Peptides with Neuropeptide-2 receptor (Y2R) agonist activity

The present invention relates to truncated analogs of PYY $_{3-36}$.of formula (I)

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}R_7\text{-}R_8\text{-}R_9\text{-}R_{10}\text{-}R_{11}\text{-}R_{12}\text{-}R_{13}\text{-}R_{14}\text{-}NH_2 \quad (I)$$

wherein X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety, R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp, R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly), R_3 is Arg, Ala, (D)Arg, N-methylArg, Phe, 3,4,5- TrifluoroPhe or 2,3,4,5,6- Pentafluoro Phe, R_4 is His, Ala, (D)His, N-methyl His, 4-MeOApc, 3-Pal or 4-Pal, R_5 is Tyr, Ala, (D) Tyr , N- methyl Tyr, Trp, Tic, Bip, Dip, (1)Nal, (2)Nal, 3,4,5- TrifluroPhe or 2,3,4,5,6-Pentafluoro Phe, R_6 is Leu, Ala, (D)Leu or N-methyl Leu, R_7 is Asn, Ala or (D)Asn, R_8 is Leu, Ala, (D)Leu or N-methyl Leu, R_9 is Val, Ala, (D) Val or N-methyl Val, R_{10} is Thr, Ala or N-methyl Thr, R_{11} is Arg, (D) Arg or N-methyl Arg, R_{12} is Gln or Ala, R_{13} is Arg, (D)Arg or N-methyl Arg, and R_{14} is Tyr, (D) Tyr or N- methyl Tyr, or a pharmaceutically acceptable salt thereof.

The analogs are agonists of neuropeptide-2 receptor and are useful for the treatment of metabolic disorders, such as, for example, obesity. All documents cited herein are hereby expressly incorporated herein by reference.

Obesity is widely recognized as a serious health problem for developed countries, and has reached epidemic status in the United States. According to recent studies, more than 50 % of the U.S. population is considered overweight, with more than 25 % diagnosed as clinically obese and at considerable risk for heart disease, non-insulin dependent diabetes

mellitus (NIDDM), hypertension, and certain cancers. This epidemic presents a significant burden on the health care system as projected obesity treatment costs of more than $70 billion annually are expected in the U.S. alone. Strategies for treating obesity include reduction of food intake and enhancing the expenditure of energy.

Neuropeptide Y (NPY), a 36 amino acid peptide neurotransmitter, is a member of the pancreatic polypeptide class of neurotransmitters/neurohormones which has been shown to be present in both the periphery and central nervous system. NPY is one of the most potent orexogenic agents known and has been shown to play a major role in the regulation of food intake in animals, including humans.

Five neuropeptide Y receptors (NPY) , the Y1-, Y2-, Y3-, Y4, and Y5- and Y6-subtypes, have been cloned, which belong to the rhodopsin-like G-protein-coupled ,7-transmembrane helix- spanning receptors (GPCR) . The NPY Y2 receptor (Y2R) is a 381 amino-acid which inhibits the activation of adenyl cyclase via G_i while having low homology with other known NPY receptors. There is a high degree of conservation between rat and human Y2 receptor with 98 % amino acid identity.

The Y2R receptor is widely distributed within the central nervous system in both rodents and humans. In the hypothalamus, Y2 mRNA is localized in the arcuate nucleus, preoptic nucleus, and dorsomedial nucleus. In the human brain, Y2R is the predominant Y receptor subtype. Within the arcuate nuclease, over 80 % of the NPY neurons co-express Y2R mRNA. Application of a Y2-selective agonist has been shown to reduce the release of NPY from hypothalamic slices in vitro, whereas the Y2 non-peptide antagonist BIIE0246 increases NPY release. These findings support the role of Y2R as a presynaptic autoreceptor that regulates the NPY release and hence may be involved in the regulation of feeding. (Poter, E.K., et al. Eur. J. Pharmac. 267,253-262 (1994)).

Peptide YY $_{3-36}$ (PYY $_{3-36}$) is a 33 amino acid linear peptide having specific neuropeptide Y2 (NPY2R) agonist activity. It has been demonstrated that Intra-arcuate (IC) or Intra-peritoneal (IP) injection of PYY $_{3-36}$ reduced feeding in rats and, as a chronic treatment, reduced body weight gain. Intra-venous (IV) infusion (0.8 pmol/kg/min) for 90 min of PYY $_{3-36}$ reduced food intake in obese and normal human subjects 33 % over 24 hours.

These finding suggest that the PYY system may be a therapeutic target for the treatment of obesity. (Bloom, S. et al, Nature Vol. 418, 8 August 2002 , P. 650-654). Further, a Cys^2-(D)Cys^{27}-cyclized version of PYY, in which residues 5-24 were replaced by a methylene-chain of 5 to 8 carbons in length, showed activation of the intestinal PYY receptor, as evidenced by reduced current across voltage-clamped mucosal preparations of rat jejunum. (Krstenansky, et al. in Peptides, Proceedings of the Twelfth American Peptide Symposium. J. Smith and J. Rivier Editors, ESCOM. Leiden Page 136-137).

Further, covalent modification of proteins with poly (ethylene glycol) or poly (ethylene oxide) (both referred to as PEG), was demonstrated with superoxide dismutase (Somack, R, et al., (1991) Free Rad Res Commun 12-13:553-562; U.S. Pat. Nos. 5,283,317 and 5,468,478) and for other types of proteins, e.g., cytokines (Saifer, M G P, et al., (1997) Polym Preprints 38:576-577; Sherman, M R, et al., (1997) in J M Harris, et al., (Eds.), Poly(ethylene glycol) Chemistry and Biological Applications. ACS Symposium Series 680 (pp. 155-169) Washington, D.C.: American Chemical Society).

A need exists, however, for novel engineered analogs of PYY having significantly lower molecular weight, while possessing equal or better potency, pharmacokinetic properties and pharmacological properties as existing Y2R agonists. A need also exists for pegylated analogs of PYY in order to, for example, increase peptide half-life and reduce immunogenicity in subjects in need of such agonists.

According to a first aspect, there is provided a neuropeptide-2 receptor agonist of the formula (I):

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}R_7\text{-}R_8\text{-}R_9\text{-}R_{10}\text{-}R_{11}\text{-}R_{12}\text{-}R_{13}\text{-}R_{14}\text{-}NH_2 \quad (I)$$

wherein:

X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine,

Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,

R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp,

R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly),

R_3 is Arg, Ala, (D)Arg, N-methylArg, Phe, 3,4,5- TrifluoroPhe or 2,3,4,5,6- Pentafluoro Phe,

R_4 is His, Ala, (D)His, N-methyl His, 4-MeOApc, 3-Pal or 4-Pal,

R_5 is Tyr, Ala, (D) Tyr , N- methyl Tyr, Trp, Tic, Bip, Dip, (1)Nal, (2)Nal, 3,4,5-TrifluroPhe or 2,3,4,5,6- Pentafluoro Phe,

R_6 is Leu, Ala, (D)Leu or N-methyl Leu,

R_7 is Asn, Ala or (D)Asn,

R_8 is Leu, Ala, (D)Leu or N-methyl Leu,

R_9 is Val, Ala, (D) Val or N-methyl Val,

R_{10} is Thr, Ala or N-methyl Thr,

R_{11} is Arg, (D) Arg or N-methyl Arg,

R_{12} is Gln or Ala,

R_{13} is Arg, (D)Arg or N-methyl Arg, and

R_{14} is Tyr, (D) Tyr or N- methyl Tyr,

or a pharmaceutically acceptable salt thereof.

According to a second aspect, there is provided a neuropeptide-2 receptor agonist of the formula (II):

$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}Arg\text{-}His\text{-}Tyr\text{-}Leu\text{-}Asn\text{-}Leu\text{-}Val\text{-}Thr\text{-}Arg\text{-}Gln\text{-}Arg\text{-}Tyr\text{-}NH_2$ (II)

wherein:

X is a moiety selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine,

Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,

R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp, and

R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly),

or a pharmaceutically acceptable salt thereof.

According to a third aspect, there is provided a neuropeptide-2 receptor agonist of the formula (III):

Y-Ile-Lys-X-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (III)

wherein:

X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, and

Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,

or a pharmaceutically acceptable salt thereof.

According to a fourth aspect, there is provided pharmaceutical compositions comprising a compound as defined above and a pharmaceutically acceptable carrier and/or adjuvant.

According to a fifth aspect, there is provided compounds as defined above for use as therapeutic active substances.

According to a sixth aspect, there is provided compounds as defined above for use as therapeutic active substances for the treatment and/or prophylaxis of diseases which are modulated by neuropeptide-2 receptor agonists.

According to a seventh aspect, there is provided the use of compounds as defined above for the preparation of medicaments for the therapeutic and/or prophylactic treatment of diseases which are modulated by neuropeptide-2 receptor agonists.

According to an eighth aspect, there is provided the use of compounds as defined above for the preparation of medicaments for the therapeutic and/or prophylactic treatment of obesity.

The compounds of the invention are also advantageous because they are truncated versions of the PYY_{3-36}. Thus, the shorter peptides not only facilitate easier synthesis and purification of the compounds, but also improve and reduce manufacturing procedures and expenses. Moreover, the compounds of the invention will interact preferably with PYY receptors and not with homologous receptors such as NPY Y1, Y4 and Y5. Unwanted agonist or antagonist side reactions are, thereby, minimized.

Figure 1 shows a HPLC chromatogram of a compound of the present invention.

Figure 2 shows a HPLC chromatogram of a compound of the present invention.

Figure 3 shows a MALDI-TOF of a compound of the present invention.

Figure 4 shows a HPLC chromatogram of another compound of the present invention.

Figure 5 shows a HPLC chromatogram of a compound of the present invention.

Figure 6 shows a MALDI-TOF of a compound of the present invention.

Figure 7 shows a chart of the effects on food intake after administration of a compound of the present invention.

Figure 8 shows a chart of the effects on food intake after administration of another compound of the present invention.

Figure 9 shows a chart of the effects on food intake after administration of a yet another compound of the present invention.

Figure 10 shows a chart of the effects on food intake after administration of a still another compound of the present invention.

All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right, unless noted otherwise. A short line between two amino acid residues indicates a peptide bond. Where the amino acid has isomeric forms, it is the L form of the amino acid that is represented unless otherwise expressly indicated. For convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. These abbreviations are familiar to those skilled in the art, but for clarity are listed below:

Asp=D=Aspartic Acid; Ala=A=Alanine; Arg=R=Arginine; Asn=N=Asparagine; Gly=G=Glycine; Glu=E=Glutamic Acid; Gln=Q=Glutamine; His=H=Histidine; Ile=I=Isoleucine; Leu=L=Leucine; Lys=K=Lysine; Met=M=Methionine;

Phe=F=Phenylalanine; Pro=P=Proline; Ser=S=Serine; Thr=T=Threonine; Trp=W=Tryptophan; Tyr=Y=Tyrosine; and Val=V=Valine.

Also for convenience, and readily known to one skilled in the art, the following abbreviations or symbols are used to represent the moieties, reagents and the like used in this invention:

Pqa	N-piperazin-1-yl-4-(3H)-quanazolinone-3-acetic –acid;
Cms	N-(5-O-carboxymethyl)-seratonin;
3,4,5, F3-Phe	3,4,5 –Trifluoro phenylalanine;
2,3,4,5,6, F5- Phe	2,3,4,5,6-Pentafluoro phenylalanine;
4-MeO-Apc	4- Methoxy-1-amino 4-phenylcyclohexane carboxylic acid;
3-Pal	3-Pyridyl alanine;
4-Pal	4-Pyridyl Alanine;
Aib	Amino isobutyric acid;
1-1-Aic	1 Amino-indane 1- carboxylic acid;
2-2-Aic	2 Amino-indane 2- carboxylic acid;
Ach	1-Amino cyclohexyl carboxylic acid;
Acp	1-Amino cyclopentyl carboxylic acid;
Fmoc	9-Fluorenylmethyloxycarbonyl;
Allyl	Allyl ester;
Aloc	Allyloxycarbonyl;
Mtt	4- Methyltrityl;
2Pip	2-Phenylisopropyl ester;
Pmc	2,2,5,7,8-Pentamethylchroman-6-sulfonyl;
CH_2Cl_2	Methylene chloride;
A2O	Acetic anhydride;
CH_3CN	Acetonitrile;
DMAc	Dimethylacetamide;
DMF	Dimethylformamide;
DIPEA	N, N-Diisopropylethylamine;
TFA	Trifluoroacetic acid;
HOBT	N-Hydroxybenzotriazole;

DIC	N, N'-Diisopropylcarbodiimide;
BOP	Benzotriazol-1-yloxy-tris- (dimethylamino) phosphonium-Hexafluorophosphate;
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-Hexafluorophosphate;
NMP	1-methyl 2-Pyrolidenone;
Bip	Biphenylalanin or 4-Phenyl-phentylalanine;
Dip	Diphenylalanine;
Tic	1,2,3,4,-tetrahydroisoquinoline-3 carboxylic acid;
FAB-MS	Fast atom bombardment mass spectrometry; and
ES-MS	Electro spray mass spectrometry.

