbonyl

C18DA(tBu): 18-(tert-butoxy)-18-oxooctadecanoic acid

DPBS: Dulbecco's phosphate-buffered saline

DCM: dichloromethane

DIC: diisopropylcarbodiimide

DIPEA: diisopropylethylamine

Dde: (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl

DMF: N,N-dimethylformamid

DODT: 3,6-dioxa-1,8-octanedithiol

Fmoc: 9H-fluoren-9-ylmethoxycarbonyl

Fmoc-OEG-OH: 2-[2-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethoxy]ethoxy] acetic acid

Fmoc-OEG-OEG-OH: 2-[2-[2-[[2-[2-[2-(9H-fluoren-9-ylmethoxycarbonyl-amino)ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

HTRF: homogeneous time resolved fluorescence

IBMX: 3-isobutyl-1-methylxanthine

iVal: 3-methylbutanoyl (isovalerianoyl)

MRT: mean residence time

NMP: 1-methyl-pyrrolidine-2-one

Oxyma: 2-cyano-2-(hydroxyimino)acetic acid ethyl ester

OEG: 2-[2-(2-aminoethoxy)ethoxy]acetic acid

Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl

Rt: retention time

RT: room temperature

SPPS: solid-phase peptide synthesis

tBu: tert-butyl

Trt: trityl

TES: triethylsilane

TFA: trifluoroacetic acid

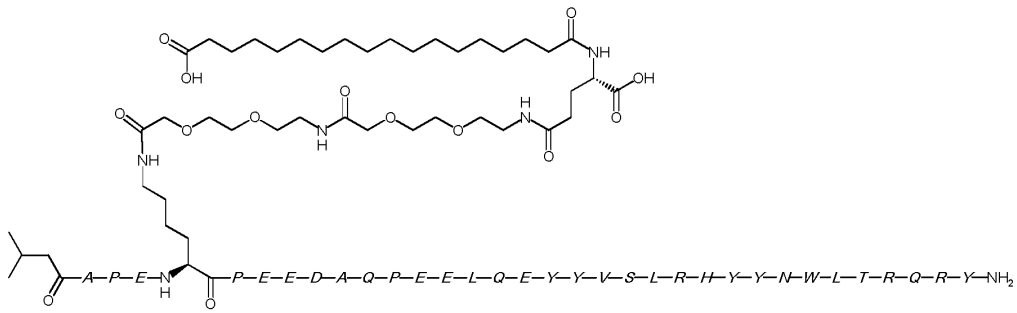
The following compounds were synthesised. All compounds were obtained as TFA salts:

Compound 1

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13Q,18Q,19E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAQPEELQEYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.17 min; m/3: 1668.0 m/4: - m/5: -

Compound 2

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,5Hyp,7K,9E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-AHypEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5003.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.29 min; m/3: 1668.0 m/4: - m/5: -

Compound 3

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPAELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4943.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.86 min; m/3: - m/4: 1237.0 m/5: -

Compound 4

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,28Y,30W,31L,32Q]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEELQRYYVSLRHYYNWLQRQRY-NH2

MW (calculated): 5014.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.31 min; m/3: 1672.0 m/4: - m/5: -

Compound 5

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,12V,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEVSPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5029.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.02 min; m/3: - m/4: 1258.0 m/5: -

Compound 6

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,24A,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQRYYVSARHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.32 min; m/3: - m/4: 1241.0 m/5: -

Compound 7

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19A,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQAYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4902.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.00 min; m/3: - m/4: 1226.0 m/5: -

Compound 8

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASEEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4991.6 Da

Synthesis and purification methods: S02; P02

LCMS: A02; Rt: 12.75 min; m/3: 1665.3 m/4: 1248.9 m/5: 999.2

Compound 9

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17I,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEIQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5029.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.60 min; m/3: 1677.0 m/4: - m/5: -

Compound 10

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,17T,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEADASPEETQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4917.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.21 min; m/3: - m/4: 1230.0 m/5: -

Compound 11

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17I,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEIQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.79 min; m/3: - m/4: 1241.0 m/5: -

Compound 12

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13P,14G,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDAPGEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4957.6 Da

Synthesis and purification methods: S01; P01

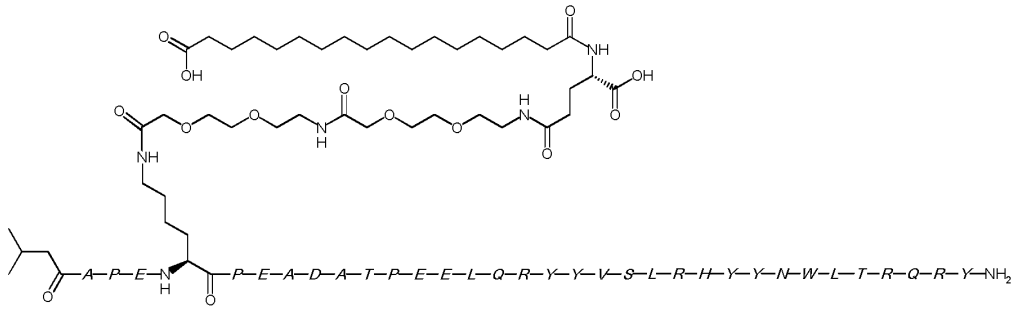
LCMS: A01; Rt: 10.87 min; m/3: - m/4: 1240.0 m/5: -

Compound 13

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAAPEELQQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4943.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.70 min; m/3: - m/4: 1237.0 m/5: -

Compound 14
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,10A,13T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEADATPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4943.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.96 min; m/3: 1648.0 m/4: - m/5: -

Compound 15
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,17I,18Q,21E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEIQRYEVSLRHYYNWLTRQRY-NH2
MW (calculated): 4953.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.22 min; m/3: 1652.0 m/4: - m/5: -

Compound 16

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,22V,23T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEETQRYYVTLRHYYNWLTRQRY-NH2

MW (calculated): 4989.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.28 min; m/3: - m/4: 1248.0 m/5: -

Compound 17

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPAELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4929.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.77 min; m/3: 1643.0 m/4: - m/5: -

Compound 18

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.27 min; m/3: - m/4: 1251.0 m/5: -

Compound 19

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEADASPEELQQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4901.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.90 min; m/3: 1634.0 m/4: - m/5: -

Compound 20
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAAPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5013.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.72 min; m/3: - m/4: 1253.0 m/5: -

Compound 21
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQKYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4959.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.62 min; m/3: - m/4: 1241.0 m/5: -

Compound 22
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYTSLRHYYNWLTRQRY-NH2

MW (calculated): 4989.6 Da

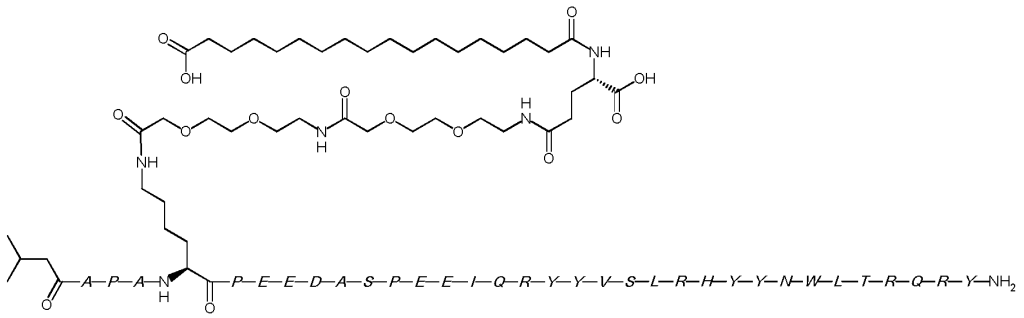
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.63 min; m/3: 1664.0 m/4: - m/5: -

Compound 23

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEIQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4929.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.82 min; m/3: - m/4: 1233.0 m/5: -

Compound 24

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,26K,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRKYYNWLTRQRY-NH2

MW (calculated): 4978.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.70 min; m/3: 1661.0 m/4: - m/5: -

Compound 25

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEEASAEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4975.6 Da

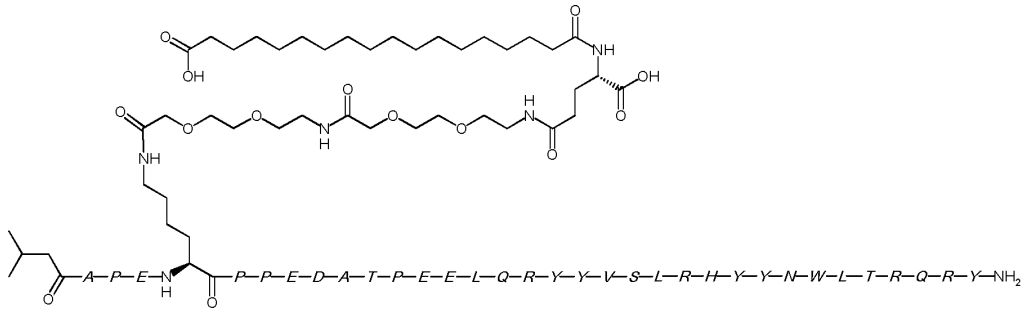
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.74 min; m/3: - m/4: 1245.0 m/5: -

Compound 26

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,13T,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PPEDATPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4969.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.74 min; m/3: - m/4: 1243.0 m/5: -

Compound 27

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PPEDAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4997.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.11 min; m/3: 1667.0 m/4: - m/5: -

Compound 28

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12T,18Q,22V,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDTSPEELQRYYVELRHYYNWLTRQRY-NH2

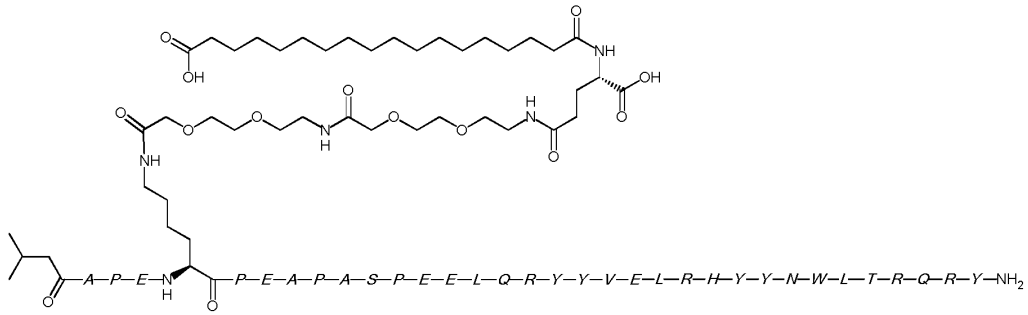
MW (calculated): 5059.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.77 min; m/3: 1688.0 m/4: - m/5: -

Compound 29

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,11P,18Q,22V,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEAPASPEELQRYYVELRHYYNWLTRQRY-NH2



MW (calculated): 4953.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.97 min; m/3: - m/4: 1239.0 m/5: -

Compound 30

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,14E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEADASEEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4961.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.47 min; m/3: - m/4: 1241.0 m/5: -

Compound 31

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,22V,23A,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEETQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.26 min; m/3: 1654.0 m/4: - m/5: -

Compound 32

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12T,18Q,21E,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDTSPEELQRYEVSLRHYYNWLTRQRY-NH2

MW (calculated): 4983.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.46 min; m/3: - m/4: 1247.0 m/5: -

Compound 33

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11P,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEPASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5011.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.85 min; m/3: - m/4: 1254.0 m/5: -

Compound 34

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,24T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSTRHYYNWLTRQRY-NH2

MW (calculated): 4975.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.20 min; m/3: 1660.0 m/4: - m/5: -

Compound 35

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAAPEETQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.45 min; m/3: - m/4: 1240.0 m/5: -

Compound 36

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8A,9E,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

AEEDAEPEELQRYYVSLRHYYNWLTRQRY-NH2

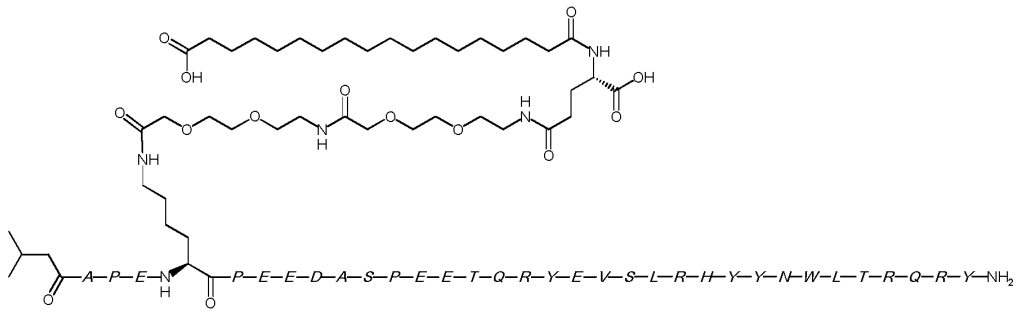
MW (calculated): 5003.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.34 min; m/3: - m/4: 1252.0 m/5: -

Compound 37

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,21E,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEETQRYEVSLRHYYNWLTRQRY-NH2



MW (calculated): 4941.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 8.97 min; m/3: 1649.0 m/4: - m/5: -

Compound 38

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,18Q,22V,23A,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PPEDASPEELQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4939.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.75 min; m/3: - m/4: 1236.0 m/5: -

Compound 39

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,10A,13E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PPADAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4939.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.18 min; m/3: - m/4: 1236.0 m/5: -

Compound 40

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,17T,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDATPEETQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4989.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 8.84 min; m/3: 1664.0 m/4: - m/5: -

Compound 41

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,13T,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEATPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5015.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.93 min; m/3: - m/4: 1255.0 m/5: -

Compound 42

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,22V,24T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEAASPEELQRYYVSTRHYYNWLTRQRY-NH2

MW (calculated): 4931.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.35 min; m/3: - m/4: 1234.0 m/5: -

Compound 43

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,11P,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEAPASPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4911.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.86 min; m/3: 1638.0 m/4: - m/5: -

Compound 44

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,28Y,30W,31I]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQRYYVSLRHYYNWITRQRY-NH2

MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.66 min; m/3: 1668.0 m/4: - m/5: -

Compound 45

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,16S,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPESLQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.96 min; m/3: - m/4: 1240.0 m/5: -

Compound 46
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5001.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.80 min; m/3: 1668.0 m/4: - m/5: -

Compound 47
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,10A,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PPADASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4897.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.60 min; m/3: - m/4: 1224.0 m/5: -

Compound 48
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12T,13E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDTEPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5059.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.08 min; m/3: 1688.0 m/4: - m/5: -

Compound 49

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQQYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.95 min; m/3: - m/4: 1251.0 m/5: -