As used herein, the term "alkyl" means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical which may be substituted or unsubstituted. Where cyclic, the alkyl group is preferably C_3 to C_{12}, more preferably C_5 to C_{10}, more preferably C_5 to C_7. Where acyclic, the alkyl group is preferably C_1 to C_{10}, more preferably C_1 to C_6, more preferably methyl, ethyl, propyl (n-propyl or isopropyl), butyl (n-butyl, isobutyl or tertiary-butyl) or pentyl (including n-pentyl and isopentyl), more preferably methyl. It will be appreciated therefore that the term "alkyl" as used herein includes alkyl (branched or unbranched), substituted alkyl (branched or unbranched), alkenyl (branched or unbranched), substituted alkenyl (branched or unbranched), alkynyl (branched or unbranched), substituted alkynyl (branched or unbranched), cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, cycloalkynyl and substituted cycloalkynyl.

As used herein, the term "lower alkyl" means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical wherein said cyclic lower alkyl group is C_5, C_6 or C_7, and wherein said acyclic lower alkyl group is C_1, C_2, C_3 or C_4, and is preferably selected from methyl, ethyl, propyl (n-propyl or isopropyl) or butyl (n-butyl, isobutyl or tertiary-butyl). It will be appreciated therefore that the term "lower alkyl" as used herein includes lower alkyl (branched or unbranched), lower alkenyl (branched or unbranched), lower alkynyl (branched or unbranched), cyclolower alkyl, cyclolower alkenyl and cyclolower alkynyl.

As used herein, the term "aryl" means a substituted or unsubstituted carbocyclic aromatic group, such as phenyl or naphthyl, or a substituted or unsubstituted heteroaromatic group containing one or more, preferably one, heteroatom, such as pyridyl, pyrrolyl, furanyl, thienyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl pyrazolyl, imidazolyl, triazolyl, pyrimidinyl pyridazinyl, pyrazinyl, triazinyl, indolyl, indazolyl, quinolyl, quinazolyl, benzimidazolyl, benzothiazolyl, benzisoxazolyl and benzisothiazolyl.

The alkyl and aryl groups may be substituted or unsubstituted. Where substituted, there will generally be 1 to 3 substituents present, preferably 1 substituent. Substituents may include: carbon-containing groups such as alkyl, aryl, arylalkyl (e.g. substituted and unsubstituted phenyl, substituted and unsubstituted benzyl); halogen atoms and halogen-containing groups such as haloalkyl (e.g. trifluoromethyl); oxygen-containing groups such as alcohols (e.g. hydroxyl, hydroxyalkyl, aryl(hydroxyl)alkyl), ethers (e.g. alkoxy, aryloxy, alkoxyalkyl, aryloxyalkyl), aldehydes (e.g. carboxaldehyde), ketones(e.g. alkylcarbonyl, alkylcarbonylalkyl, arylcarbonyl, arylalkylcarbonyl, arycarbonylalkyl), acids (e.g. carboxy, carboxyalkyl), acid derivatives such as esters(e.g. alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, alkylcarbonyloxyalkyl), amides (e.g. aminocarbonyl, mono- or di-alkylaminocarbonyl, aminocarbonylalkyl, mono-or di-alkylaminocarbonylalkyl, arylaminocarbonyl), carbamates (e.g. alkoxycarbonylamino, arloxycarbonylamino, aminocarbonyloxy, mono-or di-alkylaminocarbonyloxy, arylminocarbonloxy) and ureas (e.g. mono- or di- alkylaminocarbonylamino or arylaminocarbonylamino); nitrogen-containing groups such as amines (e.g. amino, mono- or di-alkylamino, aminoalkyl, mono- or di-alkylaminoalkyl), azides, nitriles (e.g. cyano, cyanoalkyl), nitro; sulfur-containing groups such asthiols, thioethers, sulfoxides and sulfones (e.g. alkylthio, alkylsulfinyl, alkylsulfonyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, arylthio, arysulfinyl, arysulfonyl, arythioalkyl, arylsulfinylalkyl, arylsulfonylalkyl); and heterocyclic groups containing one or more, preferably one, heteroatom, (e.g. thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, thiadiazolyl, aziridinyl, azetidinyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, tetrahydrofuranyl, pyranyl, pyronyl, pyridyl, pyrazinyl, pyridazinyl, piperidyl, hexahydroazepinyl, peperazinyl, morpholinyl, thianaphthyl, benzofuranyl, isobenzofuranyl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, benzopyranyl,

coumarinyl, isocoumarinyl, quinolinyl, isoquinolinyl, naphthridinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxalinyl, chromenyl, chromanyl, isochromanyl, phthalazinyl and carbolinyl).

The lower alkyl groups may be substituted or unsubstituted, preferably unsubstituted. Where substituted, there will generally be 1 to 3 substitutents present, preferably 1 substituent. Substituents include the substituent groups listed above other than alkyl, aryl and arylalkyl.

As used herein, the term "alkoxy" means alkyl-O- and "alkoyl" means alkyl-CO-. Alkoxy substituent groups or alkoxy-containing substituent groups may be substituted by one or more alkyl groups.

As used herein, the term "halogen" means a fluorine, chlorine, bromine or iodine radical, preferably a fluorine, chlorine or bromine radical, and more preferably a fluorine or chlorine radical.

As used herein, the term "pharmaceutically acceptable salt" means any pharmaceutically acceptable salt of the compound of formula (I). Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, dichloroacetic, formic, fumaric, gluconic, glutamic, hippuric, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, oxalic, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, oxalic, p-toluenesulfonic and the like. Particularly preferred are fumaric, hydrochloric, hydrobromic, phosphoric, succinic, sulfuric and methanesulfonic acids. Acceptable base salts include alkali metal (e.g. sodium, potassium), alkaline earth metal (e.g. calcium, magnesium) and aluminium salts.

In an embodiment of the present invention, a neuropeptide-2 receptor agonist of the formula (I) is provided:

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}R_7\text{-}R_8\text{-}R_9\text{-}R_{10}\text{-}R_{11}\text{-}R_{12}\text{-}R_{13}\text{-}R_{14}\text{-}NH_2 \quad (I)$$

wherein X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety, R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp, R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly), R_3 is Arg, Ala, (D)Arg, N-methylArg, Phe, 3,4,5- TrifluoroPhe or 2,3,4,5,6- Pentafluoro Phe, R_4 is His, Ala, (D)His, N-methyl His, 4-MeOApc, 3-Pal or 4-Pal, R_5 is Tyr, Ala, (D) Tyr , N- methyl Tyr, Trp, Tic, Bip, Dip, (1)Nal, (2)Nal, 3,4,5- TrifluroPhe or 2,3,4,5,6-Pentafluoro Phe, R_6 is Leu, Ala, (D)Leu or N-methyl Leu, R_7 is Asn, Ala or (D)Asn, R_8 is Leu, Ala, (D)Leu or N-methyl Leu, R_9 is Val, Ala, (D) Val or N-methyl Val, R_{10} is Thr, Ala or N-methyl Thr, R_{11} is Arg, (D) Arg or N-methyl Arg, R_{12} is Gln or Ala, R_{13} is Arg, (D)Arg or N-methyl Arg, and R_{14} is Tyr, (D) Tyr or N- methyl Tyr, or a pharmaceutically acceptable salt thereof.

In another embodiment of the present invention, a neuropeptide-2 receptor agonist of the formula (II) is provided:

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}Arg\text{-}His\text{-}Tyr\text{-}Leu\text{-}Asn\text{-}Leu\text{-}Val\text{-}Thr\text{-}Arg\text{-}Gln\text{-}Arg\text{-}Tyr\text{-}NH_2 \quad (II)$$

(SEQ ID NO: 1)

wherein X is a moiety selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety, R_1 is Ile, Ala, (D) Ile, N-methyl Ile,

Aib, 1-1Aic, 2-2 Aic, Ach or Acp, and R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly), or a pharmaceutically acceptable salt thereof.

In yet another embodiment of the present invention, a neuropeptide-2 receptor agonist of the formula (III) is provided:

Y-Ile-Lys-X-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (III)
(SEQ ID NO: 2)

wherein X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, and Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety, or a pharmaceutically acceptable salt thereof.

Preferred neuropeptide-2 receptor agonist as defined abover are those, wherein X is Pqa. Other preferred neuropeptide-2 receptor agonist as defined above are those, wherein X is Cms. Preferably, Y is H or (C_1-C_6) alkyl, more preferably Y is a (C_1-C_6) alkyl moiety.

Preferred neuropeptide-2 receptor agonists as defined above are those selected from the group consisting of
IK-Pqa -RHYLNLVTRQRY,
Ac-IK-Pqa -RHYLNLVTRQRY,
IK-Pqa -RHYLNLVTRARY,
IK-Pqa -RHYLNLVARQRY,
IK-Pqa -RHYLNLATRQRY,
IK-Pqa -RHYLNAVTRQRY,
IK-Pqa -RHYLALVTRQRY,
IK-Pqa -RHYANLVTRQRY,
IK-Pqa -RHALNLVTRQRY,
IK-Pqa -RAYLNLVTRQRY,
IK-Pqa -AHYLNLVTRQRY,

IA-Pqa -RHYLNLVTRQRY,

Ac-IA-Pqa -RHYLNLVTRQRY,

AK-Pqa -RHYLNLVTRQRY,

IK-Pqa -RHYLNLVTRQR(D)Y,

IK-Pqa -RHYLNLVTRQ(D)RY,

IK-Pqa -RHYLNLVT(D)RQRY,

IK-Pqa -RHYLNL(D)VTRQRY,

IK-Pqa -RHYLN(D)LVTRQRY,

IK-Pqa -RHYL(D)NLVTRQRY,

IK-Pqa -RHY(D)LNLVTRQRY,

IK-Pqa -RH(D)YLNLVTRQRY,

IK-Pqa -R(D)HYLNLVTRQRY,

IK-Pqa -(D)RHYLNLVTRQRY,

I(D)K-Pqa -RHYLNLVTRQRY,

(D)IK-Pqa -RHYLNLVTRQRY,

IK-Pqa -RHYLNLVTRQR(N-methyl)Y,

IK-Pqa -RHYLNLVTRQ(N-methyl)RY,

IK-Pqa -RHYLNLVT(N-methyl)RQRY,

IK-Pqa -RHYLNLV(N-methyl)TRQRY,

IK-Pqa -RHYLNL(N-methyl)VTRQRY,

IK-Pqa -RHYLN(N-methyl)LVTRQRY,

IK-Pqa -RHY(N-methyl)LNLVTRQRY,

IK-Pqa -RH(N-methyl)YLNLVTRQRY,

IK-Pqa -R(N-methyl)HYLNLVTRQRY,

IK-Pqa -(N-methyl)RHYLNLVTRQRY,

I(N-methyl)K-Pqa -RHYLNLVTRQRY,

(N-methyl)IK-Pqa -RHYLNLVTRQRY,

INle-Pqa -RHYLNLVTRQRY,

Ac-INle-Pqa -RHYLNLVTRQRY,

Ac-INle-Pqa -FHYLNLVTRQRY,

IK-Pqa -RHWLNLVTRQRY,

IK-Pqa -AHWLNLVTRQRY,

Ac-INle-Pqa -RHYLNLVTRQR(D)Y,

Ac-INle-Pqa -RHYLNLVTRQR(N-methyl)Y,

Ac-INle-Pqa -RHYLys(28)NLVAsp(32)RQRY (cyclo Lys-Asp),

IK-Cms -RHYLNLVTRQRY,

IKG-Cms -RHYLNLVTRQRY,

Ac-INle-Cms-RHYLys(28)NLVAsp(32)RQRY(cyclo Lys-Asp),

Ac-INle-Pqa -RHTicLNLVTRQRY,

Ac-INle-Pqa -RHBipLNLVTRQRY,

Ac-INle-Pqa -RHDipLNLVTRQRY,

Ac-INle-Pqa -RH(1)NalLNLVTRQRY,

Ac-INle-Pqa -RH(2)NalLNLVTRQRY,

Ac-INle-Pqa -RH(3.4,5 Trifluoro Phe)LNLVTRQRY,

Ac-INle-Pqa -RH(2,3.4,5,6 Pentafluoro Phe)LNLVTRQRY,

Ac-INle-Pqa -R(4-MeOApc)YLNLVTRQRY,

Ac-INle-Pqa -R(3-Pal)YLNLVTRQRY,

Ac-INle-Pqa -R(4-Pal)YLNLVTRQRY,

Ac-INle-Pqa -(3,4,5 Trifluro Phe)HYLNLVTRQRY,

Ac-INle-Pqa -(2,3,4,5 ,6 Pentafluro Phe)HYLNLVTRQRY,

Ac-Aib-Nle-Pqa-RHYLNLVTRQRT,

Ac1-1-Aic-Nle-Pqa -RHYLNLVTRQRT,

Ac1-1-Aic-Nle-Pqa -RHYLNLVTRQRT,

Ac-2-2Aic-Nle-Pqa -RHYLNLVTRQRT,

Ac-Ach-Nle-Pqa-RHYLNLVTRQRT,

Ac-Acp-Nle-Pqa -RHYLNLVTRQRT,

H- INle-Pqa -RHYLNLVTRQRY,

(PEG-10,000) INle-Pqa -RHYLNLVTRQRY, and

(PEG-30,000) INle-Pqa -RHYLNLVTRQRY.