Compound 50

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,22V,28Y,30W,31I]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASAEELQRYYVSLRHYYNWITRQRY-NH2

MW (calculated): 4961.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.33 min; m/3: - m/4: 1241.0 m/5: -

Compound 51

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8A,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)AEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4975.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.07 min; m/3: 1659.0 m/4: - m/5: -

Compound 52

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8A,9E,14E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) AEEDASEEELQRYYVSLRHYYNWLTRQRY-NH2

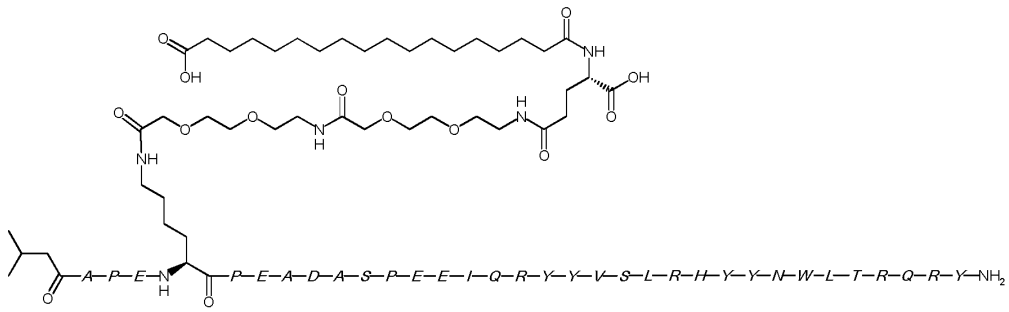
MW (calculated): 4993.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.76 min; m/3: - m/4: 1249.0 m/5: -

Compound 53

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,17I,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEADASPEEIQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4929.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.72 min; m/3: - m/4: 1233.0 m/5: -

Compound 54

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.77 min; m/3: - m/4: - m/5: 997.0

Compound 55
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,17A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEAQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4945.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.25 min; m/3: 1649.0 m/4: - m/5: -

Compound 56
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,10A,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEADAEPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.17 min; m/3: - m/4: 1244.0 m/5: -

Compound 57
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,14A,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASAEEIQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4961.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.50 min; m/3: - m/4: 1241.0 m/5: -

Compound 58

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.87 min; m/3: - m/4: 1241.0 m/5: -

Compound 59

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEETEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4948.5 Da

Synthesis and purification methods: S02; P02

LCMS: A02; Rt: 13.67 min; m/3: 1651.0 m/4: 1238.0 m/5: 990.5

Compound 60

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEAASPEELEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4916.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.30 min; m/3: - m/4: 1230.0 m/5: -

Compound 61

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,17I,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PPEDASPEEIQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4955.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.51 min; m/3: - m/4: 1240.0 m/5: -

Compound 62

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18E,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PGEDASPEELERYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4916.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.91 min; m/3: - m/4: - m/5: 983.0

Compound 63

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,26A,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRAYYNWLTRQRY-NH2

MW (calculated): 4921.5 Da

Synthesis and purification methods: S01; P01

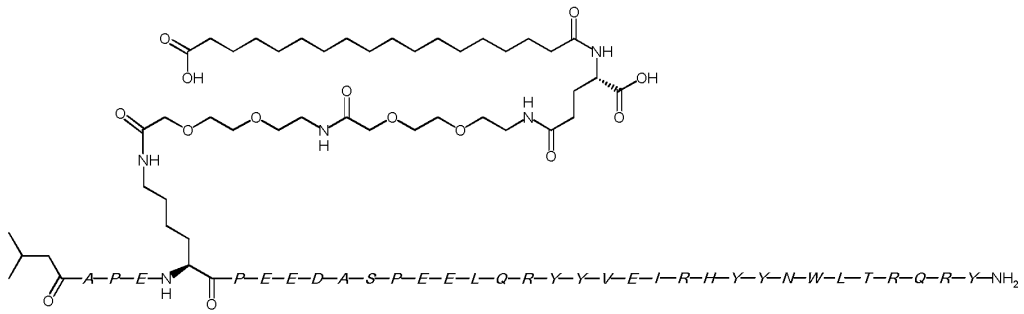
LCMS: A01; Rt: 10.10 min; m/3: 1641.0 m/4: - m/5: -

Compound 64

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PGEDASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4915.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.65 min; m/3: 1639.0 m/4: - m/5: -

Compound 65
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,23E,24I,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVEIRHYYNWLTRQRY-NH2



MW (calculated): 5029.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.82 min; m/3: - m/4: 1258.0 m/5: -

Compound 66
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASAEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4949.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.61 min; m/3: - m/4: 1238.0 m/5: -

Compound 67

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,13A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDSAPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.60 min; m/3: - m/4: 1248.0 m/5: -

Compound 68

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,19A,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQAYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4916.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.13 min; m/3: - m/4: 1230.0 m/5: -

Compound 69

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDSSAEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4977.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.26 min; m/3: - m/4: 1245.0 m/5: -

Compound 70

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,21E,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEAASPEELQRYEVSLRHYYNWLTRQRY-NH2

MW (calculated): 4909.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.55 min; m/3: 1638.0 m/4: - m/5: -

Compound 71

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,15S,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPSELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4959.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.96 min; m/3: - m/4: 1242.0 m/5: -

Compound 72

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,12G,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEGSPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.87 min; m/3: - m/4: 1248.0 m/5: -

Compound 73

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,26A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQRYYVSLRAYYNWLTRQRY-NH2

MW (calculated): 4935.6 Da

Synthesis and purification methods: S03; P02

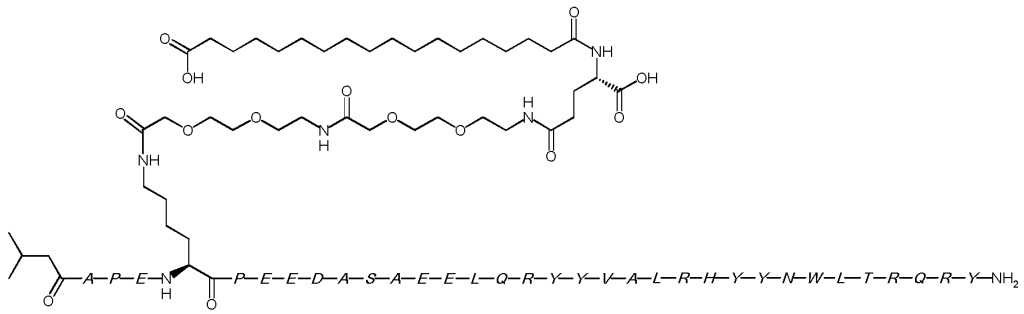
LCMS: A02; Rt: 12.23 min; m/3: 1646.8 m/4: 1235.0 m/5: 988.0

Compound 74

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASAEELQRYYVALRHYYNWLTRQRY-NH2



MW (calculated): 4945.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.72 min; m/3: 1650.0 m/4: - m/5: -

Compound 75

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,22V,23T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASAEELQRYYVTLRHYYNWLTRQRY-NH2

MW (calculated): 4975.6 Da

Synthesis and purification methods: S01; P01

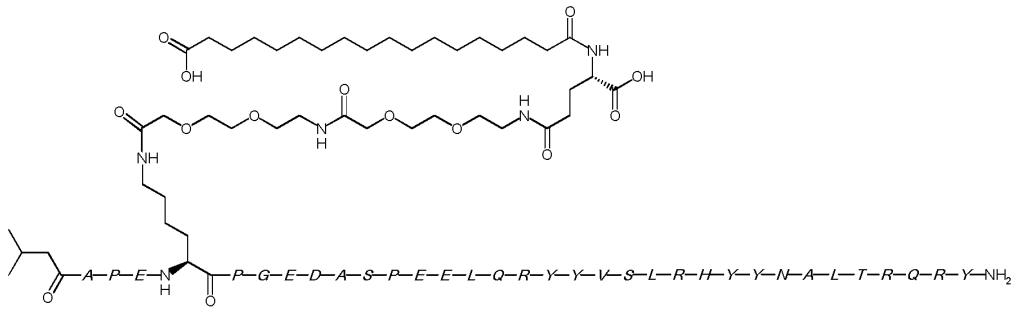
LCMS: A01; Rt: 10.74 min; m/3: - m/4: 1244.0 m/5: -

Compound 76

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18Q,22V,28Y,30A,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PGEDASPEELQRYYVSLRHYYNALTRQRY-NH2



MW (calculated): 4800.4 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.12 min; m/3: - m/4: 1201.0 m/5: -

Compound 77

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASEEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5061.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.44 min; m/3: - m/4: 1266.0 m/5: -

Compound 78

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,19Q,22V,23T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAEPEELQQYYVTLRHYYNWLTRQRY-NH2

MW (calculated): 5043.7 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 12.75 min; m/3: 1682.6 m/4: 1262.1 m/5: 1009.7

Compound 79

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,14E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASEEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5033.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.66 min; m/3: - m/4: 1260.0 m/5: -

Compound 80

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,17T,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEETQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4989.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.19 min; m/3: - m/4: 1248.0 m/5: -

Compound 81

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,24I,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSIRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.72 min; m/3: 1664.0 m/4: - m/5: -

Compound 82

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,16A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEALQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4913.6 Da

Synthesis and purification methods: S01; P01

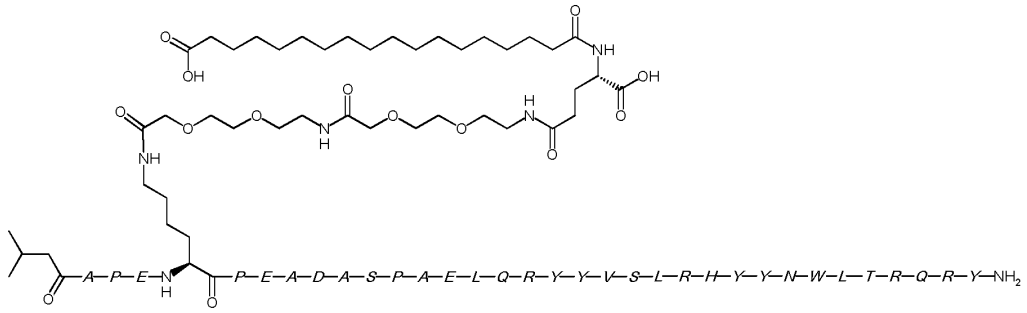
LCMS: A01; Rt: 10.28 min; m/3: - m/4: 1229.0 m/5: -

Compound 83

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEADASPAELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4871.5 Da

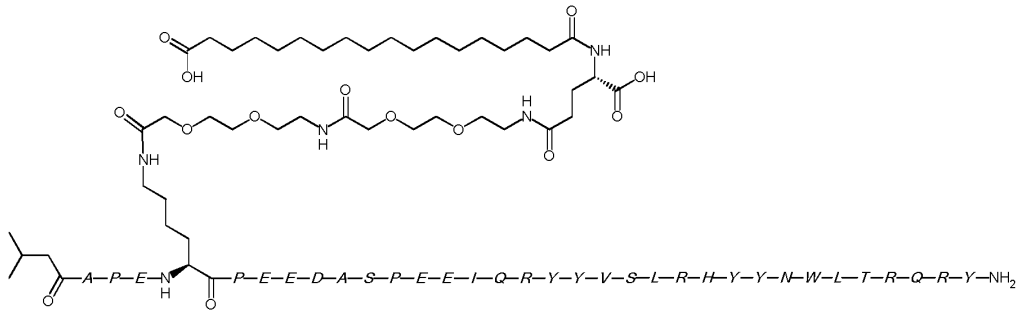
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.85 min; m/3: - m/4: 1219.0 m/5: -

Compound 84

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEEIQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.60 min; m/3: 1664.0 m/4: - m/5: -

Compound 85

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,21A,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYAVSLRHYYNWLTRQRY-NH2

MW (calculated): 4895.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.82 min; m/3: 1633.0 m/4: - m/5: -

Compound 86

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,18Q,21E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEADASPEELQRYEVSLRHYYNWLTRQRY-NH2

MW (calculated): 4895.5 Da

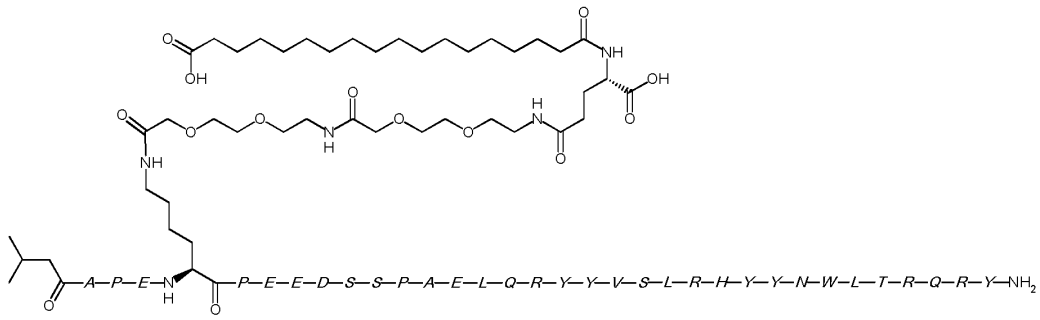
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.44 min; m/3: - m/4: 1224.0 m/5: -

Compound 87

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDSSPAELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4945.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.82 min; m/3: - m/4: 1237.0 m/5: -

Compound 88

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PPEDASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 4997.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.62 min; m/3: - m/4: 1250.0 m/5: -

Compound 89

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5043.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.08 min; m/3: - m/4: 1261.0 m/5: -

Compound 90

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,21E,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYEVSLRHYYNWLTRQRY-NH2

MW (calculated): 4953.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.46 min; m/3: 1651.0 m/4: - m/5: -

Compound 91

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13P,14A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDAPAEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5013.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 11.44 min; m/3: - m/4: 1254.0 m/5: -

Compound 92

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12T,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDTSPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5017.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.76 min; m/3: - m/4: 1256.0 m/5: -

Compound 93

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASAEELQKYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4933.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 12.03 min; m/3: 1646.0 m/4: 1234.3 m/5: 987.4

Compound 94

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEETQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5017.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.15 min; m/3: - m/4: 1256.0 m/5: -

Compound 95

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,15A,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPAEIQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4929.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.69 min; m/3: 1644.0 m/4: - m/5: -

Compound 96

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEADASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4929.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.70 min; m/3: 1644.0 m/4: - m/5: -

Compound 97
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.86 min; m/3: - m/4: 1244.0 m/5: -

Compound 98
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22S,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYSALRHYYNWLTRQRY-NH2
MW (calculated): 4959.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.71 min; m/3: 1654.0 m/4: - m/5: -