More preferred neuropeptide-2 receptor agonists as defined above are those selected from the group consisting of

IK-Pqa -RHYLNLVTRQRY,

Ac-IK-Pqa -RHYLNLVTRQRY,

IK-Pqa -RHYLNLVTRQR(N-methyl)Y,

IK-Pqa -RHYLNLVTRQ(N-methyl)RY,

INle-Pqa -RHYLNLVTRQRY,
Ac-INle-Pqa -RHYLNLVTRQRY, and
(PEG-30,000) INle-Pqa -RHYLNLVTRQRY.

All of the above mentione neuropeptide-2 receptor agonists individually constitute separate preferred embodiments.

In a further embodiment of the present invention, a method of treating obesity in a patient in need thereof is provided, the method having the steps of administering to said patient a therapeutically effective amount of a neuropeptide-2 receptor agonist of the formula (I):

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}R_7\text{-}R_8\text{-}R_9\text{-}R_{10}\text{-}R_{11}\text{-}R_{12}\text{-}R_{13}\text{-}R_{14}\text{-}NH_2 \quad (I)$$

wherein X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety, R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp, R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly), R_3 is Arg, Ala, (D)Arg, N-methylArg, Phe, 3,4,5- TrifluoroPhe or 2,3,4,5,6- Pentafluoro Phe, R_4 is His, Ala, (D)His, N-methyl His, 4-MeOApc, 3-Pal or 4-Pal,
R_5 is Tyr, Ala, (D) Tyr , N- methyl Tyr, Trp, Tic, Bip, Dip, (1)Nal, (2)Nal, 3,4,5-TrifluroPhe or 2,3,4,5,6- Pentafluoro Phe, R_6 is Leu, Ala, (D)Leu or N-methyl Leu, R_7 is Asn, Ala or (D)Asn, R_8 is Leu, Ala, (D)Leu or N-methyl Leu, R_9 is Val, Ala, (D) Val or N-methyl Val, R_{10} is Thr, Ala or N-methyl Thr, R_{11} is Arg, (D) Arg or N-methyl Arg, R_{12} is Gln or Ala, R_{13} is Arg, (D)Arg or N-methyl Arg, and R_{14} is Tyr, (D) Tyr or N-methyl Tyr, or a pharmaceutically acceptable salt thereof.

Preferred is a method as described above, wherein said neuropeptide-2 receptor agonist is administered to said patient once every three days. Preferably, said neuropeptide-2 receptor agonist is administered to said patient once a week.

Preferred is a method as described above, wherein said neuropeptide-2 receptor agonist is administered to said patient orally, intranasally, intravenously, subcutaneously, parenterally, transdermally, intraperitoneally or rectally. Preferably, said neuropeptide-2 receptor agonist is administered intranasally. It is also preferred, that said neuropeptide-2 receptor agonist is administered subcutaneously.

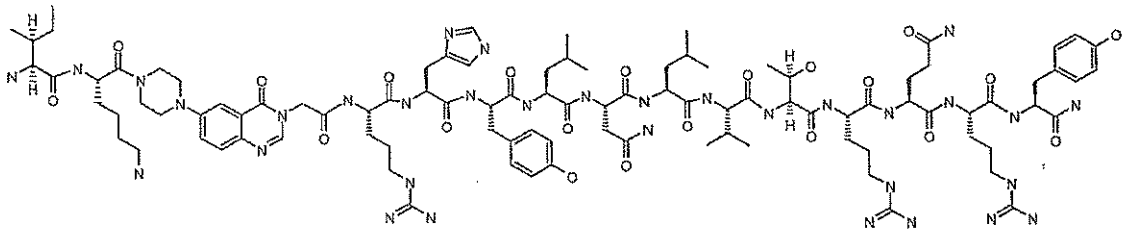
In a preferred method as described above, said neuropeptide-2 receptor agonist is administered at a dosage of from about 2.5 to about 10 mg/kg, more preferably from about 2.5 to about 5 mg/kg. A method is also preferred, wherein said neuropeptide-2 receptor agonist is administered at a dosage of from about 5 to about 10 mg/kg.

In the methods as described above, neuropeptide-2 receptor agonist are preferred, wherein X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine.

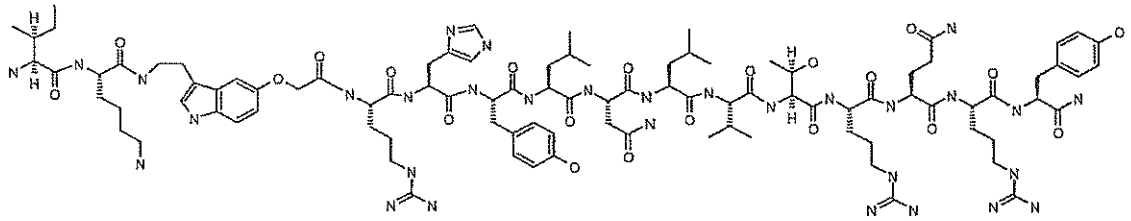
In a yet further embodiment of the present invention, a neuropeptide-2 receptor agonist is provided having a PYY $_{3-36}$ derivative with amino acid residues 5-24 are replaced by a moiety of about 8 to about 11 Angstroms in length.

The invention is now further illustrated by the following detailed description and the accompanying figures from which further embodiments, features and advantages may be taken.

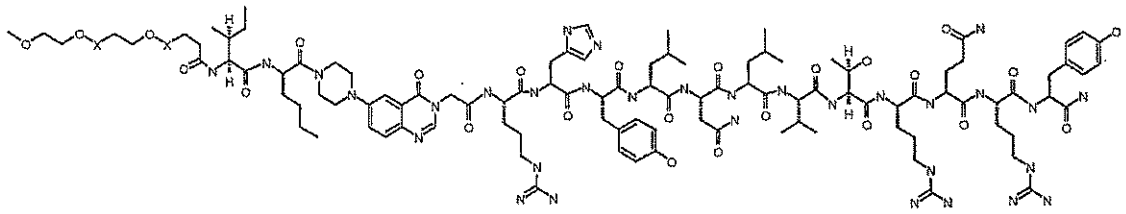
The present invention pertains to analogs of PYY_{3-36}, wherein amino acid residues 5-24 are replaced by a moiety such as, for example, Pqa or Cms. For example, the invention provides compounds of the formula PYY $_{3-4}$-Pqa- PYY $_{25-36}$:



and the formula PYY $_{3-4}$-Cms- PYY $_{25-36}$:



The invention further provides compounds of the formula:



In the structure above, the group -X-CH_2-CH_2-O-X-, according to common nomenclature, refers to the repeating glycol units of a polyethyleneglycol moiety, i.e. to a group -(CH_2-CH_2-O)$_n$-. "n" is chosen accordingly to achieve the desired weight of the desired polyethylene glycol unit, preferably about 10'000 (n is approximately 227) or about 30'000 (n is approximately 682). Using the NMR-derived structure of native PYY $_{3-36}$ as a guide, analogs are herein presented having a moiety replace amino acid residues 5-24 of PYY $_{3-36}$. Examples of such moieties include N-piperazin-1-yl-4-(3H)-quinazolinone-3-acetic acid (Pqa) and N-(5-O-carboxymethyl)-seratonin (Cms). The moiety is typically rigid and advantageously stabilizes the molecule's tertiary structure, thereby yielding truncated analogs with the desired potency and pharmacokinetic and pharmacological properties. The moiety is typically about 8 to about 11 Angstroms in length, preferably about 9 to about 11 Angstroms in length, more preferably about 9 to about 10 Angstroms in length, even more preferably about 8 to about 10 Angstroms in length, still more preferably about 8 to about 9 Angstroms in length.

It is to be understood that the invention is not limited to the particular embodiments of the invention described herein, as variations of the particular embodiments may be made and still fall within the scope of the appended claims. It is also to be understood that the terminology employed is for the purpose of describing particular embodiments, and is not intended to be limiting. Instead, the scope of the present invention will be established by the appended claims.

Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

The present representative compounds may be readily synthesized by any known conventional procedure for the formation of a peptide linkage between amino acids. Such conventional procedures include, for example, any solution phase procedure permitting a condensation between the free alpha amino group of an amino acid or residue thereof having its carboxyl group and other reactive groups protected and the free primary carboxyl group of another amino acid or residue thereof having its amino group or other reactive groups protected.

Such conventional procedures for synthesizing the novel compounds of the present invention include for example any solid phase peptide synthesis method. In such a method the synthesis of the novel compounds can be carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods. Such methods are disclosed in, for example, Merrifield, R. B., J. Amer. Chem. Soc. 85, 2149-2154 (1963); Barany *et al.*, The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross, E. and Meienhofer, J., Eds. Academic Press 1-284 (1980), which are incorporated herein by reference.

Common to chemical syntheses of peptides is the protection of reactive side chain groups of the various amino acid moieties with suitable protecting groups, which will prevent a chemical reaction from occurring at that site until the protecting group is ultimately removed. Usually also common is the protection of the alpha amino group on an amino acid or fragment while that entity reacts at the carboxyl group, followed by the selective

removal of the alpha amino protecting group at allow a subsequent reaction to take place at that site. While specific protecting groups have been disclosed in regard to the solid phase synthesis method, it should be noted that each amino acid can be protected by a protective group conventionally used for the respective amino acid in solution phase synthesis.

Alpha amino groups may be protected by a suitable protecting group selected from aromatic urethane-type protecting groups, such as allyloxycarbony, benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-biphenyl-isopropyloxycarbonyl, 9-fluorenylmethyloxycarbonyl (Fmoc) and p-methoxybenzyloxycarbonyl (Moz); aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), diisopropylmethyloxycarbonyl, isopropyloxycarbonyl, and allyloxycarbonyl. Herein, Fmoc is most preferred for alpha amino protection.

Guanidino groups may be protected by a suitable protecting group selected from nitro, p-toluenesulfonyl (Tos), (Z,) pentamethylchromanesulfonyl (Pmc), 4-Methoxy-2,3,6,-trimethylbenzzenesulfonyl (Mtr), (Pmc), and (Mtr) are most preferred for arginine (Arg).

The ε- amino groups may be protected by a suitable protecting group selected from 2-chloro benzyloxycarbonyl (2-Cl-Z), 2- Bromo benztloxycarbonyl (2-Br-Z) - and t-butyloxycarbonyl (Boc). Boc is the most preferred for (Lys).

Hydroxyl groups (OH) may be protected by a suitable protecting group selected from benzyl (Bzl), 2, 6 dichlorobenztl (2, 6 diCl-Bzl), and tert. Butyl (t-Bu), (tBu) is most preferred for (Tyr), (Ser) and (Thr).

The β- and γ- amide groups may be protected by a suitable protecting group selected from 4- methyltrityl (Mtt), 2, 4, 6-trimethoxybenzyl (Tmob), 4, 4-DimethoxyditylBis-(4-methoxyphenyl)-methyl (Dod) and Trityl (Trt). Trt is the most preferred for (Asn) and (Gln).

The indole group may be protected by a suitable protecting group selected from formyl (For), Mesityl -2- sulfonyl (Mts) and t-butyloxycarbonyl (Boc). Boc is the most preferred for (Trp).

The imidazol group may be protected by a suitable protecting group selected from Benzyl (Bzl), -t-butyloxycarbonyl (Boc), and Trityl (Trt). Trt is the most preferred for (His).

All solvents, isopropanol (iPrOH), methylene chloride (CH_2Cl_2), dimethylformamide (DMF) and N-methylpyrrolinone (NMP) were purchased from Fisher or Burdick & Jackson and were used without additional distillation. Trifluoroacetic acid was purchased from Halocarbon or Fluka and used without further purification.