Compound 99
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5003.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.61 min; m/3: 1668.0 m/4: - m/5: -

Compound 100
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,6A,7K,9P,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PPEDASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4897.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.76 min; m/3: - m/4: 1225.0 m/5: -

Compound 101
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13T,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDATPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5043.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.92 min; m/3: - m/4: 1261.0 m/5: -

Compound 102
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,18Q,22V,23T,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVTLRHYYNWLTRQRY-NH2
MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.88 min; m/3: 1668.0 m/4: - m/5: -

Compound 103

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,14E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)

PEEDASEEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4961.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.03 min; m/3: 1654.0 m/4: - m/5: -

Compound 104

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASAEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4963.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.39 min; m/3: - m/4: 1241.0 m/5: -

Compound 105

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,19E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAEPEELQEYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5002.6 Da

Synthesis and purification methods: S01; P01

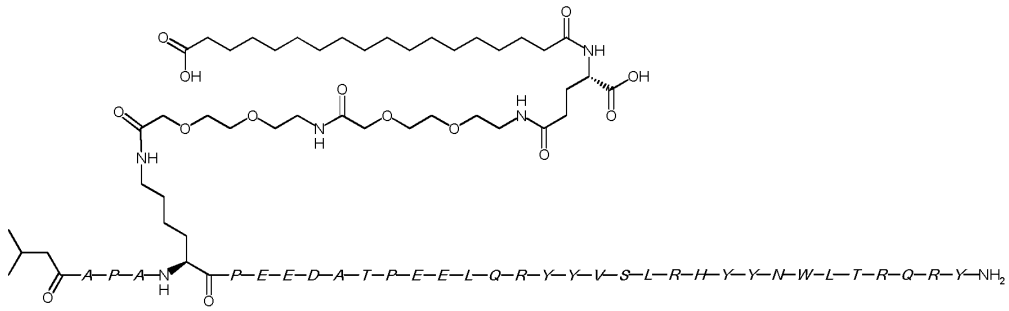
LCMS: A01; Rt: 10.41 min; m/3: - m/4: 1251.0 m/5: -

Compound 106

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,13T,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)

PEEDATPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4943.6 Da

Synthesis and purification methods: S01; P01

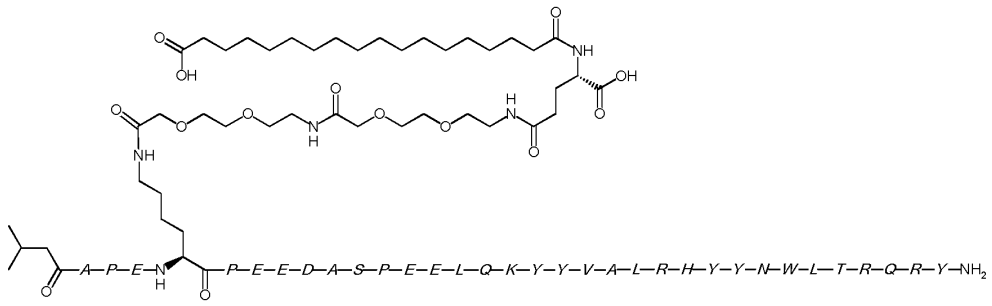
LCMS: A01; Rt: 10.03 min; m/3: - m/4: 1237.0 m/5: -

Compound 107

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19K,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEELQKYYVALRHYYNWLTRQRY-NH2



MW (calculated): 4943.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 11.80 min; m/3: 1648.6 m/4: 1236.9 m/5: 989.5

Compound 108

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5029.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 11.47 min; m/3: 1678.1 m/4: 1258.1 m/5: 1006.7

Compound 109

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4943.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.90 min; m/3: 1649.0 m/4: - m/5: -

Compound 110

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEEIQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5029.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.10 min; m/3: - m/4: 1258.0 m/5: -

Compound 111

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAEPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5071.7 Da

Synthesis and purification methods: S01; P01

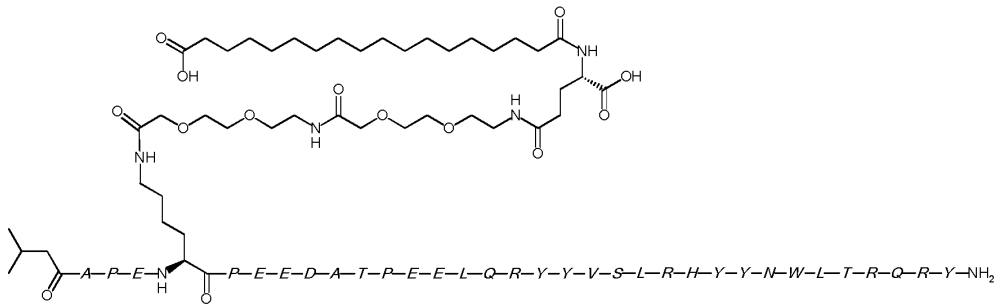
LCMS: A01; Rt: 10.17 min; m/3: - m/4: 1268.0 m/5: -

Compound 112

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDATPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.85 min; m/3: - m/4: 1252.0 m/5: -

Compound 113

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8A,9E,13T,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

AEEDATPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4975.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.22 min; m/3: 1659.0 m/4: - m/5: -

Compound 114

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,28H,29W,30Y,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEELQRYYVSLRHYHWYLTRQRY-NH2

MW (calculated): 5010.6 Da

Synthesis and purification methods: S02; P02

LCMS: A02; Rt: 12.38 min; m/3: 1671.2 m/4: 1253.9 m/5: -

Compound 115

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,16A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEEASPEALQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4943.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.09 min; m/3: - m/4: 1237.0 m/5: -

Compound 116

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAAAEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): .0 Da

Synthesis and purification methods: S01; P01

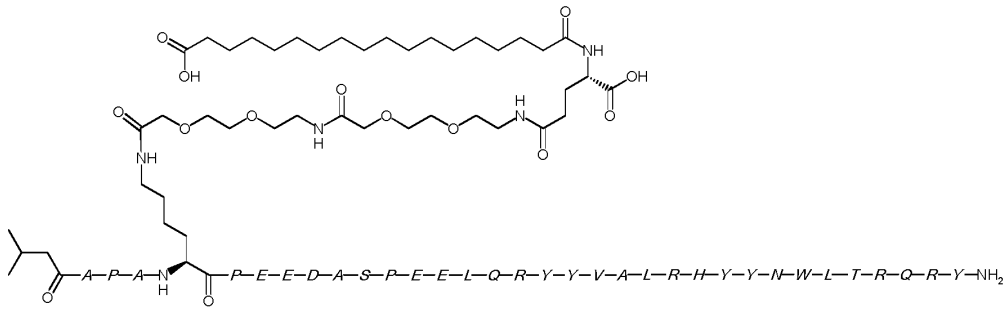
LCMS: A01; Rt: 11.36 min; m/3: - m/4: 1238.0 m/5: -

Compound 117

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEELQRYYVALRHYYNWLTRQRY-NH2



MW (calculated): 4913.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.08 min; m/3: - m/4: 1228.0 m/5: -

Compound 118

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASAAELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4903.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.49 min; m/3: 1636.0 m/4: - m/5: -

Compound 119

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,23T,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEEASPEELQRYYVTLRHYYNWLTRQRY-NH2

MW (calculated): 5015.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.99 min; m/3: - m/4: 1254.0 m/5: -

Compound 120
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,13E,14E,18Q,19E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PGEDAEEEELQEYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4962.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.78 min; m/3: - m/4: 1241.0 m/5: -

Compound 121
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,6A,7K,9E,10A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEADASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4871.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.95 min; m/3: 1624.0 m/4: m/5: -

Compound 122
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAAPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.55 min; m/3: 1658.0 m/4: - m/5: -

Compound 123

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,28W,29Y,30H,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRHYWYHLTRQRY-NH2

MW (calculated): 5010.6 Da

Synthesis and purification methods: S02; P02

LCMS: A02; Rt: 9.68 min; m/3: 1671.7 m/4: 1253.7 m/5: -

Compound 124

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDAAPEEIQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4971.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.55 min; m/3: - m/4: 1244.0 m/5: -

Compound 125

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18Q,22V,28Y,30W,31A]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PGEDASPEELQRYYVSLRHYYNWATRQRY-NH2

MW (calculated): 4873.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.06 min; m/3: 1625.0 m/4: - m/5: -

Compound 126

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,12S,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDSSPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4945.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.89 min; m/3: 1649.0 m/4: - m/5: -

Compound 127
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4903.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.80 min; m/3: - m/4: 1226.0 m/5: -

Compound 128
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13P,14A,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAPAEEIQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 11.28 min; m/3: 1658.0 m/4: - m/5: -

Compound 129
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,22T,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEETQRYYTALRHYYNWLTRQRY-NH2
MW (calculated): 4961.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.22 min; m/3: - m/4: 1241.0 m/5: -

Compound 130
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,12T,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDTSAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4991.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.30 min; m/3: 1665.0 m/4: - m/5: -

Compound 131
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,8A,9E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
AEEDASPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5002.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.10 min; m/3: - m/4: 1251.0 m/5: -

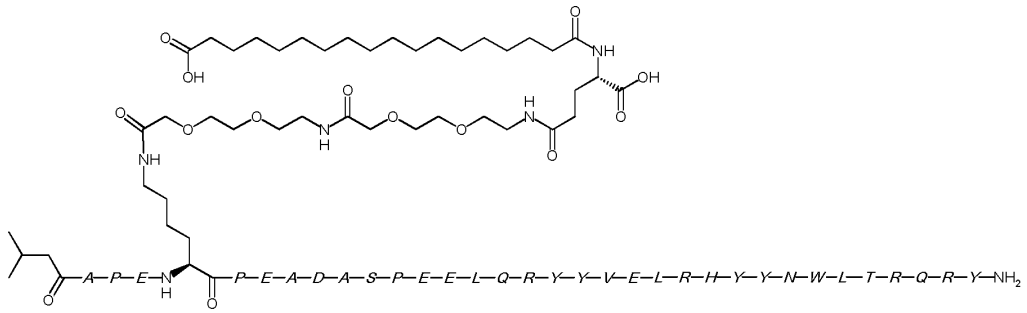
Compound 132
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,18Q,19K,22V,23T,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQKYYVTLRHYYNWLTRQRY-NH2
MW (calculated): 4973.6 Da

Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 11.78 min; m/3: 1659.4 m/4: 1244.6 m/5: 995.3

Compound 133
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17V,18Q,22V,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEEVQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5015.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.45 min; m/3: - m/4: 1254.0 m/5: -

Compound 134
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17I,18Q,22T,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEEIQRYYTELRHYYNWLTRQRY-NH2
MW (calculated): 5031.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.54 min; m/3: - m/4: 1258.0 m/5: -

Compound 135
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEADASPEELQRYYVELRHYYNWLTRQRY-NH2



MW (calculated): 4971.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.81 min; m/3: - m/4: 1244.0 m/5: -

Compound 136

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,12S,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEADSSPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4945.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.79 min; m/3: 1649.0 m/4: - m/5: -

Compound 137

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,28Y,30W,31I]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEELQRYYVSLRHYYNWITRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.56 min; m/3: 1664.0 m/4: - m/5: -

Compound 138

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APAK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4901.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.03 min; m/3: - m/4: 1226.0 m/5: -

Compound 139

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,12S,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEESSPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5017.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.79 min; m/3: - m/4: 1256.0 m/5: -

Compound 140

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,18E,19Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PGEDASPEELEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4888.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.01 min; m/3: - m/4: 1223.0 m/5: -

Compound 141

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,22V,24A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEELQRYYVSARHYYNWLTRQRY-NH2
MW (calculated): 4901.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.46 min; m/3: - m/4: 1227.0 m/5: -

Compound 142
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9P,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PPEEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4969.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.79 min; m/3: - m/4: 1243.0 m/5: -

Compound 143
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13T,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDATPEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 4985.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.10 min; m/3: 1662.0 m/4: - m/5: -

Compound 144
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,8A,9E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
AEEDASPEELQQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4933.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.14 min; m/3: 1645.0 m/4: - m/5: -

Compound 145

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,17I,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDSSPEEIQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5003.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.74 min; m/3: - m/4: 1251.0 m/5: -

Compound 146

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,24T,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEEASPEELQRYYVSTRHYYNWLTRQRY-NH2

MW (calculated): 4989.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.22 min; m/3: - m/4: 1248.0 m/5: -

Compound 147

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,19K,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEETQKYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4947.5 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 9.18 min; m/3: 1650.3 m/4: 1238.1 m/5: 990.4

Compound 148

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13P,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAPAEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4971.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 11.20 min; m/3: - m/4: 1242.0 m/5: -

Compound 149

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 4985.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.99 min; m/3: - m/4: 1248.0 m/5: -

Compound 150

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEELQKYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5001.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 11.23 min; m/3: 1668.7 m/4: 1251.5 m/5: 1001.2

Compound 151

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEAEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4943.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.87 min; m/3: - m/4: 1237.0 m/5: -

Compound 152
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8A,9E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
AEEDASPEEIQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4961.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.95 min; m/3: 1654.0 m/4: - m/5: -

Compound 153
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8V,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
VEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5003.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.62 min; m/3: - m/4: - m/5: 1001

Compound 154
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,21E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEEASPEELQRYEVSLRHYYNWLTRQRY-NH2
MW (calculated): 4967.6 Da
Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 10.32 min; m/3: 1657.2 m/4: 1243.0 m/5: 994.4

Compound 155
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4975.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.19 min; m/3: 1659.0 m/4: - m/5: -

Compound 156
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,14E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEELEQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4992.5 Da
Synthesis and purification methods: S02; P02
LCMS: A02; Rt: 13.23 min; m/3: 1665.6 m/4: 1248.9 m/5: 999.2

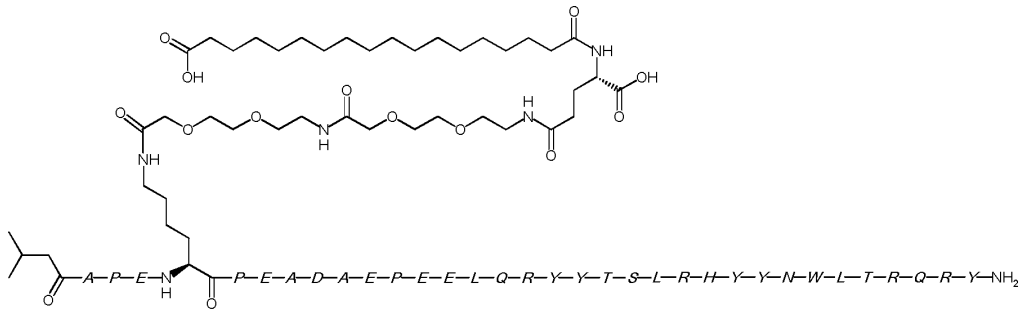
Compound 157
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,6A,7K,9E,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4917.5 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.28 min; m/3: 1640.0 m/4: - m/5: -

Compound 158
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,18Q,22V,28W,30Y,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRHYWNYLTRQRY-NH2
MW (calculated): 4987.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.09 min; m/3: - m/4: 1248.0 m/5: -

Compound 159
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,10A,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEADASPEELEQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4902.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.13 min; m/3: - m/4: 1226.0 m/5: -

Compound 160
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,10A,13E,18Q,22T,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEADAEPEELQRYYTSLRHYYNWLTRQRY-NH2



MW (calculated): 4973.6 Da

Synthesis and purification methods: S01; P01

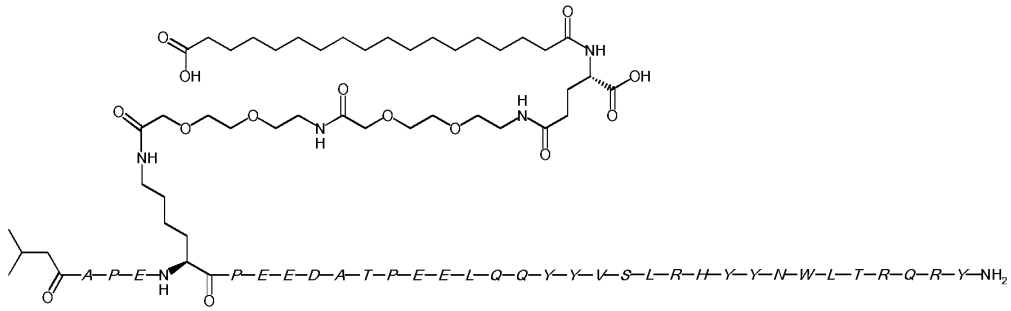
LCMS: A01; Rt: 10.10 min; m/3: - m/4: 1244.0 m/5: -

Compound 161

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDATPEELQQYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4973.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 11.90 min; m/3: 1658.2 m/4: 1244.5 m/5: 995.7

Compound 162

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,21E,22V,26A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYEVSLRAYYNWLTRQRY-NH2
MW (calculated): 4887.5 Da
Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 11.22 min; m/3: 1630.5 m/4: 1223.0 m/5: 978.3

Compound 163
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,11A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 4927.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.16 min; m/3: - m/4: 1234.0 m/5: -

Compound 164
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,14E,18Q,19E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEELQEYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4992.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.65 min; m/3: 1665.0 m/4: - m/5: -

Compound 165
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,18Q,21Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYQVSLRHYYNWLTRQRY-NH2
MW (calculated): 4952.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.26 min; m/3: - m/4: 1239.0 m/5: -

Compound 166

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9P,10A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PPADASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 4939.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.58 min; m/3: 1647.0 m/4: - m/5: -

Compound 167

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,19Q,21E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASPEELQQYEVSLRHYYNWLTRQRY-NH2

MW (calculated): 4925.5 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 10.95 min; m/3: 1643.1 m/4: 1232.5 m/5: 985.5

Compound 168

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,22V,28Y,30W,31I]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDAEPEELQRYYVSLRHYYNWITRQRY-NH2

MW (calculated): 5029.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.66 min; m/3: 1678.0 m/4: - m/5: -

Compound 169

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4985.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.96 min; m/3: 1663.0 m/4: - m/5: -

Compound 170

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4974.6 Da

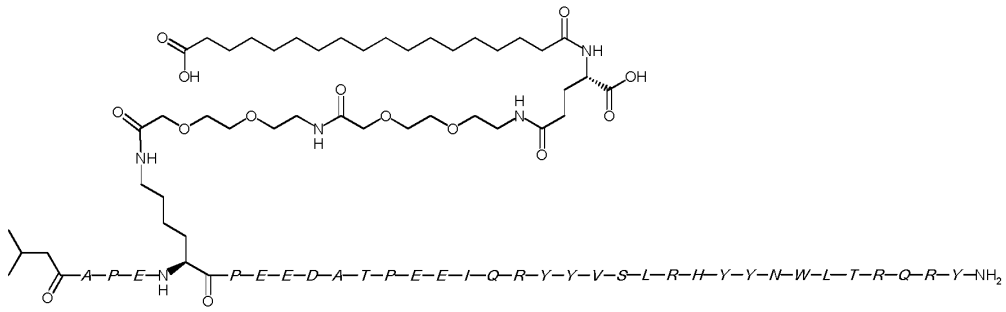
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.18 min; m/3: - m/4: 1244.0 m/5: -

Compound 171

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDATPEEIQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 5001.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.82 min; m/3: - m/4: 1251.0 m/5: -

Compound 172
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 5013.6 Da
Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 12.87 min; m/3: 1672.6 m/4: 1254.6 m/5: 1003.6

Compound 173
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.91 min; m/3: - m/4: 1245.0 m/5: -

Compound 174
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.27 min; m/3: - m/4: 1244.0 m/5: -

Compound 175

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDAAPEELQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4955.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.87 min; m/3: 1653.0 m/4: - m/5: -

Compound 176

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,14E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASEEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5019.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.40 min; m/3: 1674.0 m/4: - m/5: -

Compound 177

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,12S,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDSSPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 5045.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.85 min; m/3: - m/4: 1262.0 m/5: -

Compound 178

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,17T,18E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDATPEETERYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4990.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.35 min; m/3: - m/4: 1249.0 m/5: -

Compound 179
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPAELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4871.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.96 min; m/3: 1625.0 m/4: m/5: -

Compound 180
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17T,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEETQQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4947.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.27 min; m/3: 1650.0 m/4: - m/5: -

Compound 181
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,10A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEADASPEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 4913.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.93 min; m/3: - m/4: 1229.0 m/5: -

Compound 182
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,12G,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDGSPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5015.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 11.02 min; m/3: 1672.0 m/4: - m/5: -

Compound 183
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9P,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PPEDASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4955.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.46 min; m/3: 1652.0 m/4: - m/5: -

Compound 184
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,11A,18Q,22V,23T,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEELQRYYVTLRHYYNWLTRQRY-NH2
MW (calculated): 4957.6 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.13 min; m/3: - m/4: 1241.0 m/5: -

Compound 185
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEEASPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5043.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.83 min; m/3: 1683.0 m/4: - m/5: -

Compound 186
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASAEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 5003.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.55 min; m/3: - m/4: 1252.0 m/5: -

Compound 187
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,27Q,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVSLRHQYNWLTRQRY-NH2
MW (calculated): 4952.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.22 min; m/3: 1652.0 m/4: - m/5: -

Compound 188

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5031.7 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.87 min; m/3: - m/4: 1257.0 m/5: -

Compound 189

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEAELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4961.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.40 min; m/3: - m/4: 1241.0 m/5: -

Compound 190

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,13P,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEEAPPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5011.7 Da

Synthesis and purification methods: S01; P01

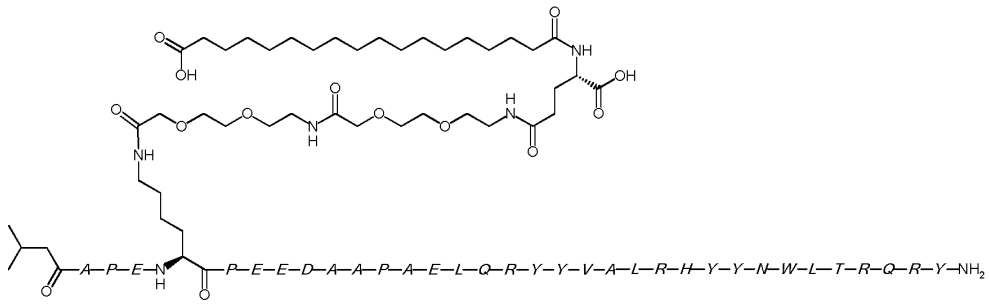
LCMS: A01; Rt: 10.32 min; m/3: - m/4: 1254.0 m/5: -

Compound 191

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22I,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDASPEELQRYYISLRHYYNWLTRQRY-NH2

MW (calculated): 5001.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.81 min; m/3: 1668.0 m/4: - m/5: -

Compound 192
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13A,15A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAAPAELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4913.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.60 min; m/3: - m/4: 1229.0 m/5: -

Compound 193
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEEIQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5019.6 Da
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.37 min; m/3: 1674.0 m/4: - m/5: -

Compound 194

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,5Hyp,7K,9E,18Q,22V,28Y,30W,31T,32L]hPYY(4-36)

iVal-AHypEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEELQRYYVSLRHYYNWTLRQRY-NH2

MW (calculated): 5003.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 8.84 min; m/3: - m/4: 1252.0 m/5: -

Compound 195

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,16A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASPEALQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 4971.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.11 min; m/3: 1658.0 m/4: - m/5: -

Compound 196

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,6A,7K,9P,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)PPEDAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4939.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.28 min; m/3: - m/4: 1236.0 m/5: -

Compound 197

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,10A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEADASPEELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 4913.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.00 min; m/3: 1639.0 m/4: m/5: -

Compound 198
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,16A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEALQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.31 min; m/3: - m/4: 1244.0 m/5: -

Compound 199
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAEPEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5017.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.37 min; m/3: - m/4: 1255.0 m/5: -

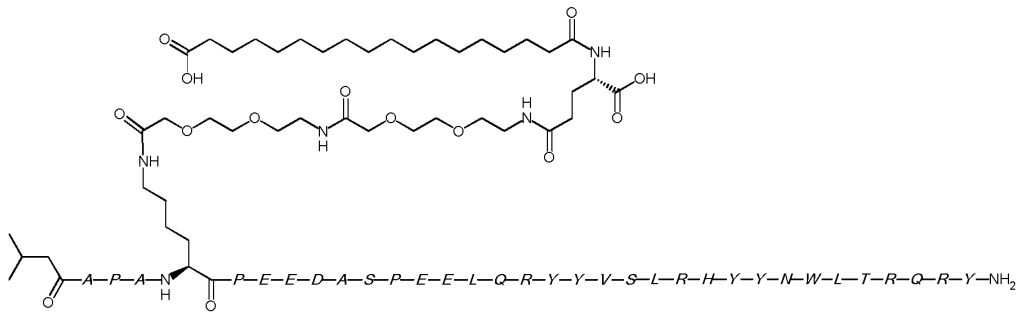
Compound 200
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8Hyp,9E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
HypEEDASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5003.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.53 min; m/3: - m/4: 1250.0 m/5: -

Compound 201
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9Q,13E,18Q,19E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PQEDAEPEELQEYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5001.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.24 min; m/3: - m/4: 1251.0 m/5: -

Compound 202
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13A,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAAPEELQKYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4943.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.48 min; m/3: - m/4: 1237.0 m/5: -

Compound 203
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,6A,7K,9E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4929.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.83 min; m/3: 1644.0 m/4: - m/5: -

Compound 204

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,17V,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEEASPEEVQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.62 min; m/3: 1663.0 m/4: - m/5: -

Compound 205

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEEASPEELQKYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4973.6 Da

Synthesis and purification methods: S03; P02

LCMS: A02; Rt: 11.05 min; m/3: 1659.2 m/4: 1244.4 m/5: 995.5

Compound 206

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18E,19Q,21E,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELEQYEVSLRHYYNWLTRQRY-NH2
MW (calculated): 4926.5 Da
Synthesis and purification methods: S02; P02
LCMS: A02; Rt: 11.60 min; m/3: 1643.3 m/4: 1232.8 m/5: 986.2

Compound 207
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,10A,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEADAEPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4913.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.37 min; m/3: - m/4: 1229.0 m/5: -

Compound 208
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5007.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.63 min; m/3: 1670.0 m/4: - m/5: -

Compound 209
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDSEPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5045.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.09 min; m/3: - m/4: 1263.0 m/5: -

Compound 210
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,8I,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-) IEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5017.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 11.05 min; m/3: - m/4: 1255.0 m/5: -

Compound 211
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,13T,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDATAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4975.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.92 min; m/3: - m/4: 1245.0 m/5: -

Compound 212
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,12T,13A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDTAPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5001.6 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.56 min; m/3: - m/4: 1251.0 m/5: -

Compound 213
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,12T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEETSPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5031.7 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.79 min; m/3: 1678.0 m/4: - m/5: -

Compound 214
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEELQRYYVALRHYYNWLTRQRY-NH2
MW (calculated): 5003.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.70 min; m/3: 1668.0 m/4: - m/5: -

Compound 215
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,15A,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPAELQRYYVELRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.86 min; m/3: - m/4: 1244.0 m/5: -

Compound 216

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4902.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.24 min; m/3: 1635.0 m/4: - m/5: -

Compound 217

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,15A,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPAELQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4913.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.99 min; m/3: 1639.0 m/4: m/5: -

Compound 218

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,12S,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDSSPEELQRYYVALRHYYNWLTRQRY-NH2

MW (calculated): 4987.6 Da

Synthesis and purification methods: S01; P01

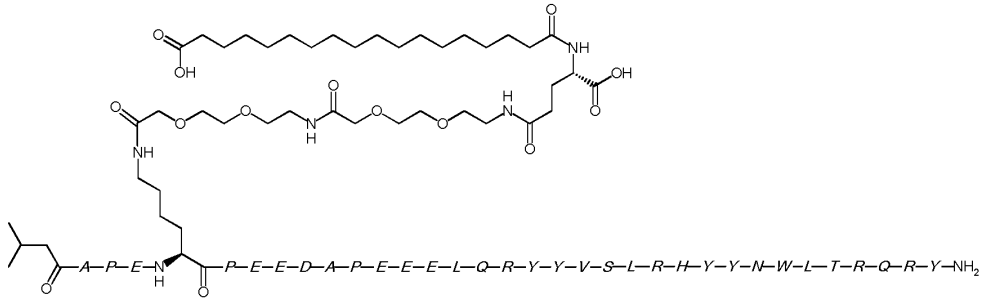
LCMS: A01; Rt: 10.00 min; m/3: - m/4: 1247.0 m/5: -

Compound 219

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,13A,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEEAAPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4985.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.41 min; m/3: - m/4: 1247.0 m/5: -

Compound 220
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,14A,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4917.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.70 min; m/3: - m/4: 1230.0 m/5: -

Compound 221
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13P,14E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDAPEEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 5029.6 Da
Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 11.00 min; m/3: - m/4: 1258.0 m/5: -

Compound 222

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,14A,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEDASAEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4933.5 Da

Synthesis and purification methods: S02; P02

LCMS: A02; Rt: 13.32 min; m/3: 1645.5 m/4: 1234.4 m/5: 987.4

Compound 223

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,11A,13E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEAAEPEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4985.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.21 min; m/3: - m/4: 1247.0 m/5: -