Diisopropylcarbodiimide (DIC) and diisopropylethylamine (DIPEA) was purchased from Fluka or Aldrich and used without further purification. Hydroxybenzotriazole (HOBT) dimethylsulfide (DMS) and 1, 2-ethanedithiol (EDT) were purchased from Sigma Chemical Co. and used without further purification. Protected amino acids were generally of the L configuration and were obtained commercially from Bachem, or Neosystem. Purity of these reagents was confirmed by thin layer chromatography, NMR and melting point prior to use. Benzhydrylamine resin (BHA) was a copolymer of styrene - 1% divinylbenzene (100-200 or 200-400 mesh) obtained from Bachem or Advanced Chemtech. Total nitrogen content of these resins were generally between 0.3 - 1.2 meq/g.

High performance liquid chromatography (HPLC) was conducted on a LDC apparatus consisting of Constametric I and III pumps, a Gradient Master solvent programmer and mixer, and a Spectromonitor III variable wavelength UV detector. Analytical HPLC was performed in reversed phase mode using Vydac C_{18} columns (0.4 x 30 cm). Preparative HPLC separations were run on Vaydac columns (2 x 25 cm).

In a preferred embodiment, peptides were prepared using solid phase synthesis by the method generally described by Merrifield, (J. Amer. Chem. Soc., 85, 2149 (1963)), although other equivalent chemical synthesis known in the art could be used as previously mentioned. Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected alpha-amino acid to a suitable resin. Such a starting material can be prepared by attaching an alpha-amino-protected amino acid by an ester linkage to a p-

benzyloxybenzyl alcohol (Wang) resin, or by an amide bond between an Fmoc-Linker, such as p- ((R, S)-α-(1-(9H-fluoren-9-yl)-methoxyformamido)-2,4-dimethyloxybenzyl)-phenoxyacetic acid (Rink linker) to a benzhydrylamine (BHA) resin. Preparation of the hydroxymethyl resin is well known in the art. Fmoc-Linker-BHA resin supports are commercially available and generally used when the desired peptide being synthesized has an unsubstituted amide at the C-terminus.

Typically, the amino acids or mimetic are coupled onto the Fmoc-Linker-BHA resin using the Fmoc protected form of amino acid or mimetic, with 2 - 5 equivalents of amino acid and a suitable coupling reagent. After couplings, the resin may be washed and dried under vacuum. Loading of the amino acid onto the resin may be determined by amino acid analysis of an aliquot of Fmoc-amino acid resin or by determination of Fmoc groups by UV analysis. Any unreacted amino groups may be capped by reacting the resin with acetic anhydride and diispropylethylamine in methylene chloride.

The resins are carried through several repetitive cycles to add amino acids sequentially. The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, piperazine or morpholine (20-40% v/v) in DMF may be used for this purpose. Preferably 40% piperidine in DMF is utilized

Following the removal of the alpha amino protecting group, the subsequent protected amino acids are coupled stepwise in the desired order to obtain an intermediate, protected peptide-resin. The activating reagents used for coupling of the amino acids in the solid phase synthesis of the peptides are well known in the art. For example, appropriate reagents for such syntheses are benzotriazol-1-yloxy-tri- (dimethylamino) phosphonium hexafluorophosphate (BOP), Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP) 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and diisopropylcarbodiimide (DIC). Preferred here are HBTU and DIC. Other activating agents are described by Barany and Merrifield (in The Peptides, Vol. 2, J. Meienhofer, ed., Academic Press, 1979, pp 1-284) may be utilized. Various reagents such as 1 hydroxybenzotriazole (HOBT), N-hydroxysuccinimide (HOSu) and 3, 4-dihydro-3-hydroxy-4-oxo-1, 2, 3-benzotriazine (HOOBT) may be added to the coupling mixtures in order to optimize the synthetic cycles. Preferred here is HOBT.

The protocol for a typical synthetic cycle is as follows:

Protocol 1

Step	Reagent	Time
1	DMF	2 x 30 sec
2	20% piperidine/DMF	1 min
3	20% piperidine/DMF	15 min
4	DMF	2 x 30 sec
5	iPrOH	2 x 30 sec
6	DMF	3 x 30 sec
7	coupling	60 min - 18 hours
8	DMF	2 x 30 sec
9	iPrOH	1 x 30 sec
10	DMF	1 x 30 sec
11	CH_2Cl_2	2 x 30 sec

Solvents for all washings and couplings were measured to volumes of 10 - 20 ml/g resins. Coupling reactions throughout the synthesis were monitored by the Kaiser Ninhydrin test to determine extent of completion (Kaiser et at. Anal.Biochem.34, 595-598 (1970)). Slow reaction kinetics was observed for Fmoc-Arg (Pmc) and for couplings to secondary amines by sterically hindered acids. Any incomplete coupling reactions were either recoupled with freshly prepared activated amino acid or capped by treating the peptide resin with acetic anhydride as described above. The fully assembled peptide-resins were dried in vacuum for several hours.

For each compound, the blocking groups were removed and the peptide cleaved from the resin. For example, the peptide-resins were treated with 100 µL ethanedithiol, 100 µl dimethylsulfide, 300 µL anisole, and 9.5 mL trifluoroacetic acid, per gram of resin, at room temperature for 180 min. Or alternately the peptide-resins were treated with 1.0 mL triisopropyl silane and 9.5 mL trifluoroacetic acid, per gram of resin, at room temperature for 180 min. The resin is filtered off and the filtrates are precipitated in chilled ethyl ether. The precipitates are centrifuged and the ether layer is decanted.

The residue was washed with two or three volumes of Et_2O and recentrifuged. The crude products were dried under vacuum.

Purification of the crude peptides were performed on Shimadzu LC-8A system by high performance liquid chromatography (HPLC) on a reverse phase Vydac C-18 Column (50x250 mm. 300 A, 10-15 μm). The peptides were injected to the columns in a minimum volume of either 0.1 AcOH/H_2O or CH3CH/H_2O. Gradient elution was generally started at 2% B buffer, 2% -70% B over 70 minutes, (buffer A: 0.1% TFA/H_2O, buffer B: 0.1% TFA/CH_3CN) at a flow rate of 50 ml/min. UV detection was made at 220/280 nm. The fractions containing the products were separated and their purity was judged on Shimadzu LC-10AT analytical system using reverse phase Ace C18 column (4.6 x50mm) at a flow rate of 2 ml/min., gradient (2-70 %) over 10 min.(buffer A: 0.1% TFA/H_2O, buffer B: 0.1% TFA/CH_3CN)). Fractions judged to be of high purity were pooled and lyophilized.

Purity of the final products was checked by analytical HPLC on a reversed phase column as stated above. Purity of all products was judged to be approximately 95 - 99%. All final products were also subjected to fast atom bombardment mass spectrometry (FAB-MS) or electrospray mass spectrometry (ES-MS). All products yielded the expected parent M+H ions within acceptable limits.

Agonists of Y2R cause reduction of food intake in mouse models of human obesity. Therefore, administration of these compounds agonize Y2 receptor activity, which is important for the reduction of food intake and regulation of body weight. According to the in vivo activity experiments in Example 78, reduction of food intake was demonstrated for selected analogs of the invention (those of Examples 5, 44, 73 and 74) and the results summarized in Figures 7, 8, 9 and 10.

The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those formed with pharmaceutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic

acids, such as hydrohalic acids (e.g., hydrochloric acid), sulfuric acid, or phosphoric acid and the like. Any procedure for obtaining a pharmaceutically acceptable salt known to a skilled artisan can be used.

As described above, the novel compounds of the present invention have been found to be agonists of neuropeptide-2 receptor. The compounds of the present invention can therefore be used in the treatment and/or prophylaxis of diseases which are modulated by neuropeptide-2 receptor agonists, particularly obesity.

The invention therefore also relates to pharmaceutical compositions comprising a compound as defined above and a pharmaceutically acceptable carrier and/or adjuvant.

The invention likewise embraces compounds as described above for use as therapeutically active substances, especially as therapeutically active substances for the treatment and/or prophylaxis of diseases which are modulated by neuropeptide -2 receptor agonists, particularly as therapeutically active substances for the treatment and/or prophylaxis of obesity.

In another preferred embodiment, the invention relates to a method for the therapeutic and/or prophylactic treatment of diseases which are modulated by neuropeptide -2 receptor agonists, particularly for the therapeutic and/or prophylactic treatment of obesity, which method comprises administering a compound as defined above to a human being or animal.

The invention also embraces the use of compounds as defined above for the therapeutic and/or prophylactic treatment of diseases which are modulated by neuropeptide -2 receptor agonists, particularly for the therapeutic and/or prophylactic treatment of obesity.

The invention also relates to the use of compounds as described above for the preparation of medicaments for the therapeutic and/or prophylactic treatment of diseases which are modulated by neuropeptide -2 receptor agonists, particularly for the therapeutic and/or prophylactic treatment of obesity. Such medicaments comprise a compound as described above.

In the practice of the method of the present invention, an effective amount of any one of the peptides of this invention or a combination of any of the peptides of this invention or a pharmaceutically acceptable salt thereof, is administered via any of the usual and acceptable methods known in the art, either singly or in combination. The compounds or compositions can thus be administered orally (e.g., buccal cavity), sublingually, parenterally (e.g., intramuscularly, intravenously, or subcutaneously), rectally (e.g., by suppositories or washings), transdermally (e.g., skin electroporation) or by inhalation (e.g., by aerosol), and in the form or solid, liquid or gaseous dosages, including tablets and suspensions. The administration can be conducted in a single unit dosage form with continuous therapy or in a single dose therapy ad libitum. The therapeutic composition can also be in the form of an oil emulsion or dispersion in conjunction with a lipophilic salt such as pamoic acid, or in the form of a biodegradable sustained-release composition for subcutaneous or intramuscular administration.

Thus, the method of the present invention is practiced when relief of symptoms is specifically required or perhaps imminent. Alternatively, the method of the present invention is effectively practiced as continuous or prophylactic treatment.

Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids or gases; thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (e.g. binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, aerosols, and the like. The carrier can be selected from the various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulations for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, talc, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical additives

such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers and the like. Suitable pharmaceutical carriers and their formulation are described in Remington's Pharmaceutical Sciences by E. W. Martin. Such compositions will, in any event, contain an effective amount of the active compound together with a suitable carrier so as to prepare the proper dosage form for proper administration to the recipient.

The dose of a compound of the present invention depends on a number of factors, such as, for example, the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the active compound as determined by the attending physician or veterinarian is referred to herein, and in the claims, as an "effective amount". For example, the dose for intranasal administration is typically in the range of about 1 to about 100 mg/kg body weight; and the dose for subcutaneous administration is typically in the range of about 0.001 to about 50 mg/kg body weight.

Preferably, the dose of a compound of the present invention is from about 2.5 to about 10 mg/kg. Most preferably, the dosages are about 2.5, about 5 and about 10 mg/kg.

The invention will now be further described in the Examples which follow, which are intended as an illustration only and do not limit the scope of the invention.

Examples

EXAMPLE 1

Preparation of Fmoc-Linker-BHA Resin

Benzhydrylamine copolystyrene-1% divinylbenzene cross-linked resin (10.0 g, 9.3 mequiv, 100-200 ASTM mesh, Advanced ChemTech) was swelled in 100 mL CH2Cl2, filtered and washed successively with 100 ml each of CH_2Cl_2, 6% DIPEA/CH_2Cl_2 (two times), CH_2Cl_2 (two times). The resin was treated with p- ((R, S)-α-(1-(9H-fluoren-9-yl)-methoxyformamido)-2, 4-dimethoxybenzyl)-phenoxyacetic acid (Fmoc-Linker) (7.01 g, 13.0 mmole), N-hydroxybenzotriazole (2.16g, 16.0mmole), and diisopropyl-carbodiimide (2.04 ml, 13.0 mmol) in 100 mL 25% DMF/CH_2Cl_2 for 24 hours at room temperature. The resin was filtered and washed successively with 100 ml each of CH_2Cl_2 (two times), isopropanol (two times), DMF, and CH_2Cl_2 (three times). A Kaiser Ninhydrin analysis was negative. The resin was dried under vacuum to yield 16.12 g of Fmoc-Linker-BHA resin. A portion of this resin (3.5 mg) was subjected to Fmoc deprotection and quantitative UV analysis which indicated a loading of 0.56 mmol/g.

EXAMPLE 2

Protocol for the synthesis of peptides by Applied Biosystem 433A synthesizer using Fluorenylmethyloxycarbonyl (Fmoc) chemistry.