Compound 224

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,11E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PGEEASPEELEQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4902.5 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 10.12 min; m/3: 1634.0 m/4: - m/5: -

Compound 225

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,13E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PGEDAEPEELEQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4930.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.96 min; m/3: 1644.0 m/4: - m/5: -

Compound 226
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,11A,17T,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEAASPEETQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4931.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.26 min; m/3: 1645.0 m/4: - m/5: -
Compound 227

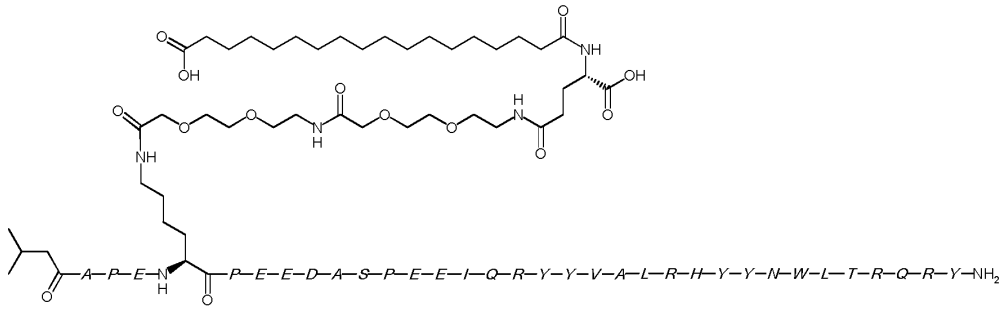
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,8A,9E,18E,19Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
AEEDASPEELEQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4934.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.38 min; m/3: - m/4: 1235.0 m/5: -

Compound 228
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,18Q,19Q,22V,26A,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQQYYVSLRAYYNWLTRQRY-NH2
MW (calculated): 4893.5 Da
Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 12.98 min; m/3: 1632.6 m/4: 1224.3 m/5: 979.6

Compound 229
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,24A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVSARHYYNWLTRQRY-NH2
MW (calculated): 4945.5 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.27 min; m/3: 1650.0 m/4: - m/5: -

Compound 230
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,17I,18Q,22V,23A,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEEIQRYYVALRHYYNWLTRQRY-NH2



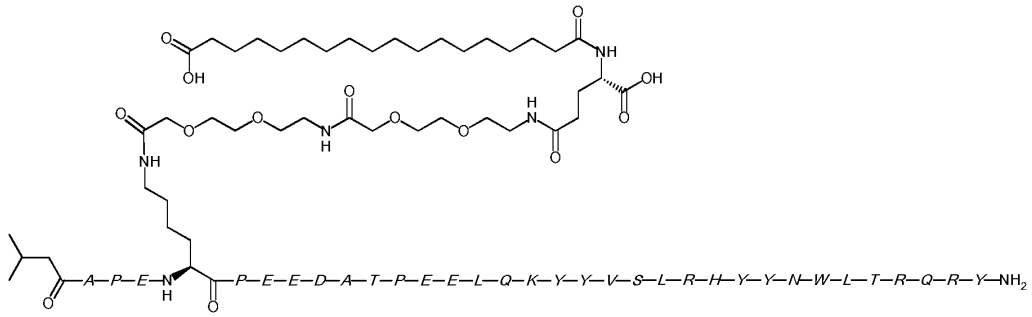
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.81 min; m/3: - m/4: 1243.0 m/5: -

Compound 231

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13T,18Q,19K,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDATPEELQKYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4973.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.79 min; m/3: - m/4: 1245.0 m/5: -

Compound 232

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9P,18Q,22V,23E,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)

PPEDASPEELQRYYVELRHYYNWLTRQRY-NH2

MW (calculated): 4939.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.71 min; m/3: - m/4: 1235.0 m/5: -

Compound 233

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11A,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEAASPEELQQYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 4915.5 Da

Synthesis and purification methods: S03; P02

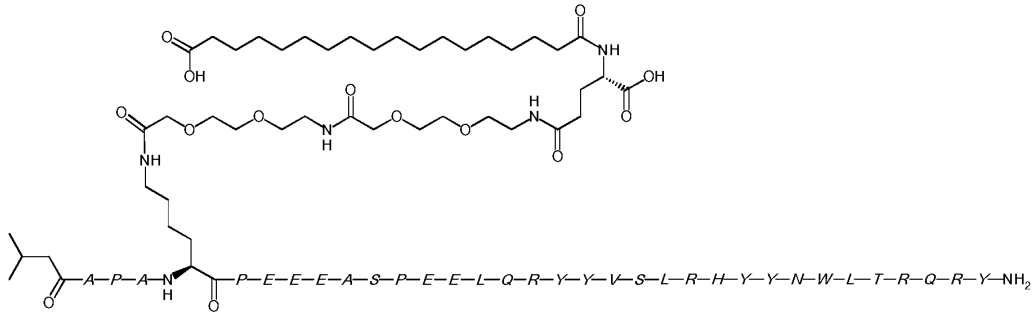
LCMS: A02; Rt: 12.58 min; m/3: 1639.6 m/4: 1230.1 m/5: 984.0

Compound 234

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,6A,7K,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APAK(C18DA-gGlu-OEG1-OEG2-)

PEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2



MW (calculated): 4943.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.96 min; m/3: 1649.0 m/4: - m/5: -

Compound 235

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14Hyp,18Q,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)

PEEDASHypEELQRYYVSLRHYYNWLTRQRY-NH2

MW (calculated): 5003.6 Da

Synthesis and purification methods: S01; P01

LCMS: A01; Rt: 9.28 min; m/3: - m/4: 1252.0 m/5: -

Compound 236

N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,13P,14A,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEEAPAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4985.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 11.15 min; m/3: - m/4: 1247.0 m/5: -

Compound 237
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,18Q,22V,23E,24V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEELQRYYVEVRHYYNWLTRQRY-NH2
MW (calculated): 5015.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.60 min; m/3: 1672.0 m/4: - m/5: -

Compound 238
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,8T,9E,11E,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
TEEEASPEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5005.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.89 min; m/3: 1669.0 m/4: - m/5: -

Compound 239
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,14E,18Q,21E,22V,28Y,30W,31L]hPYY(4-36)

iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASEEELQRYEVSLRHYYNWLTRQRY-NH2
MW (calculated): 4985.5 Da
Synthesis and purification methods: S02; P02
LCMS: A02; Rt: 11.55 min; m/3: 1662.9 m/4: 1247.5 m/5: 998.0

Compound 240
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,16A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEEDASPEALQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4929.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.96 min; m/3: 1643.0 m/4: - m/5: -

Compound 241
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,11E,17I,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEEIQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 5001.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 9.70 min; m/3: - m/4: 1251.0 m/5: -

Compound 242
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy)acetamido]-[4A,7K,9E,10A,14A,18Q,22V,28Y,30W,31L]hPYY(4-36)
iVal-APEK(C18DA-gGlu-OEG1-OEG2-)
PEADASAEELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4903.5 Da

Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.72 min; m/3: - m/4: 1226.0 m/5: -

Compound 243
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,11E,18Q,19Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-)PEEEASPEELQQYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4973.6 Da
Synthesis and purification methods: S03; P02
LCMS: A02; Rt: 11.78 min; m/3: 1659.0 m/4: 1244.6 m/5: 995.5

Compound 244
N{alpha-4}-(3-methylbutanoyl)-N{epsilon-7}-[2-(2-{2-[2-(2-{2-[(4S)-4-carboxy-4-(17-carboxyheptadecanamido)butanamido]ethoxy}ethoxy)acetamido]ethoxy}ethoxy) acetamido]-[4A,7K,9E,13E,15A,18Q,22V,28Y,30W,31L]hPYY(4-36) iVal-APEK(C18DA-gGlu-OEG1-OEG2-) PEEDAEPAELQRYYVSLRHYYNWLTRQRY-NH2
MW (calculated): 4971.6 Da
Synthesis and purification methods: S01; P01
LCMS: A01; Rt: 10.08 min; m/3: 1658.0 m/4: - m/5: -

The following compound disclosed as Compound 38 in WO 2016/198682 A1 was synthesized as reference:

Ref. 1
iVal-RPEK(C18DA-gGlu-OEG1-OEG2-)PGEDASPEELQRYYVSLRHYYNWLTRQRY-NH2 (SEQ ID NO: 249)

Further compounds were synthesized as references:

Ref. 2

iVal-RPEK(C18DA-gGlu-OEG1-OEG2-)
PGEDASPEELQRYYVSLAHYYNWLTRQRY-NH2 (SEQ ID NO: 250)

Ref. 3
iVal-RPEK(C18DA-gGlu-OEG1-OEG2-)
PGEDASPEELQRYYVSLRAYYNWLTRQRY-NH2 (SEQ ID NO: 251)

Ref. 4
iVal-RPEK(C18DA-gGlu-OEG1-OEG2-)
PGEDASPEELQRYYVSLRHYYNWLTAQRY-NH2 (SEQ ID NO: 252)

Ref. 5
iVal-RPEK(C18DA-gGlu-OEG1-OEG2-)
PGEDASPEELQRYYVSLRHYYNWLTRQAY-NH2 (SEQ ID NO: 253)

The structure of Ref. 1 is – except for alanine at position 4 – identical to Compound 64. This R4A mutation leads to a compound that shows much higher solubility at pH 6.2 (0.5 mg/ml for Ref. 1 vs. 8.7 mg/ml for compound 64). At the same time high binding affinity and receptor activity are maintained for compound 64 (see Table 2). Other arginine to alanine mutations at different positions (R25A in Ref. 2; R33A in Ref. 4; R35A in Ref. 5) or histidine to alanine mutation at position 26 (H26A in Ref. 3) negatively affect binding affinity and receptor activity or physical stability of the resulting compounds.

Comp.	Seq. mod. vs. Ref. 1	hY2 RLB (nM) (Example 1)	hY2 cAMP (nM) (Example 2)	Sol pH 6 (mg/ml) [final pH] (Example 3)	Sol pH 7 (mg/ml) [final pH] (Example 3)	DLS pH 7.2 (Example 4)
Ref. 1	-	0.47	0.075	0.5 [6.2]	8.7 [6.9]	n.d.
Comp. 64	R4A	0.76	0.157	8.7 [6.2]	9.6 [6.8]	OK
Ref. 2	R25A	8.38	1.121	9.2 [6.0]	9.0 [6.7]	n.d.

Ref. 3	H26A	1.69	0.375	10.0 [6.0]	9.7 [6.7]	Not OK
Ref. 4	R33A	30.94	12.615	10.0 [6.1]	10.0 [6.8]	n.d.
Ref. 5	R35A	316.00	293.059	9.7 [6.1]	10.0 [6.8]	n.d.

Table 2. n.d.: not determined

EXAMPLES

The following examples demonstrate certain specific embodiments of the present invention. The following examples were carried out using standard techniques that are well known and routine to those of skill in the art, except where otherwise described in detail.

Example 1: Radioligand binding competition assays (RLB)

The filtration RLB assay was carried out in 96-well plates in a final volume of 100 µl per well. Freeze-dried test peptides were dissolved in 100% dimethyl sulfoxide (DMSO) to stock solutions of 1mM and serial dilutions were performed in assay buffer (50mM HEPES, 5mM $MgCl_2$, 1mM $CaCl_2$, pH 7.4) containing 0.2% ovalbumin. 10µl/well of the test peptide solution was added to the plates to give final concentrations ranging from 1µM to 3pM. Subsequently, 10 µl of human ^{125}I-PYY(1-36) (Perkin Elmer) in assay buffer containing 0.2% ovalbumin was added to wells to give a final concentration of 0.02nM. Next, 80µL membranes (HTS066M, ChemiSCREEN™ Human Neuropeptide Y2 Receptor Membrane Preparation, CHEMICON) were added to each well to give a final protein concentration of 0.5µg/well. The plates were sealed and incubated at room temperature for 2 hours in a plate shaker set at 400 rpm. The incubation was stopped by vacuum filtration onto 0.5% poly-ethylene amine (PEI) presoaked GF/C filters using a 96-well FilterMate™ harvester (Perkin Elmer) followed by four washes with 300µl/well ice-cold wash buffer (50mM HEPES, 500mM NaCl, pH7.4). Filter plates were then dried for 60 min at room temperature and the bottom of the plates was sealed with backing tape UniFilter-96. Finally, 50µl/well scintillation counter cocktail (Microscint20, Packard) was added and the radioactivity was

counted in the Packard TopCount NXT scintillation counter. IC_{50} values (the half maximal inhibitory concentration of the agonist) were calculated by nonlinear regression analysis of sigmoidal dose-response curves. Ki values for binding affinity were acquired by the Cheng-Prusoff equation ($Ki=IC_{50}/(1+[L]/Kd)$, where Kd is the previously measured receptor specific dissociation constant (for NPY2R=0.07nM) and [L] is ^{125}I-PYY(1-36) radioligand concentration.

The RLB results are summarized in Table 3, below.

Compound	Ki [nM]
1	2.50
2	1.81
3	3.14
4	1.43
5	14.77
6	6.52
7	1.02
9	1.22
10	1.28
11	0.74
12	12.73
13	1.29
14	0.31
15	2.89
16	3.09
17	0.93
18	3.71
19	0.39
20	2.57
21	1.52
22	1.36
23	0.63
24	2.22
25	4.36
26	0.77
27	0.96
28	6.03
29	0.29
30	0.47
31	0.90
32	4.84
33	0.45
34	6.40
35	1.21
36	10.64
37	4.85
38	0.49
39	0.76
40	1.21
41	3.22
42	5.92
43	0.40
44	4.44
45	3.97
46	3.30

47	0.42
48	38.88
49	0.89
50	2.48
51	39.36
52	8.53
53	0.24
54	0.95
55	3.35
56	1.26
57	2.23
58	2.66
60	5.95
61	0.40
63	3.13
64	0.76
65	1.02
66	2.23
67	4.37
68	8.47
69	5.74
70	4.86
71	5.22
74	0.57
75	1.92
77	6.17
79	4.52
80	6.96
81	3.36
82	1.53

83	0.57
84	1.19
85	1.56
86	1.19
87	1.97
88	0.83
89	9.61
90	1.60
91	4.66
92	1.57
93	1.01
94	2.07
95	0.66
96	0.76
97	1.12
98	1.30
99	4.88
100	0.44
101	3.29
102	0.87
103	0.95
104	1.37
105	6.94
106	0.66
107	1.10
108	0.99
109	0.94
110	0.77
111	5.43
112	1.36

115	1.94
116	10.03
117	0.34
118	1.37
119	3.32
120	4.81
121	0.61
122	1.84
124	0.50
126	1.18
127	1.01
128	2.10
129	1.14
130	11.10
131	8.54
132	1.18
133	2.31
134	1.17
135	1.31
136	0.89
137	2.35
138	1.42
139	3.83
140	2.66
141	3.12
142	1.17
143	0.83
144	8.92
145	1.55
146	5.05

147	1.19
148	1.75
149	2.75
150	2.02
151	1.43
152	5.18
153	14.43
155	2.05
157	0.71
158	4.43
159	0.83
160	2.00
163	0.96
164	4.33
165	2.49
166	0.66
168	6.36
169	2.34
170	12.29
171	0.34
173	1.71
174	3.89
175	1.24
176	3.05
177	2.55
178	2.05
179	1.00
180	3.85
181	0.67
182	3.45

183	0.70
184	2.06
185	3.58
186	3.36
187	2.76
188	3.09
189	2.08
190	6.05
191	1.70
192	1.65
193	3.81
194	13.36
195	0.75
196	0.98
197	0.87
198	2.40
199	5.69
200	2.34
202	0.60
203	0.75
204	3.35
205	1.90
207	1.07
208	3.80
209	8.07
210	10.48
211	3.43
212	20.28

213	6.54
214	2.56
215	0.48
216	0.93
217	1.01
218	1.49
219	7.60
220	2.99
221	5.49
223	4.77
224	4.64
225	5.89
226	1.21
227	12.28
229	2.84
230	0.71
231	1.96
232	0.81
234	1.95
235	1.04
236	5.58
237	0.76
238	25.11
240	1.36
241	2.95
242	0.81
243	2.07
244	1.84

Table 3

In general, the data from the RLB assay is predictive for the acute food intake inhibition in mice (Experiment 6).

Example 2: HTRF cAMP Gi assay

The Homogenous Time Resolved fluorescence (HTRF) technology optimized for Gi coupled receptors has thoroughly been described in Cisbio cAMP Gi kit manual. Briefly, the production of intracellular cAMP will generate a competition between unlabeled cAMP and exogenously added d2-cAMP for anti-cAMP antibodies conjugated to cryptate. CHO-K1 cells stably expressing the human NPY1, NPY2, NPY4 and NPY5 receptors were used with the cells brought to life from a frozen stock immediately before assay performance. 2000 cells per well were applied for all four NPY receptor subtype assays. A 384-well format was used applying a total volume of 20 µl using 5 µl cells, 2.5 µl peptide agonist, 2.5 µl forskolin and 5 µl of each of the fluorophores. Cells were incubated with agonist peptides (11 points concentration response curves) and forskolin (~90% activity level, 3-11 µM forskolin) for 40 min at 37°C using DPBS containing 0.5 mM IBMX as stimulation buffer. After addition of HTRF® detection reagents and incubation with shaking (2400 rpm) for one hour at room temperature signals at 620 and 665 nm (raw counts: ratio of 665/620) were detected. Concentration-response evaluation of compounds was performed with 11 concentrations of agonist peptides (covering 3 decades). EC50 values were calculated by nonlinear regression using sigmoid concentration-response with variable slope.

The *in vitro* activity results (expressed as EC_{50} values) are summarized in Table 4, below.

Compound NO	hY2R (nM)	hY1R (nM)	hY4R (nM)	hY5R (nM)
hPYY3-36	0.160	250	>5000	73.2
11	0.334	>5000	>5000	>5000
18	0.167	>5000	>5000	>5000
23	0.150	>5000	>5000	>5000
41	0.159	>5000	>5000	>5000
46	0.152	>5000	>5000	>5000

53	0.119	>5000	>5000	>5000
54	0.131	>5000	>5000	>5000
74	0.129	>5000	>5000	>5000
104	0.096	>5000	>5000	>5000
105	0.284	>5000	>5000	>5000
106	0.173	>5000	>5000	>5000
117	0.129	>5000	>5000	>5000
120	0.210	>5000	>5000	>5000
164	0.235	>5000	>5000	>5000
171	0.181	>5000	>5000	>5000
188	0.106	>5000	>5000	>5000
230	0.199	>5000	>5000	>5000
231	0.107	>5000	>5000	>5000

Table 4

Example 3: Solubility determination

Peptides (as TFA salts) were weighed out in a filter unit (Mini-UniPrep Syringeless Filter 0.45 µm, Whatman), and 0.1 M sodium diphosphate buffer at pH 6.4 or 7.0 was added to achieve 10 mg/ml final concentration. The peptide was dissolved by shaking the filter unit horizontally at 600 rpm for 2 hours at room temperature. The sample was filtered, to remove any insoluble particles and diluted to 1 mg/ml in 50% acetonitrile. The control was prepared by weighing out the corresponding peptide and dissolving it in 50% acetonitrile to final concentration of 1 mg/ml. Both the control and sample were analysed with reversed phase chromatography. The area under the peak of the sample was compared to the control and the solubility was calculated based on that ratio.
The pH was measured and recorded for each sample. Typically, buffer pH dropped by 0.2 to 0.3 pH units due to the TFA content of the peptide.

UPLC method:

System: UltiMate 3000 UPLC, ThermoFisher

Mobile phase A: 5% acetonitrile, 95% water, 0.03% trifluoracetic acid.

Mobile phase B: 95% acetonitrile, 5% water, 0.03% trifluoracetic acid.

Flow: 1 ml/min

Gradient: 0-100% mobile phase B (2 mins.)

Column: Kinetix, 5 µm C8, 100 Å, 50 x 2.1 mm

Column temperature: 50°C

The results from the solubility determination are summarized in Table 5, below.

Compound	Sol6 (mg/ml)	Sol6 pH	Sol7 (mg/ml)	Sol7 pH
3	10.0	6.1	9.7	6.8
5	8.7	6.1	9.7	6.8
8	10.0	6.0	9.9	6.7
10	9.9	6.1	10.0	6.8
11	10.0	6.1	10.0	6.8
12	10.0	6.1	9.8	6.7
13	9.6	6.1	9.1	6.7
14	9.8	6.1	9.9	6.8
16	10.0	6.1	9.9	6.7
17	10.0	6.1	9.0	6.9
19	9.9	6.1	9.9	6.8
20	10.0	6.0	9.7	6.7
23	7.6	6.1	10.0	6.8
24	9.0	6.1	9.7	6.8
26	9.7	6.2	10.0	6.9
27	9.8	6.1	10.0	6.8
29	10.0	6.2	9.1	6.8

30	9.5	6.1	9.8	6.8
31	10.0	6.1	10.0	6.7
33	10.0	6.1	9.6	6.7
35	8.7	6.1	9.3	6.8
36	10.0	6.1	10.0	6.8
38	8.9	6.2	9.2	6.8
39	9.6	6.2	9.2	6.9
40	10.0	6.1	10.0	6.7
41	9.6	6.1	8.9	6.7
43	0.2	6.2	9.9	6.9
45	9.8	6.1	9.6	6.7
47	0.7	6.2	9.3	6.8
48	9.7	6.1	9.6	6.7
49	10.0	6.1	10.0	6.7
52	7.7	6.1	9.9	6.8
53	9.7	6.2	9.5	6.8
54	9.4	6.1	10.7	6.8
56	10.0	6.1	10.0	6.8
57	9.0	6.1	8.0	6.7
59	10.0	6.1	10.0	6.8
60	9.2	6.1	10.0	6.7
61	9.2	6.2	9.7	6.8
64	8.7	6.2	9.6	6.8
65	8.2	6.0	9.7	6.8
67	9.2	6.0	9.1	6.8
68	10.0	6.1	10.0	6.7
70	9.7	6.1	9.1	6.7
72	9.2	6.1	9.5	6.7
74	8.6	6.1	9.4	6.8
78	10.0	6.1	10.0	6.7

79	10.0	6.1	10.0	6.7
80	10.0	6.1	10.0	6.7
81	10.0	6.1	9.1	6.9
82	8.9	6.2	9.9	6.8
83	0.0	6.2	3.2	6.8
87	7.9	6.2	10.0	6.8
88	10.0	6.1	9.8	6.8
89	9.9	6.1	10.0	6.7
91	9.9	6.1	9.6	6.7
93	9.9	6.1	8.5	6.7
95	9.9	6.2	9.6	6.8
96	10.0	6.1	10.0	6.9
97	9.7	6.0	9.1	6.8
100	0.8	6.2	9.0	6.8
101	10.0	6.0	9.3	6.7
102	9.3	6.0	8.6	6.8
103	10.0	6.1	10.0	6.8
106	9.5	6.2	9.9	6.8
109	9.5	6.2	9.2	6.8
110	9.4	6.0	9.5	6.7
112	9.9	6.1	9.8	6.7
113	9.8	6.1	10.0	6.7
115	9.7	6.2	9.5	6.8
117	9.4	6.1	8.8	6.8
118	8.8	6.2	8.4	6.8
119	10.0	6.0	10.0	6.7
121	0.3	6.2	9.3	6.8
122	7.3	6.1	9.7	6.7
124	9.8	6.1	10.0	6.7
126	9.5	6.2	9.0	6.8

127	8.5	6.1	7.9	6.8
128	10.0	6.1	9.6	6.8
129	9.5	6.1	10.0	6.8
131	8.6	6.1	9.1	6.8
132	9.2	6.2	9.5	6.8
133	8.7	6.0	10.0	6.7
134	10.0	6.1	9.9	6.8
135	9.6	6.1	7.9	6.8
136	9.2	6.2	10.0	6.8
138	9.7	6.1	10.0	6.7
139	10.0	6.0	8.9	6.7
142	9.1	6.1	9.7	6.8
143	10.0	6.2	9.6	6.8
144	9.7	6.1	10.0	6.8
145	8.8	6.1	9.5	6.8
148	9.5	6.1	10.0	6.7
149	9.6	6.1	9.4	6.8
150	9.8	6.0	10.0	6.7
151	9.5	6.1	9.5	6.8
152	10.0	6.1	10.0	6.8
153	10.0	6.0	10.0	6.7
155	9.8	6.0	8.9	6.8
156	10.0	6.1	9.6	6.7
157	9.1	6.1	9.4	6.8
159	10.0	6.1	9.7	6.7
160	10.0	6.1	10.0	6.7
161	10.0	6.1	8.8	6.7
163	9.2	6.1	10.0	6.8
165	10.0	6.1	9.6	6.8
166	10.0	6.2	10.0	6.8

169	9.8	6.1	10.0	6.7
171	9.7	6.1	9.5	6.8
172	10.0	6.1	10.0	6.7
173	9.9	6.1	10.0	6.7
174	8.9	6.1	8.8	6.8
175	9.0	6.1	10.0	6.8
177	7.7	6.1	7.3	6.8
178	9.8	6.1	9.7	6.7
179	0.0	6.3	0.4	6.8
181	10.0	6.2	10.0	6.8
182	9.7	6.1	9.3	6.7
183	9.3	6.2	8.0	6.9
184	9.7	6.1	10.0	6.7
185	10.0	6.1	9.5	6.8
186	8.8	6.1	9.6	6.8
189	8.3	6.1	9.8	6.8
190	10.0	6.1	10.0	6.7
192	9.1	6.2	8.9	6.8
193	9.8	6.1	9.9	6.8
195	8.8	6.1	9.7	6.7
196	9.7	6.2	9.8	6.9
197	10.0	6.1	10.0	6.8
198	9.8	6.1	9.7	6.8
199	9.2	6.1	9.1	6.8
202	9.7	6.1	9.8	6.7
203	8.8	6.2	9.6	6.9
204	8.5	6.1	9.5	6.8
205	10.0	6.1	9.9	6.7
206	10.0	6.0	10.0	6.7
207	9.1	6.2	9.7	6.9

208	5.1	6.1	9.6	6.8
209	8.9	6.0	9.5	6.7
211	9.0	6.1	6.2	6.8
213	9.5	6.1	10.0	6.8
214	10.0	6.1	10.0	6.8
215	9.7	6.1	10.0	6.8
216	9.9	6.1	9.8	6.7
217	10.0	6.2	10.0	6.7
218	9.3	6.1	9.4	6.8
220	9.8	6.2	9.1	6.8
221	9.8	6.1	10.0	6.7
222	9.0	6.1	8.7	6.7
227	9.5	6.1	9.5	6.7
229	8.8	6.0	8.2	6.9
230	8.6	6.1	8.2	6.7
231	8.0	6.1	9.9	6.7
232	10.0	6.1	9.7	6.8
233	10.0	6.1	-	-
234	9.3	6.1	7.8	6.7
236	10.0	6.1	9.7	6.7
237	10.0	6.1	9.8	6.7
239	9.9	6.0	9.6	6.7
240	10.0	6.2	10.0	6.9
241	9.8	6.1	9.5	6.7
242	8.3	6.1	8.5	6.8
244	9.3	6.1	8.8	6.8
Ref 1	0.5	6.2	8.7	6.9

Table 5

Example 4: DLS studies

Aggregation combined with particle growth in peptide solutions was detected by dynamic light scattering (DLS). Test peptides (5 mg/ml) were dissolved in 50 mM phosphate buffer with EDTA (0.05 mg/ml) which is adjusted to a final pH of 7.2. Solutions were filtered through a 0.2 µm filter and shaken with a lab shaker at approx. 150 rpm at room temperature for 5 to 7 days. Samples were analyzed with a particle size analyzer (DLS, Horiba Nano Particle Analyzer SZ-100) at day 0 and at the end of the study. Formation of aggregates was detected by an increase in particle size over time and rated as OK, indicating no increase in particle size, and NOK, indicating an increase in particle size.

The results from the DLS studies are summarized in Table 6.

Example	DLS
23	OK
53	OK
64	OK
106	OK
117	OK
171	OK
230	OK

Table 6

Example 5: Mouse PK

Pharmacokinetic parameters of the test compounds were determined after intravenous administration to NMRI mice.

Male NMRI mice were obtained either from Charles River (Germany) or from Janvier (France) weighing approximately 30 to 40 g. Mice were housed in standard cages with light cycle of 12-hour dark and 12-hour light. Standardized food and water were offered ad libitum to the animals during the whole experimental period.

The respective peptide was dissolved in 50 mM phosphate buffer (pH 7.0) containing 5% mannitol. Intravenous doses of 30 nmol/kg were given via a tail vein.

Serial blood samples were collected from the vena sephena into tubes containing EDTA as anticoagulant at different time points up to 48h post dosing. After centrifugation for approximately 5 minutes, plasma samples were transferred to 96-well PCR plates, immediately frozen and kept at approximately -20°C until analyzed for plasma concentration by liquid chromatography mass spectrometry (LC-MS/MS). Individual plasma concentration-time profiles were analyzed by a non-compartmental approach, and the resulting pharmacokinetic parameters were determined.