For a 0.25 mmol scale peptide synthesis by Applied Biosystem 433A synthesizer (Foster City, CA), the FastMoc 0.25 mmole cycles were used with either the resin sampling or non resin sampling, 41 mL reaction vessel. The Fmoc-amino acid resin was dissolved with 2.1 g NMP, 2g of 0.45M HOBT/HBTU in DMF and 2M DIEA, then transferred to the reaction vessel.

The basic FastMoc coupling cycle was represented by the module "BADEIFD," wherein each letter represents a module. For example:

B represents the module for Fmoc deprotection using 20% Piperidine/NMP and related washes and readings for 30 min (either UV monitoring or conductivity);

A represents the module for activation of amino acid in cartridges with 0.45 M HBTU/HOBt and 2.0 M DIEA and mixing with N_2 bubbling;

D represents the module for NMP washing of resin in the reaction vessel;

E represents the module for transfer of the activated amino acid to the reaction vessel for coupling;

I represents the module for a 10 minute waiting period with vortexing on and off of the reaction vessel; and

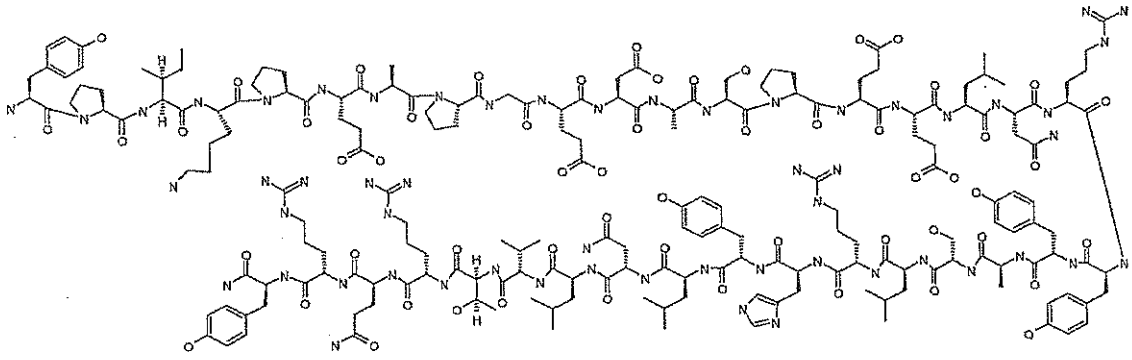
F represents the module for cleaning cartridge, coupling for approximately 10 minutes and draining the reaction vessel.

Couplings were typically extended by addition of module "I" once or multiple times. For example, double couplings were run by performing the procedure "BADEIIADEIFD." Other modules were available such as c for methylene chloride washes and "C" for capping with acetic anhydride. Individual modules were also modifiable by, for example, changing the timing of various functions, such as transfer time, in order to alter the amount of solvent or reagents transferred.

The cycles above were typically used for coupling one amino acid. For synthesizing tetra peptides, however, the cycles were repeated and strung together. For example, BADEIIADEIFD was used to couple the first amino acid, followed by BADEIIADEIFD to couple the second amino acid, followed by BADEIIADEIFD to couple the third amino acid, followed by BADEIIADEIFD to couple the fourth amino acid, followed by BIDDcc for final deprotection and washing.

EXAMPLE 3

Preparation of H-Tyr-Pro-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-NH_2 (PYY_{1-36}) (SEQ ID NO: 3)

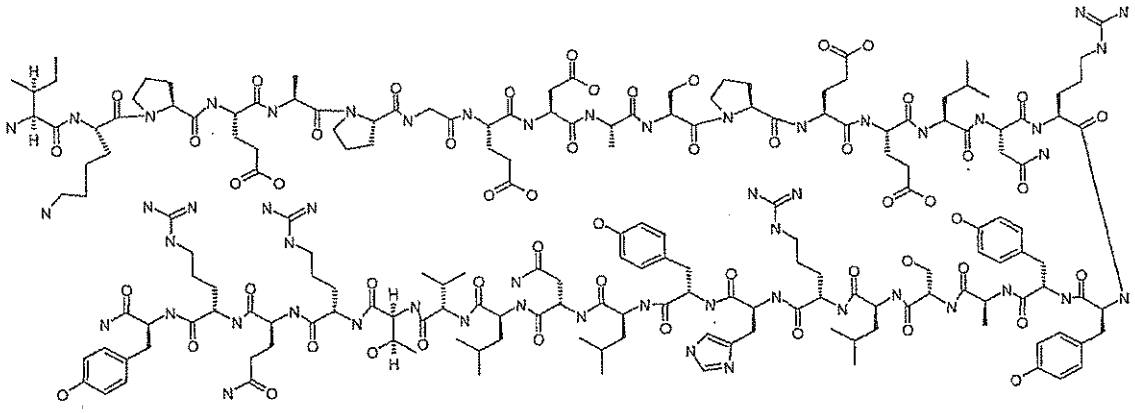


The above peptide was synthesized using Fmoc chemistry on an Applied Biosystem 433A synthesizer. The synthesizer was programmed for double coupling using the modules described in Example 2. The synthesis was carried out on a 0.25 mmol scale using the Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1. At the end of the synthesis, the resin was transferred to a reaction vessel on a shaker for cleavage. The peptide was cleaved from the resin using 13.5 mL 97% TFA/ 3%H2O and 1.5mL triisopropylsilane for 180 minutes at room temperature. The deprotection solution was added to 100 mL cold ET_2O, and washed with 1 mL TFA and 30 mL cold ET_2O to precipitate the peptide. The peptide was centrifuged 2x50 mL polypropylene tubes. The precipitates from the individual tubes were combined in a single tube and washed 3 times with cold ET_2O and dried in a desiccator under house vacuum.

The crude material was purified by preparative HPLC on a Pursuit C18-Column (250x50mm, 10µm particle size) and eluted with a linear gradient of 2-70%B (buffer A: 0.1%TFA/H2O; buffer B: 0.1% TFA/CH3CN) in 90 min., flow rate 60mL/min,and detection 220/280 nm. The fractions were collected and were checked by analytical HPLC. Fractions containing pure product were combined and lyophilized to yield 65 mg (6%) of a white amorphous powder. (ES)+-LCMS m/e calculated ("calcd") for $C_{194}H_{295}N_{55}O_{57}$ 4309.85, found 4309.15.

EXAMPLE 4

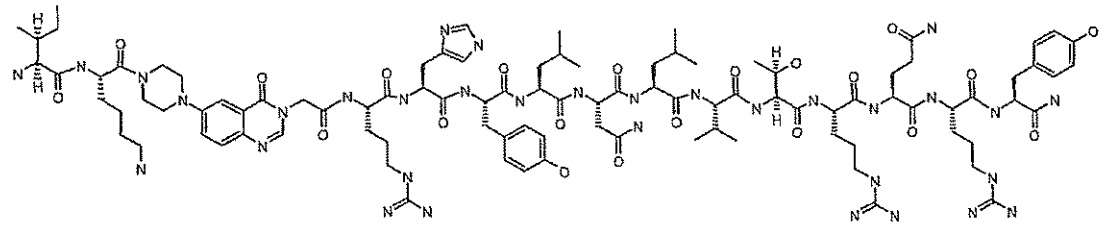
Preparation of H-Ile-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Asn-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (PYY_{3-36}) (SEQ ID NO: 4)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis and purification by following the procedure in Example 3 to yield 151 mg (15%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{180}H_{279}N_{53}O_{54}$ 4049.55 found 4050.40.

EXAMPLE 5

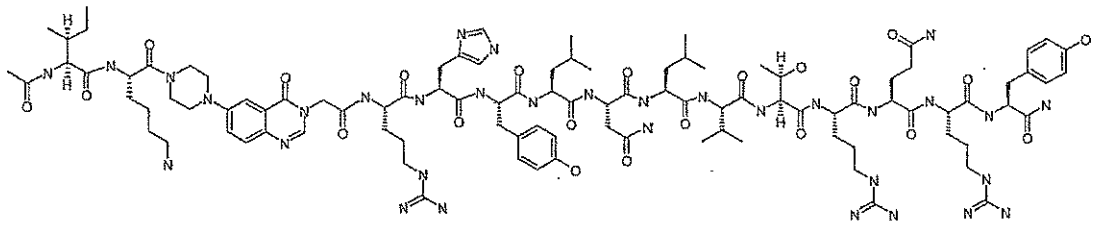
Preparation of H-Ile-Lys-*Pqa*-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis and purification by following the procedure in Example 3 to yield 148 mg (9%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{155}N_{33}O_{21}$ 2131.53 found 2130.56.

EXAMPLE 6

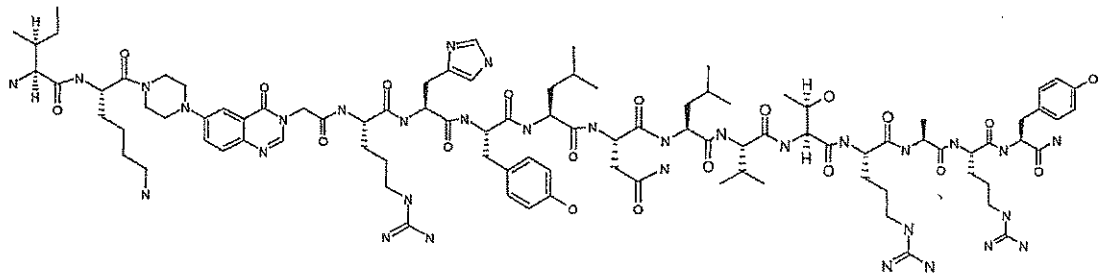
Preparation of *Ac*-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (actylation cycle was added to the ABI- protocol) and the purification by following the procedure in Example 3 to yield 150 mg (27%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{157}N_{33}O_{22}$ 2171.57 found 2171.4.

EXAMPLE 7

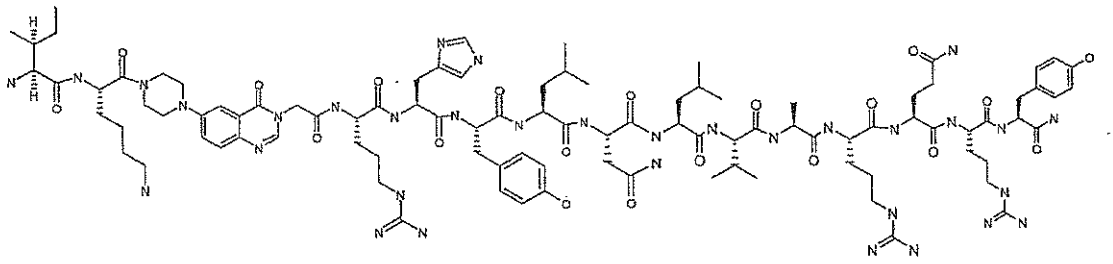
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-*Ala*-Arg-Tyr-NH_2 (SEQ ID NO: 6)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 34 of the sequence) and purification by following the procedure in Example 3 to yield 142 mg (27%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{96}H_{150}N_{32}O_{20}$ 2072.47 found 2072.4

EXAMPLE 8

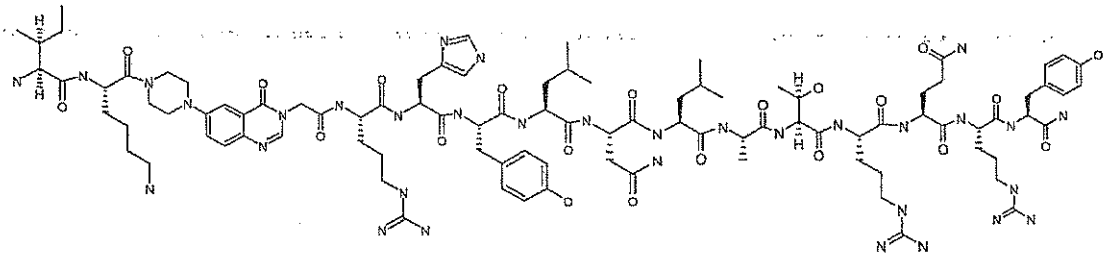
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-*Ala*-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 7)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 32 of the sequence) and purification by following the procedure in Example 3 to yield 167 mg (32%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{97}H_{151}N_{33}O_{20}$ 2099.49 found 2100.3

EXAMPLE 9

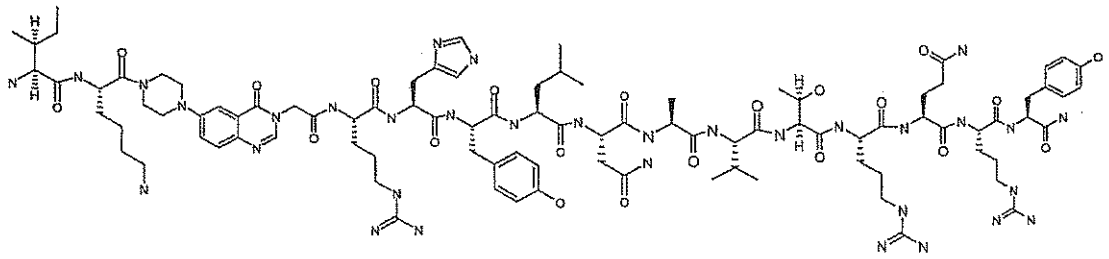
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-*Ala*-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 8)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 31 of the sequence) and purification by following the procedure in Example 3 to yield 105mg (20 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{96}H_{149}N_{33}O_{21}$ 2101.47 found 2102.1