Compound	Mouse MRT (h)	Terminal half-life (h)
Ref. 1	11	8.2

Mouse MRT of the PYY analogues according to the invention that have been measured were comparable to Ref. 1 and show very long half-lives as compared to the half-life of hPYY(3-36).

Example 6: Effect on acute food intake in normal NMRI mice

Male NMRI mice were obtained from Charles River (Charles River, Research Models & Services Germany GmbH) or from JanVier (JanVier Labs, France) at 5 weeks of age. The animals were group housed 4 mice pr. cage under a 12/12 h dark-light cycle, light off at 3 PM. Room temperature was controlled to 21 °C ± 1 °C, with 60 % ± 20% humidity. Animals had ad libitum access to regular rodent chow (KLIBA Nafag 3430 or Altromin 1324, Brogaarden, Denmark) and tap water.

Animals were transferred 5-7 days before the start of the study to a real-time food intake monitoring system, HM-2 system (MBRose, Denmark), to allow acclimatization to experimental conditions. As the animals were uniquely identified with microchips, each individual animal was identified by its microchip upon entry and exit from the food channel. Randomization of the mice for each study group (n=7-8) was based on body weight measured the day before the start of the study. A vehicle-treated (50mM phosphate buffer pH7 with 5% Mannitol) group was included in each experiment. Six hours before the start of the night phase animals were fasted. One hour before the dark phase animals

were dosed once subcutaneously (10 nmol/kg) with test peptide. Food intake was reported hourly for a period of 24h. The food intake of the treated groups was normalized (in %) to the average food intake of the group receiving vehicle (Table 7). Statistical significance was evaluated using One-way analysis of variance with Turkey's multiple comparison test. $P < 0.05$ was considered statistically significant.

Compound	Acute Food Intake 24h [%vehicle]
1	77
3	62
7	47
9	44
11	42
14	46
16	67
18	58
21	89
23	48
29	63
30	45
31	53
33	54
41	44
46	39
49	60
53	29
54	42
56	53
58	44
74	41
79	75
90	70
97	52
101	63
103	67
104	61
105	72
106	39
108	47
110	67
115	49
117	45
120	74
124	58
132	64
133	57
135	44
139	74
148	60
150	67
151	45
154	73
159	53
163	50
164	64
167	73
171	44
173	49

174	41
176	52
180	74
185	39
188	55
193	57
195	50
202	48
205	65
208	61

209	50
211	62
221	61
230	44
231	39
234	42
241	45
244	49
Ref. 1	41

Table 7

Example 7: Activity after incubation in solutions at different pH values

Peptides (as TFA salts) were weighed out in a filter unit (Mini-UniPrep Syringeless Filter 0.45 µm, Whatman), and 0.1 M sodium diphosphate buffer at pH 6.4 or 7.4, respectively, or 0.2 M TRIS buffer at pH 8.3 was added to achieve 10 mg/ml final concentration. The peptide was (partially) dissolved by shaking the filter unit horizontally at approx. 400 rpm for 2 hours at room temperature. The sample was filtered to remove any insoluble particles. The filtrate was subsequently subjected to the binding assay as described in Example 1. The apparent binding affinity (Ki) reported in Table 8 is calculated based on an assumed concentration of 10 mg/ml.

Compounds of the invention are active in the binding assay after incubation in solutions at different pH values, including pH 6. This demonstrates the fundamental feasibility of a liquid formulation of the compounds according to the invention at ~pH 6 (in the pH range from ~6 to ~8).

Contrary hereto, after incubation at ~pH 6, solutions of Ref. 1 (after filtration) show a weak response in the binding assay (apparent significant (>35x) affinity loss due to low solubility of Ref. 1 in buffered media at pH 6). Therefore, the low intrinsic solubility of Ref. 1 at ~pH 6 limits the formulation space in the physiological pH range of 6-8 of a liquid formulation of Ref. 1.

Com-pound	hY2R RLB Ki (nM) DMSO	RLB 6 (pH6.1 – 6.2) (nM)	RLB 7 (pH7.1 – 7.2) (nM)	RLB 8 (pH8.1 – 8.2) (nM)	RLB ratio (RLB6 /RLB7)	RLB ratio (RLB8 /RLB7)
Ref. 1	3.0	> 216	2.9	2.4	> 74	0.8
23	4.9	6.3	6.3	6.3	1.0	1.0
29	4.6	3.8	4.4	2.3	0.9	0.5
53	2.0	3.4	3.1	2.5	1.1	0.8
84	3.5	6.6	2.9	4.1	2.3	1.4
106	6.0	7.7	9.3	7.5	0.8	0.8
117	4.1	6.7	2.1	8.8	3.2	4.2
171	6.7	3.0	6.1	5.6	0.5	0.9
203	3.0	4.6	11.1	8.8	0.4	0.8

Table 8

WHAT WE CLAIM

1. A PYY analogue comprising an amino acid sequence corresponding to the amino acid sequence of hPYY(3-36), wherein the PYY analogue is a compound having the formula:

R^1-Z-R^2,

wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and

Z is a peptide comprising an amino acid sequence of formula II:

Ala-X5-X6-Lys-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-X22-X23-X24-Arg-X26-Tyr-X28-X29-X30-X31-X32-Arg-Gln-Arg-Tyr (II)

wherein

X5 is selected from the group consisting of Pro and Hyp;

X6 is selected from the group consisting of Ala and Glu;

X8 is selected from the group consisting of Ala, Pro, and Hyp;

X9 is selected from the group consisting of Glu, Gly, Gln and Pro;

X10 is selected from the group consisting of Ala and Glu;

X11 is selected from the group consisting of Ala, Asp, Glu and Pro;

X12 is selected from the group consisting of Ala, Gly, and Ser;

X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;

X14 is selected from the group consisting of Ala, Glu, Pro and Hyp;

X15 is selected from the group consisting of Ala, Glu and Ser;

X16 is selected from the group consisting of Glu and Ser;

X17 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;

X18 is selected from the group consisting of Glu and Gln;

X19 is selected from the group consisting of Ala, Glu, Arg, Lys and Gln

X21 is selected from the group consisting of Ala, Glu, Gln and Tyr;

X22 is selected from the group consisting of Ile, Ser, Thr and Val;

X23 is selected from the group consisting of Ala, Glu, Ser and Thr;

X24 is selected from the group consisting of Ala, Ile, Leu, Thr and Val;
X26 is selected from the group consisting of Ala, His and Lys;
X28 is selected from the group consisting of Trp and Tyr;
X29 is selected from the group consisting of Asn, and Tyr;
X30 is selected from the group consisting of His, Trp, and Tyr;
X31 is selected from the group consisting of Ala, Ile, and Leu;
X32 is selected from the group consisting of Gln, and Thr;

and wherein only one of X6 , X10 and X15 is Ala, and
at least two amino acids from X6, X10, X11, X13 and X23 are selected from the group consisting of Asp and Glu, and
wherein a half-life extending group is attached to the epsilon amino group of the lysine at position 7, the half-life extending group consists of a lipophilic substituent X and a linker U, wherein the linker U is attached to the epsilon amino group of lysine and X is attached to U, wherein X is selected from the group consisting of 15-carboxy-pentadecanoyl, 17-carboxy-heptadecanoyl (C18DA) and 19-carboxy-nonadecanoyl, and U consists of one, two or three sub-moieties independently selected from the group consisting of γ-Glu, and OEG.

2. The PYY analogue according to claim 1, wherein the PYY analogue is a compound having the formula:

R^1-Z-R^2,
wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:
R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and
Z is an amino acid sequence of formula III:

Ala-Pro-X6-Lys-Pro-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-Tyr-X21-Val-X23-Leu-Arg-His-Tyr-Tyr-Asn-Trp-Leu-Thr-Arg-Gln-Arg-Tyr (III)

wherein

X6 is selected from the group consisting of Ala and Glu;
X9 is selected from the group consisting of Glu and Gly;
X10 is selected from the group consisting of Ala and Glu;
X11 is selected from the group consisting of Ala, Asp, Glu and Pro;
X12 is selected from the group consisting of Ala and Ser;
X13 is selected from the group consisting of Ala, Glu, Ser, Thr and Pro;
X14 is selected from the group consisting of Ala, Glu and Pro;
X15 is selected from the group consisting of Ala and Glu,
X16 is selected from the group consisting of Ala and Glu;
X17 is selected from the group consisting of Ile, Leu, Thr and Val;
X18 is selected from the group consisting of Glu and Gln;
X19 is selected from the group consisting of Ala, Glu, Arg, Lys, and Gln;
X21 is selected from the group consisting of Glu and Tyr;
X23 is selected from the group consisting of Ala, Glu, Ser and Thr.

3. The PYY analogue according to claim 1, wherein the PYY analogue is a compound having the formula:

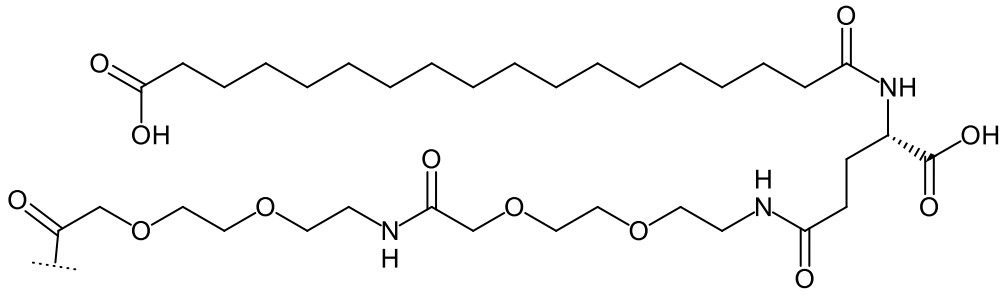
R^1-Z-R^2,
wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl or C_{1-6} alkyl:

R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and
Z is an amino acid sequence selected from the following sequences:
SEQ ID Nos: 6-8, 10-15, 17-31, 33-35, 37-46, 48-51, 53-58, 60-79, 81-86, 88-95, 97-117, 119-124, 126-133, 135-156, 158-182, 184-190, 192-197, 199, 200, 202-210, 212, 213, 215, 218-241, 243-248.

4. The PYY analogue according to claim 1, wherein X9 is selected from Glu and Gly.

5. The PYY analogue according to claim 1, wherein X9 is Glu.

6. The PYY analogue according to any one of claims 1 to 4, wherein the half-life extending group is C18DA-γ-Glu-OEG-OEG-, i.e.



.

7. The PYY analogue according to any one of the previous claims, wherein the PYY analogue is a compound selected from the group consisting of the following compounds: 2-4, 6-11, 13-27, 29-31, 33-42, 44-47, 49-54, 56-75, 77-82, 84-91, 93-113, 115-120, 122-129, 131-152, 154-178, 180-186, 188-193, 195, 196, 198-206, 208, 209, 211, 214-237, 239-244.

8. The PYY analogue according to claim 1, wherein the PYY analogue is a compound having the formula:

R^1-Z-R^2,
wherein R^1 is hydrogen, -C(O)C_{1-6} alkyl, -C(O)C_6H_6, -C(O)C_{3-6} cycloalkyl, -C(O)C_{1-6} alkyl-C_{3-6} cycloalkyl, or C_{1-6} alkyl or C_{1-6} alkyl-C_{3-6} cycloalkyl:
R^2 is OH or NHR^3, wherein R^3 is hydrogen or C_{1-3} alkyl; and
Z is an amino acid sequence of formula IV:

Ala-Pro-X6-Lys-Pro-Glu-X10-X11-Ala-X13-X14-Glu-Glu-X17-Gln-X19-Tyr-Tyr-Val-X23-Leu-Arg-His-Tyr-Tyr-Asn-Trp-Leu-Thr-Arg-Gln-Arg-Tyr (IV)

wherein
X6 is selected from the group consisting of Ala and Glu;
X10 is selected from the group consisting of Ala and Glu;
X11 is selected from the group consisting of Ala, Asp and Glu;
X13 is selected from the group consisting of Ala, Glu, Ser, and Thr;
X14 is selected from the group consisting of Ala, Pro and Hyp;
X17 is selected from the group consisting of Ile and Leu;

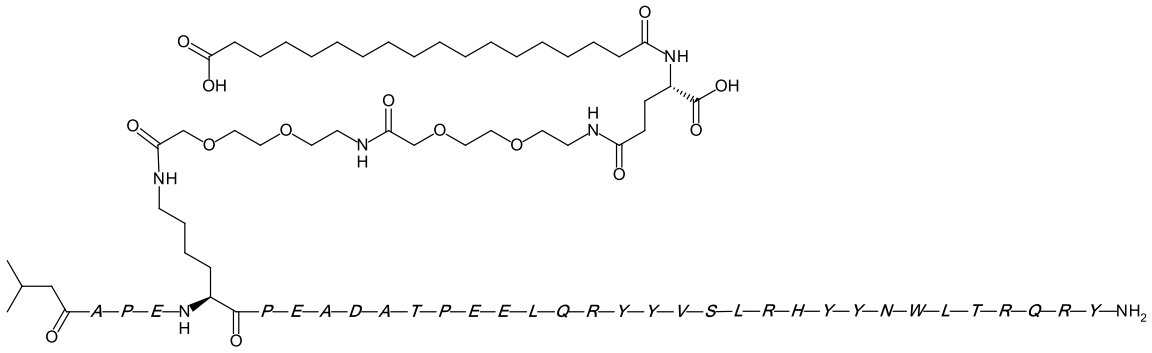
X19 is selected from the group consisting of Arg, Lys, and Gln;
X23 is selected from the group consisting of Ala, Glu, and Ser.

9. The PYY analogue according to any one of the previous claims, wherein the PYY analogue is in the form of a salt, preferably in the form of a pharmaceutically acceptable salt.

10. The PYY analogue according to any one of the previous claims, wherein the binding affinity (Ki) towards hNPY2R is below 100 nM.

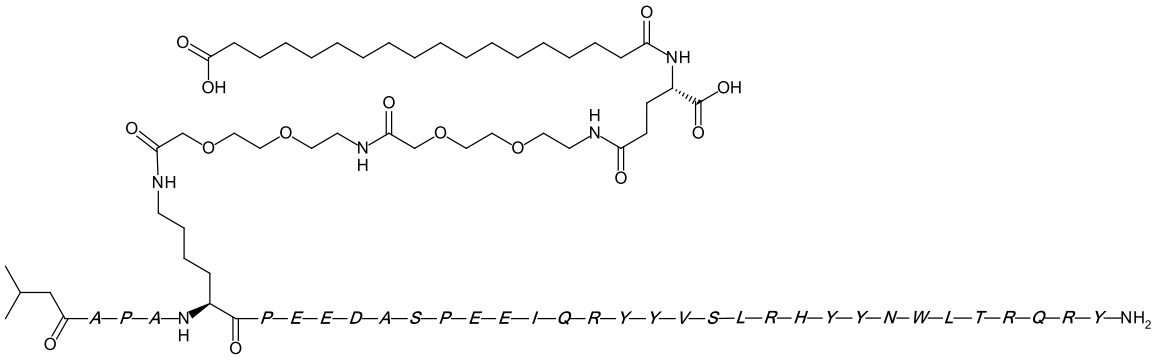
11. The PYY analogue according to any one of the previous claims, wherein the solubility of the PYY analogue is greater than 1.0 mg/ml at pH 6.