EXAMPLE 10

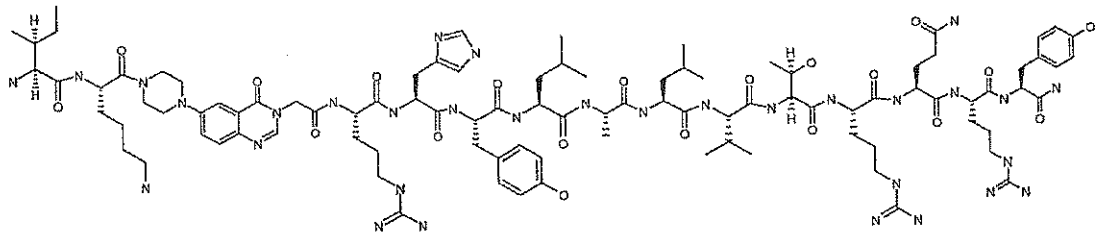
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-*Ala*-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 9)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 30 of the sequence) and purification by following the procedure in Example 3 to yield 167mg (27 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{147}N_{33}O_{21}$ 2087.44 found 2087.7

EXAMPLE 11

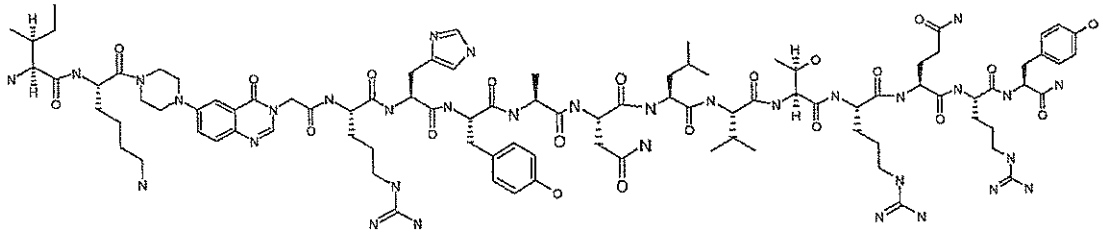
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-*Ala*-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 10)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 29 of the sequence) and purification by following the procedure in Example 3 to yield 142mg (27 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{97}H_{152}N_{32}O_{20}$ 2086.50 found 2086.50.

EXAMPLE 12

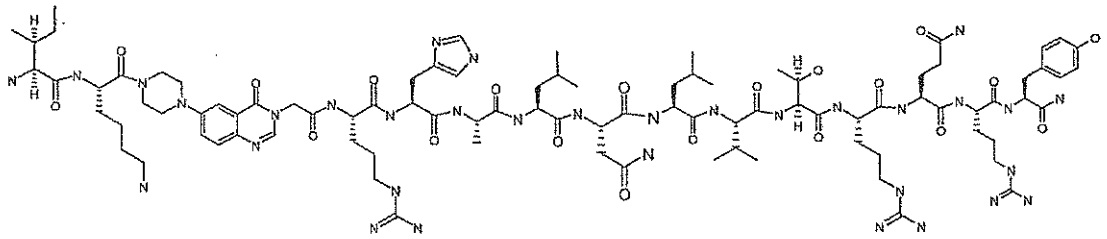
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-*Ala*-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 11)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 28 of the sequence) and purification by following the procedure in Example 3 to yield 164 mg (31%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{147}N_{33}O_{21}$ 2087.44 found 2087.40.

EXAMPLE 13

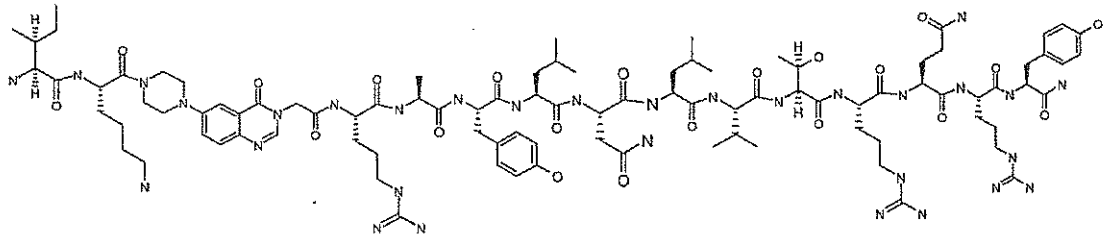
Preparation of H-Ile-Lys-Pqa-Arg-His-*Ala*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 12)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 132mg (26 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{92}H_{149}N_{33}O_{20}$ 2037.42 found 2037.60.

EXAMPLE 14

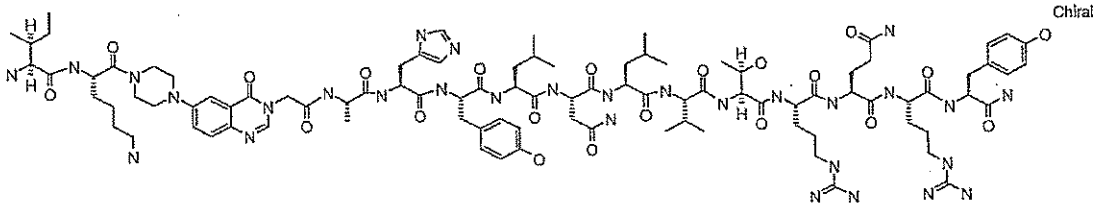
Preparation of H-Ile-Lys-Pqa-Arg-*Ala*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 13)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 76mg (15 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{151}N_{31}O_{21}$ 2063.46 found 2064.0

EXAMPLE 15

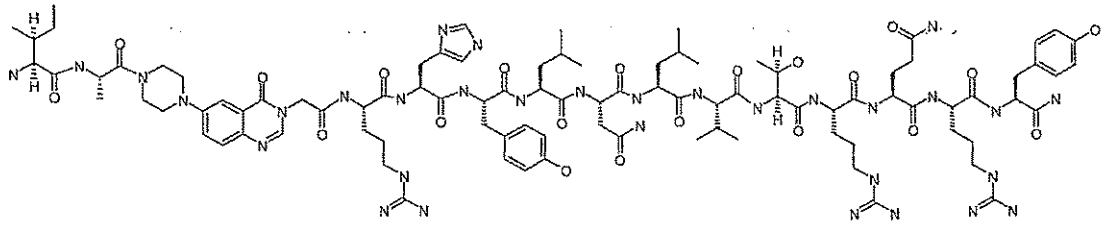
Preparation of H-Ile-Lys-Pqa-*Ala*-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 14)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 152mg (30 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{146}N_{30}O_{21}$ 2043.13 found 2043.4.

EXAMPLE 16

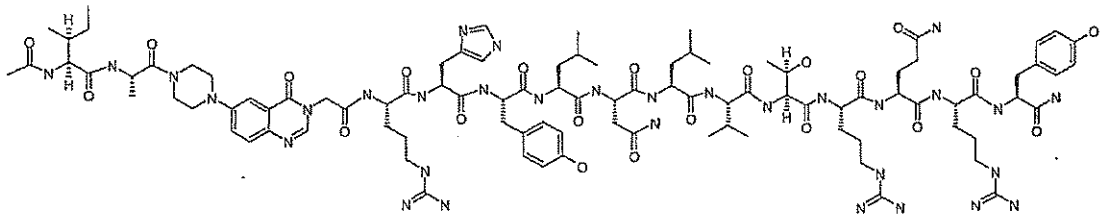
Preparation of H-Ile-*Ala*-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 4 of the sequence) and purification by following the procedure in Example 3 to yield 72mg (14%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{146}N_{32}O_{21}$ 2072.44 found 2071.2

EXAMPLE 17

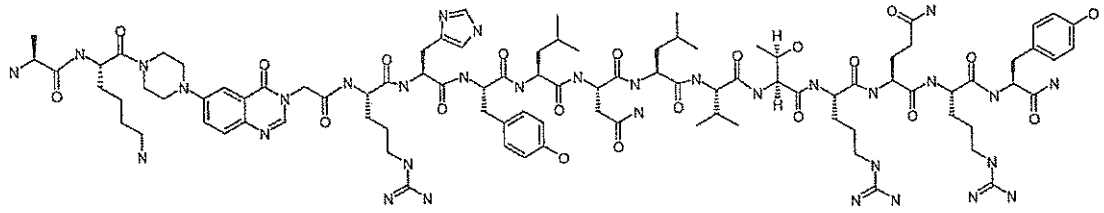
Preparation of *Ac*-Ile-Ala-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 16 was subjected to solid phase synthesis, wherein an acylation cycle was added to the protocol, and purification by following the procedure in Example 3 to yield 234mg (44%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{97}H_{148}N_{32}O_{22}$ 2114.46 found 2114.7.

EXAMPLE 18

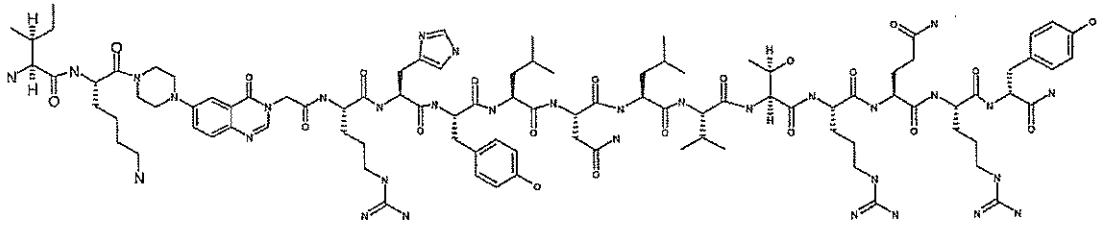
Preparation of H-*Ala*-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 196 mg (38%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{95}H_{147}N_{33}O_{21}$ 2087.45 found 2086.5.

EXAMPLE 19

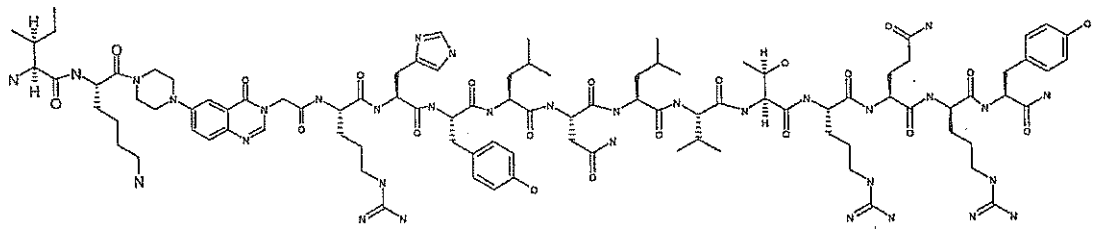
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-*(D)Tyr*-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Tyr was inserted in position 36 of the sequence) and purification by following the procedure in Example 3 to yield 114mg (21%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.52 found 2129.10.

EXAMPLE 20

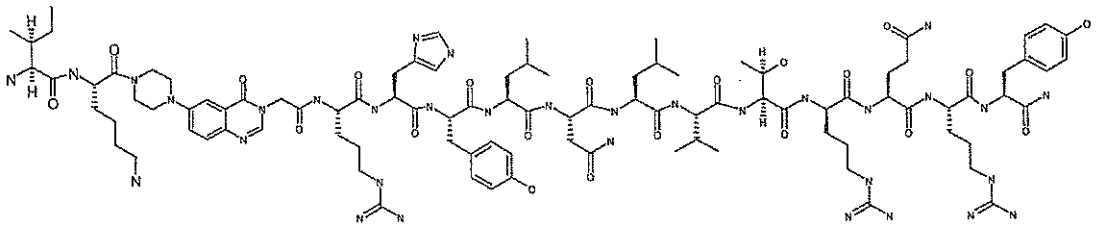
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-*(D)Arg*-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Arg was inserted in position 35 of the sequence) and purification by following the procedure in Example 3 to yield 221mg (42%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.52 found 2129.10.

EXAMPLE 21

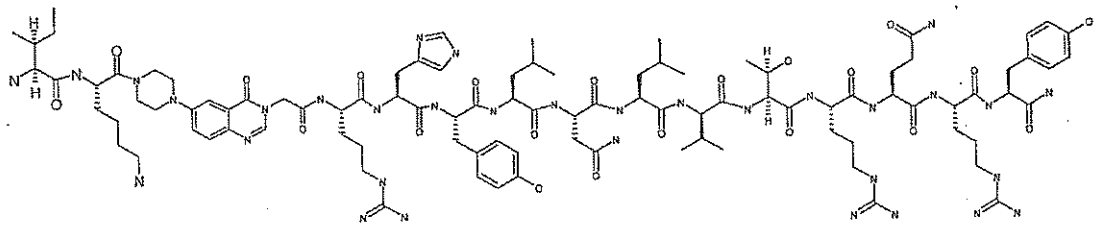
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-*(D)Arg*-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Arg was inserted in position 33) of the sequence and purification by following the procedure in Example 3 to yield 174 mg (32%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.52 found 2128.4.

EXAMPLE 22

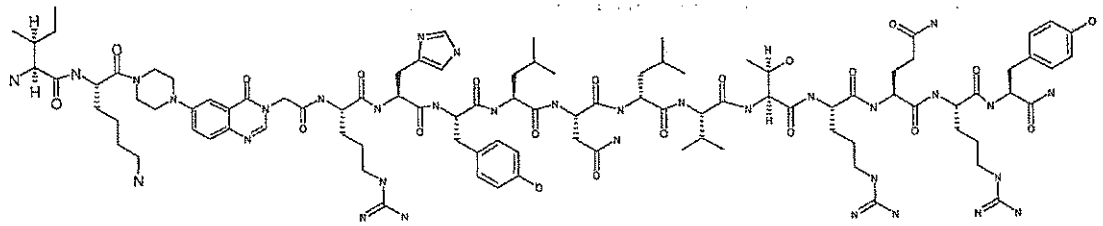
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-*(D)Val*-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Val was inserted in position 31 of the sequence) and purification by following the procedure in Example 3 to yield 67 mg (12%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.52 found 2129.10.

EXAMPLE 23

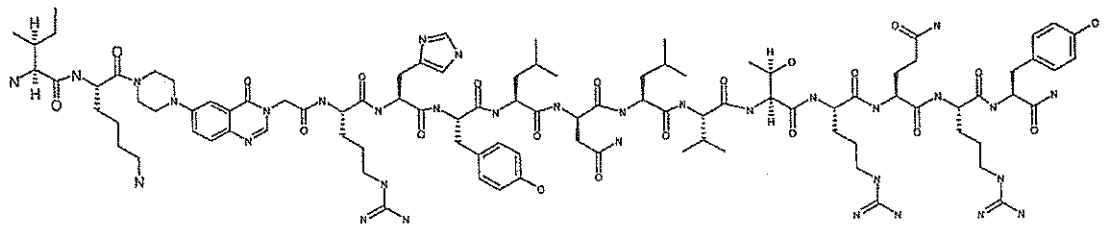
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-*(D)Leu*-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Leu was inserted in position 30 of the sequence) and purification by following the procedure in Example 3 to yield 190mg (36 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.52 found 2129.10.

EXAMPLE 24

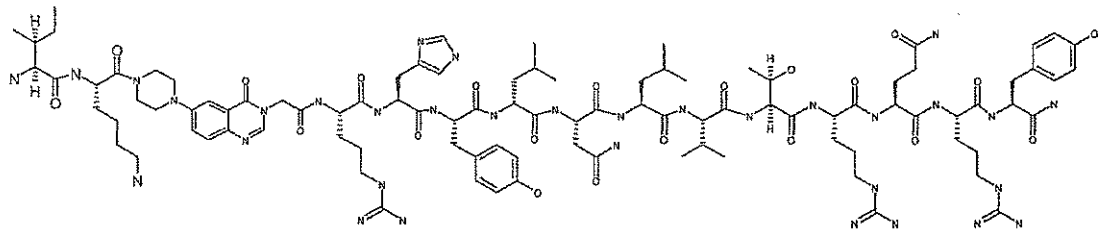
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-*(D)Asn*-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Asn was inserted in position 29 of the sequence) and purification by following the procedure in Example 3 to yield 50 mg (10%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2128.80.

EXAMPLE 25

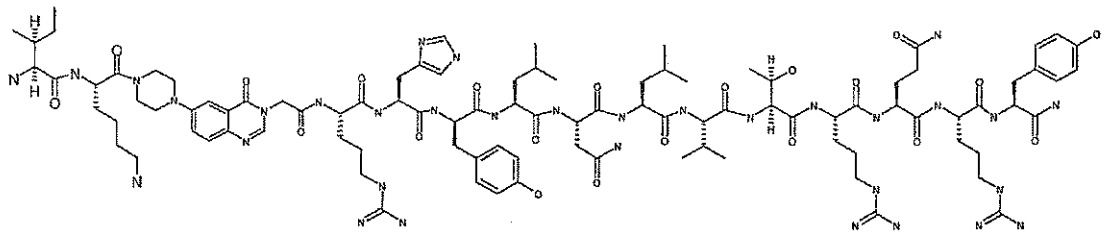
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-*(D)Leu*-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Leu was inserted in position 28 of the sequence) and purification by following the procedure in Example 3 to yield 188 mg (35%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2129.40.

EXAMPLE 26

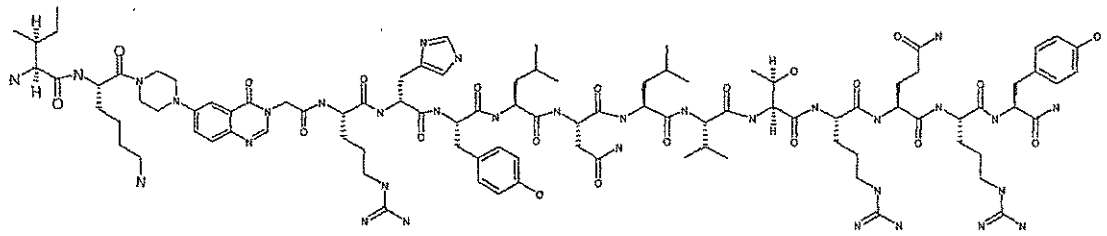
Preparation of H-Ile-Lys-Pqa-Arg-His-*(D)Tyr*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Tyr was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 119 mg (22%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2129.70.

EXAMPLE 27

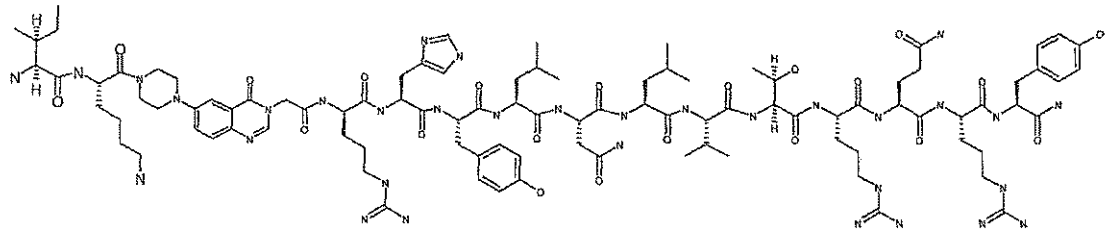
Preparation of H-Ile-Lys-Pqa-Arg-*(D)His*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) His was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 84 mg (16%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2128.80.

EXAMPLE 28

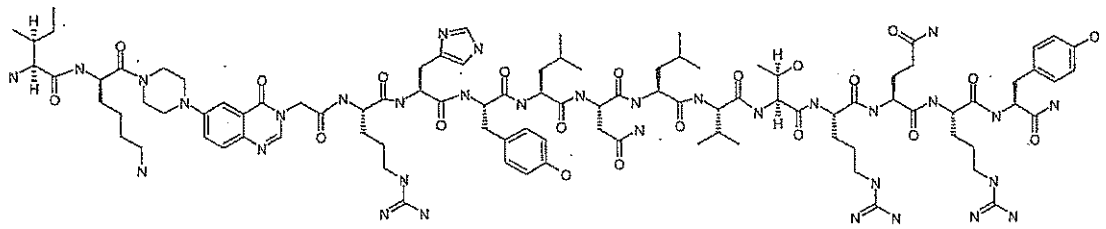
Preparation of H-Ile-Lys-Pqa-*(D)Arg*-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Arg was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 85 mg (16%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2128.80.

EXAMPLE 29

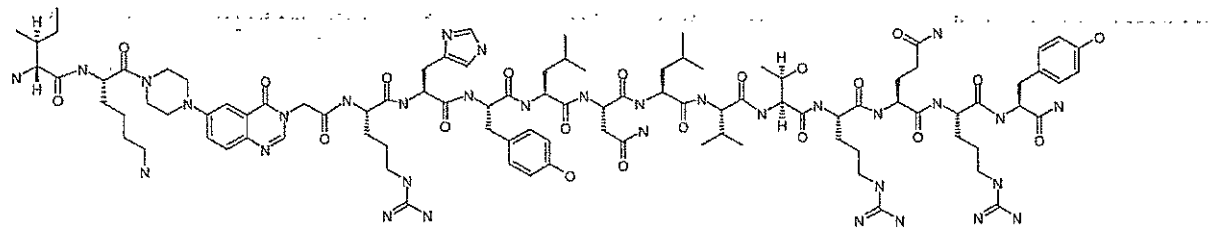
Preparation of H-Ile-*(D)Lys*-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Lys was inserted in position 4 of the sequence) and purification by following the procedure in Example 3 to yield 165 mg (31%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2129.10.

EXAMPLE 30

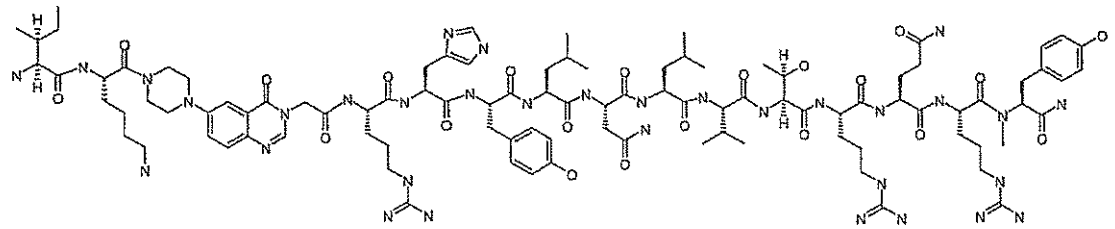
Preparation of H-*(D) Ile*-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D) Ile was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 84 mg (8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{33}O_{21}$ 2129.53 found 2129.40.

EXAMPLE 31

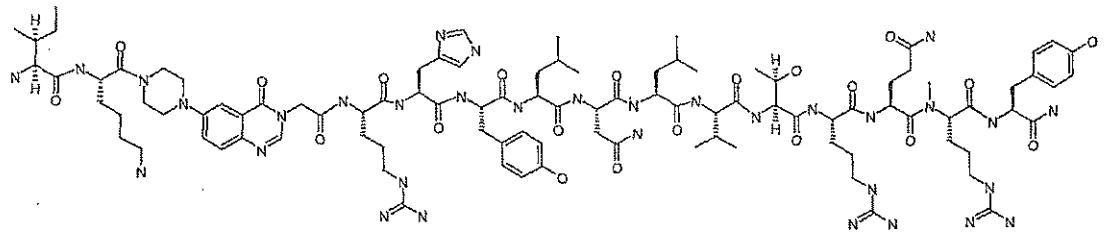
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-*(NMe)Tyr*-NH_2 (SEQ ID NO: 15)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Tyr was inserted in position 36 of the sequence) and purification by following the procedure in Example 3 to yield 90 mg (17%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{157}N_{33}O_{21}$ 2143.56 found 2143.50.

EXAMPLE 32

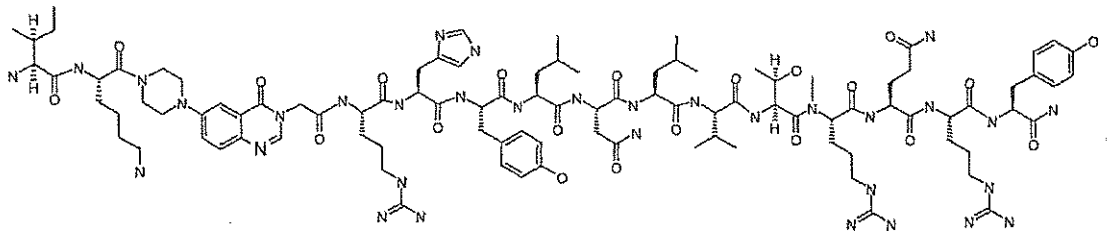
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-*(NMe)Arg*-Tyr-NH_2 (SEQ ID NO: 16)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Arg was inserted in position 35 of the sequence) and purification by following the procedure in Example 3 to yield 32 mg (6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.50.