12. The PYY analogue according to claim 1, wherein the compound is



(Compound 14)
or a pharmaceutically acceptable salt thereof.

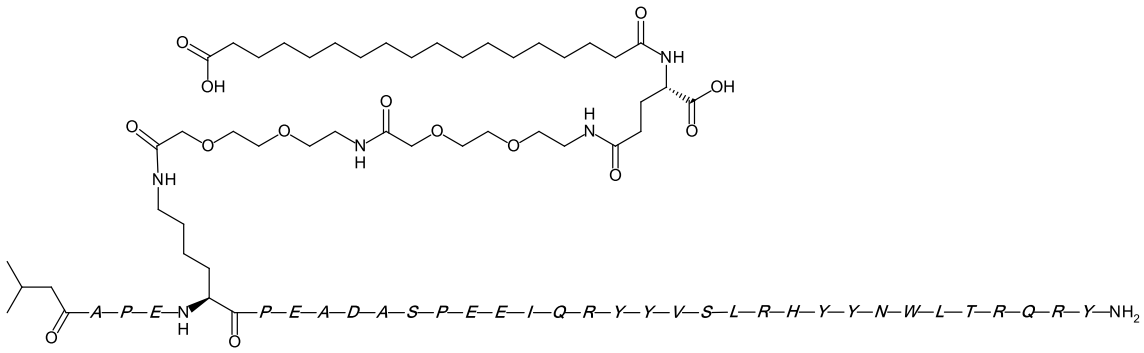
13. The PYY analogue according to claim 1, wherein the compound is



(Compound 23)

or a pharmaceutically acceptable salt thereof.

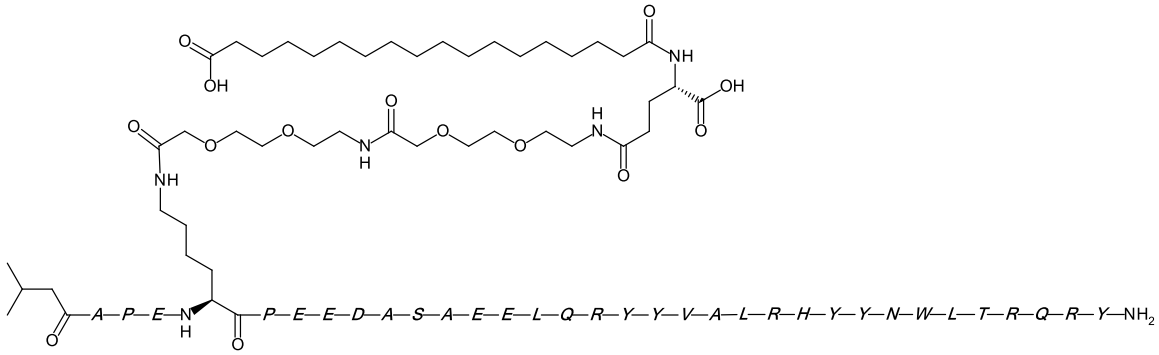
14. The PYY analogue according to claim 1, wherein the compound is



(Compound 53)

or a pharmaceutically acceptable salt thereof.

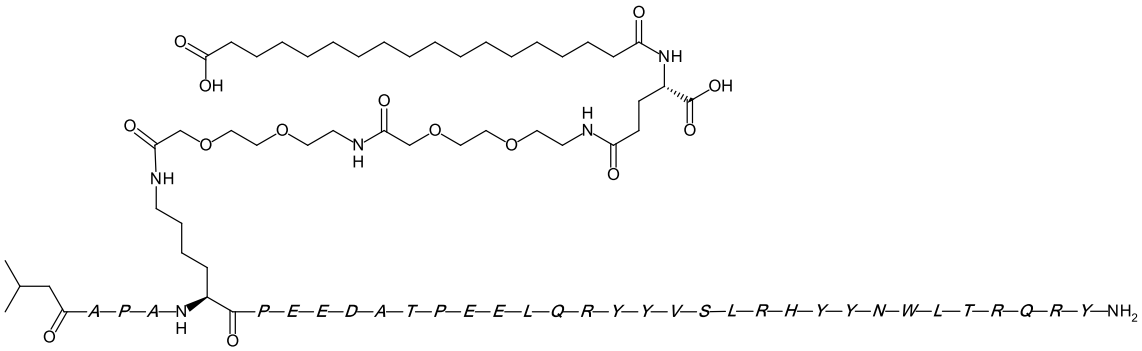
15. The PYY analogue according to claim 1, wherein the compound is



(Compound 74)

or a pharmaceutically acceptable salt thereof.

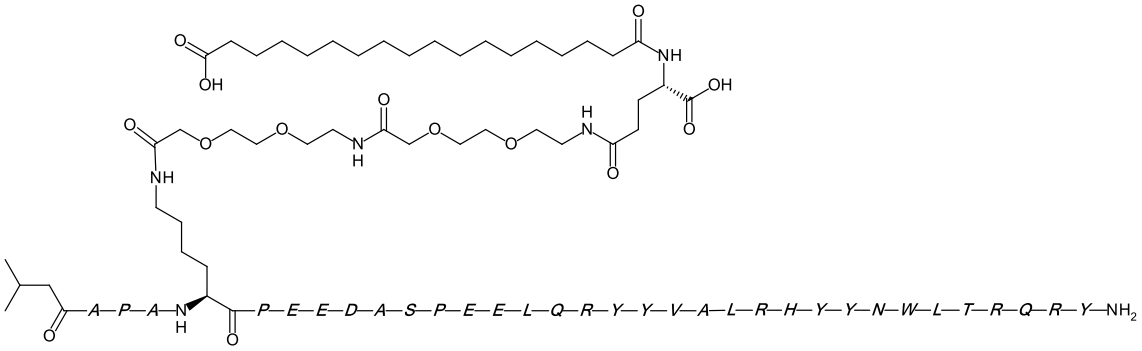
16. The PYY analogue according to claim 1, wherein the compound is



(Compound 106)

or a pharmaceutically acceptable salt thereof.

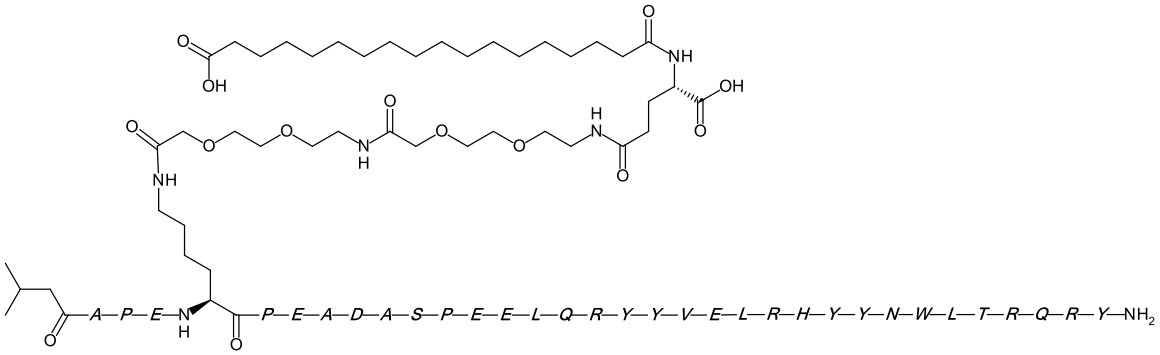
17. The PYY analogue according to claim 1, wherein the compound is



(Compound 117)

or a pharmaceutically acceptable salt thereof.

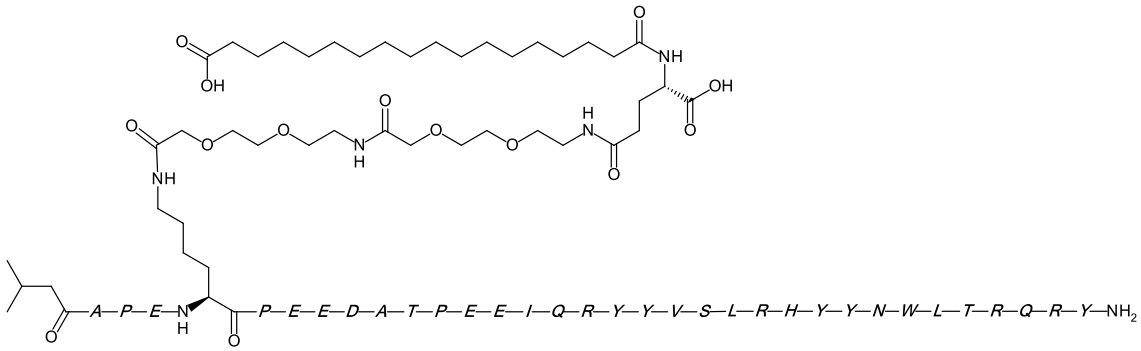
18. The PYY analogue according to claim 1, wherein the compound is



(Compound 135)

or a pharmaceutically acceptable salt thereof.

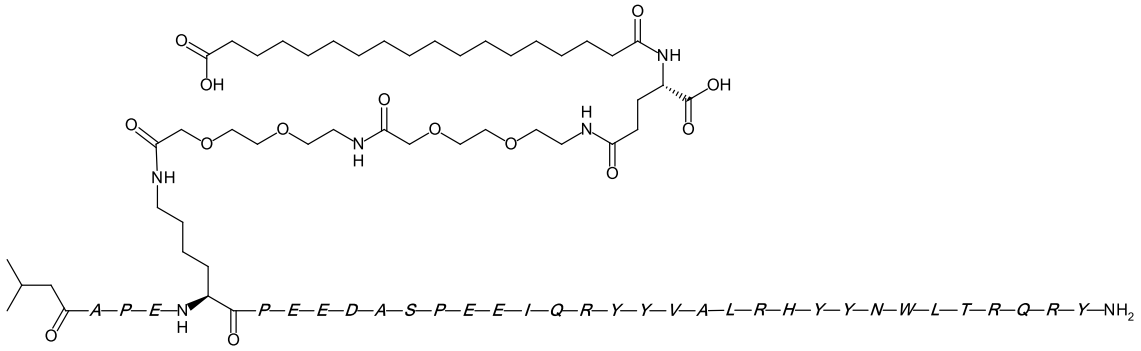
19. The PYY analogue according to claim 1, wherein the compound is



(Compound 171)

or a pharmaceutically acceptable salt thereof.

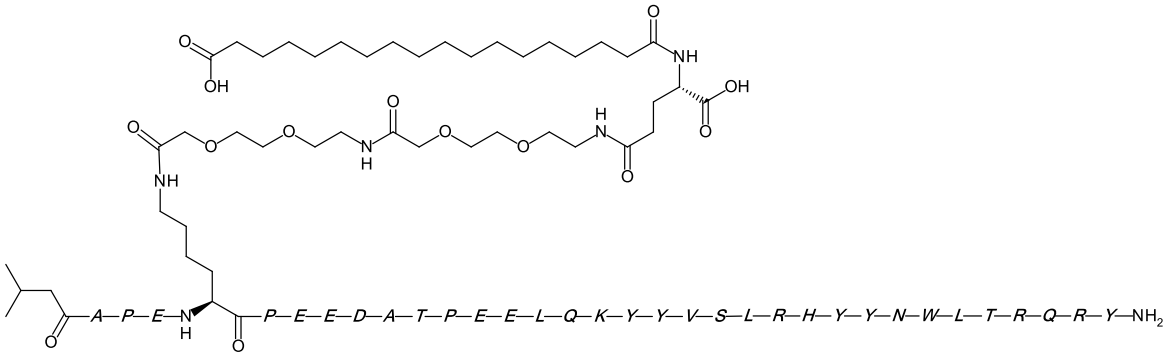
20. The PYY analogue according to claim 1, wherein the compound is



(Compound 230)

or a pharmaceutically acceptable salt thereof.

21. The PYY analogue according to claim 1, wherein the compound is



(Compound 231)

or a pharmaceutically acceptable salt thereof.

22. A pharmaceutical composition comprising at least one PYY analogue according to any one of the previous claims and one or more pharmaceutically acceptable excipient.

23. A PYY analogue according to any one of claims 1 to 21 for use in the treatment of obesity or obesity-related conditions, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

24. A PYY analogue for use according to claim 23, wherein the use is for the treatment of atherogenic dyslipidemia, hepatic steatosis, NAFLD, NASH, kidney failure or atherosclerosis.

25. A PYY analogue for use according to claim 23 or 24, wherein the PYY analogue is to be administered as part of a combination therapy together with an agent for treatment of diabetes, obesity, dyslipidemia or hypertension.

26. A PYY analogue for use according to claim 23 or 24, wherein the PYY analogue is to be administered as part of a combination therapy together with an agent for treatment of obesity, wherein the agent for obesity is a GIP or GLP-1 receptor agonist, e.g. GLP-1 or a GLP-1 analogue, exendin-4 or an exendin-4 analogue, any other GLP-1 receptor agonist including Liraglutide, Semaglutide, Dulaglutide or Albiglutide, or a glucagon-GLP-1 dual agonist, GLP-1/GIP dual agonist, or a GLP-1/GIP/glucagon triple agonist, or an amylin receptor agonist.

27. Use of a PYY analogue according to any one of claims 1 to 21 in the manufacture of a medicament for the treatment of obesity or obesity-related conditions, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea and cardiovascular disease.

28. The use according to claim 27, wherein the disease is atherogenic dyslipidemia, hepatic steatosis, NAFLD, NASH, kidney failure or atherosclerosis.

29. The use according to claim 27 or 28, wherein the PYY analogue is to be administered as part of a combination therapy together with an agent for treatment of diabetes, obesity, dyslipidemia or hypertension.

30. The use according to claim 27 or 28, wherein the PYY analogue is to be administered as part of a combination therapy together with an agent for treatment of obesity, wherein the agent for obesity is a GIP or GLP-1 receptor agonist, e.g. GLP-1 or a GLP-1 analogue, exendin-4 or an exendin-4 analogue, any other GLP-1 receptor agonist including Liraglutide, Semaglutide, Dulaglutide or Albiglutide, or a glucagon-GLP-1 dual agonist, GLP-1/GIP dual agonist, or a GLP-1/GIP/glucagon triple agonist, or an amylin receptor agonist.