EXAMPLE 33

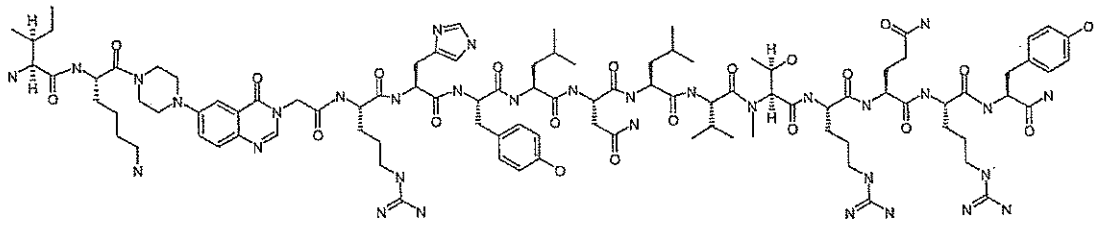
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-*(NMe)Arg*-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 17)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Arg was inserted in position 33 of the sequence) and purification by following the procedure in Example 3 to yield 40 mg (7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.20.

EXAMPLE 34

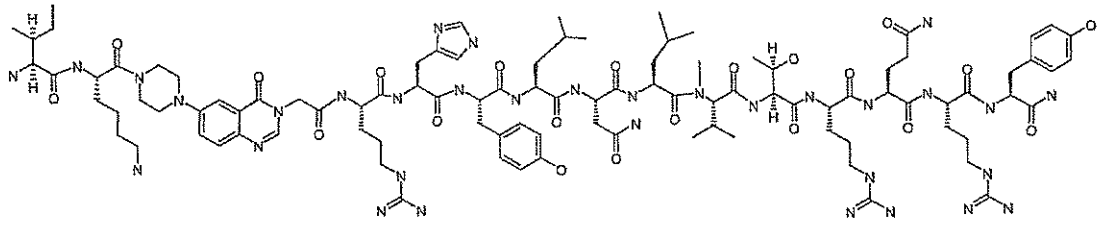
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-*(NMe)Thr*-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 18)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Thr was inserted in position 32 of the sequence) and purification by following the procedure in Example 3 to yield 115 mg (21%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.20.

EXAMPLE 35

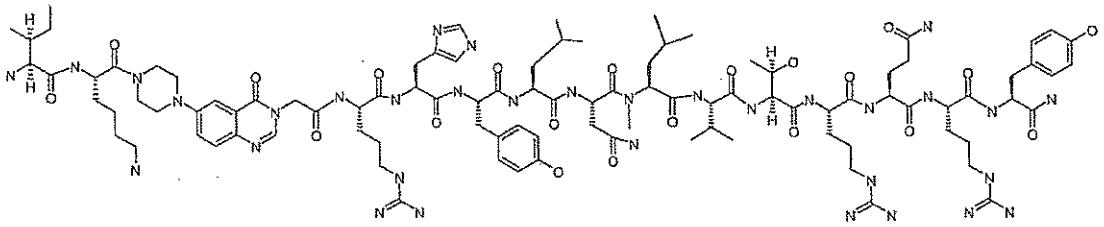
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-*(NMe)Val*-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 19)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Val was inserted in position 31 of the sequence) and purification by following the procedure in Example 3 to yield 60 mg (11%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.20.

EXAMPLE 36

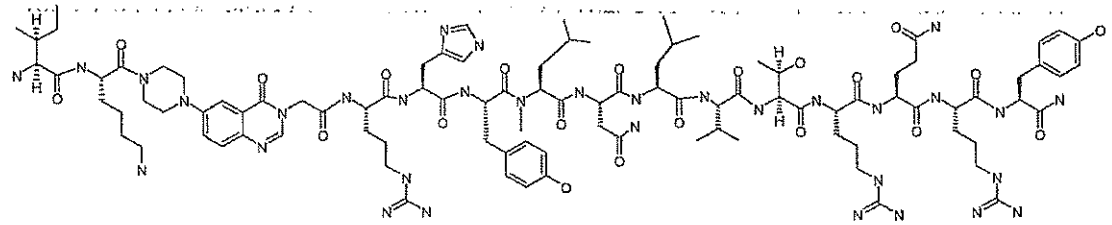
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-Leu-Asn-*(NMe)Leu*-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 20)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl leu was inserted in position 30 of the sequence) and purification by following the procedure in Example 3 to yield 91 mg (17%) of white amorphous powder. (ES)+-LCMS m/e calcd for C99H155N33O21 2143.56 found 2142.90.

EXAMPLE 37

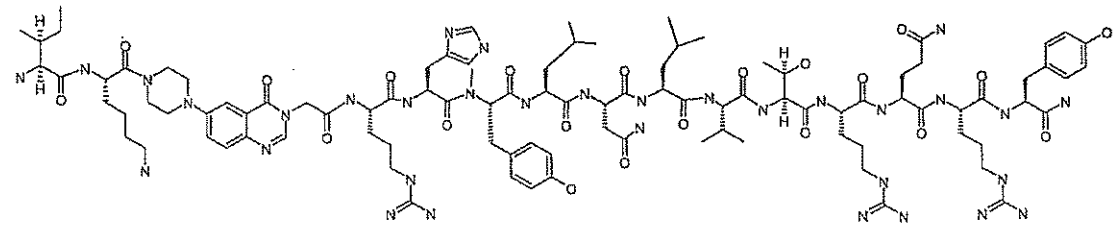
Preparation of H-Ile-Lys-Pqa-Arg-His-Tyr-*(NMe)Leu*-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 21)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl leu was inserted into position 28 of the sequence) and purification by following the procedure in Example 3 to yield 153 mg (28%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2142.90.

EXAMPLE 38

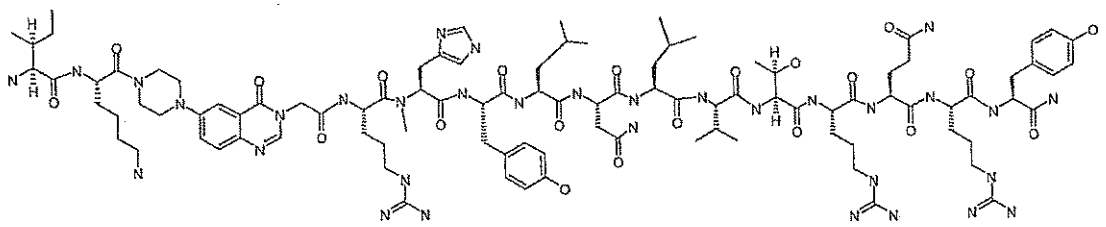
Preparation of H-Ile-Lys-Pqa-Arg-His-*(NMe)Tyr*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 22)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Tyr was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 76 mg (14%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2142.90.

EXAMPLE 39

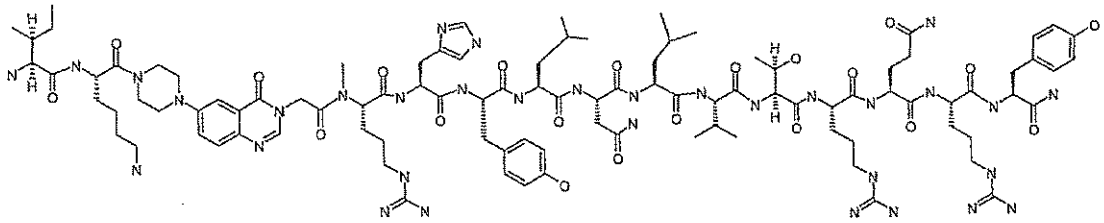
Preparation of H-Ile-Lys-Pqa-Arg-*(NMe)His*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 23)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl His was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 93 mg (7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.53 found 2143.50.

EXAMPLE 40

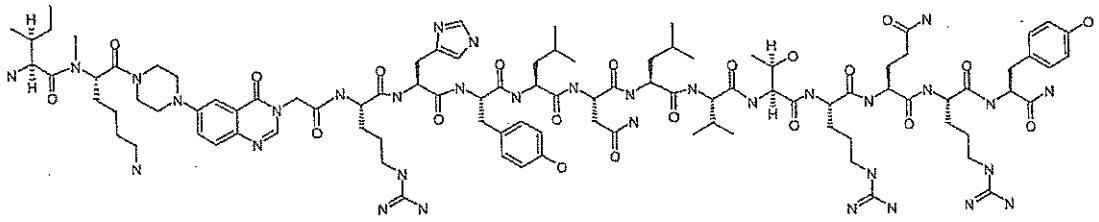
Preparation of H-Ile-Lys-Pqa-*(NMe)Arg*-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 24)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Arg was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 33 mg (6 %) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.50.

EXAMPLE 41

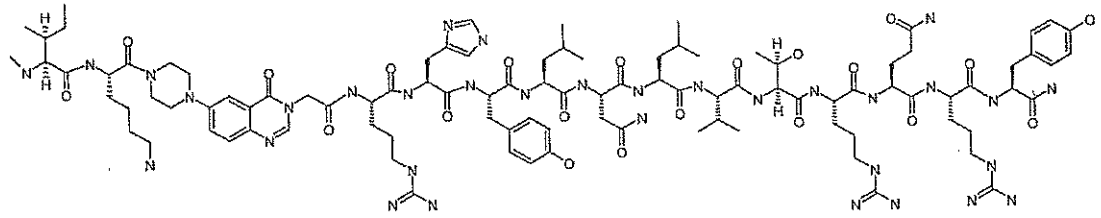
Preparation of H-Ile-*NMeLys*-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Lys was inserted in position 4 of the sequence) and purification by following the procedure in Example 3 to yield 156 mg (29%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.20.

EXAMPLE 42

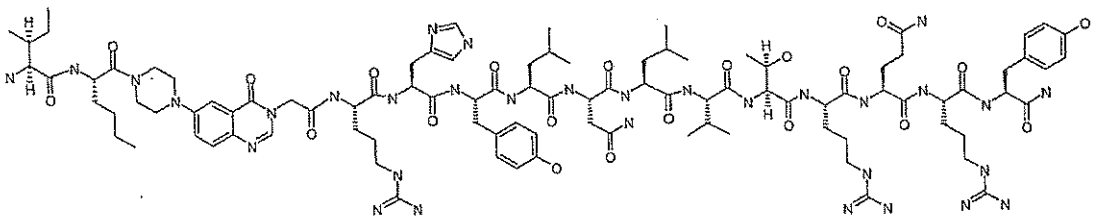
Preparation of *H-NMeIle*-Lys-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Ile was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 203 mg (38%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{99}H_{155}N_{33}O_{21}$ 2143.56 found 2143.20.

EXAMPLE 43

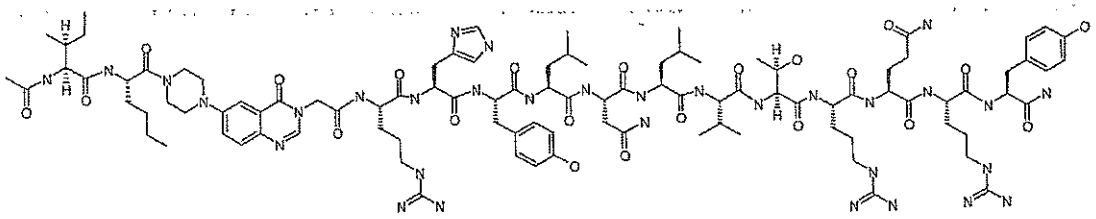
Preparation of H-Ile-*Nle*-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Nle was inserted in position 4 of the sequence) and purification by following the procedure in Example 3 to yield 60 mg (11%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{153}N_{32}O_{21}$ 2114.52 found 2113.80.

EXAMPLE 44

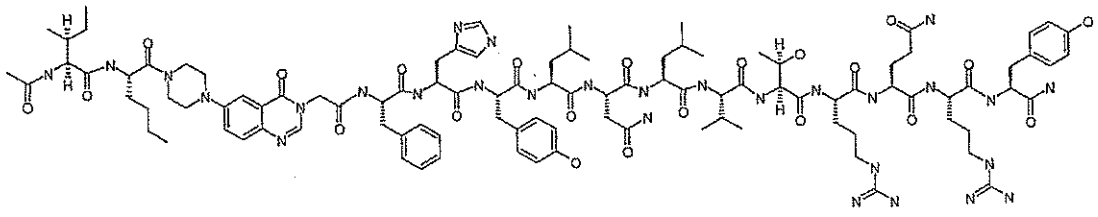
Preparation of *Ac*-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (actylation cycle was added to the protocol) and purification by following the procedure in Example 3 to yield 41 mg (8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{154}N_{32}O_{22}$ 2156.56 found 2156.10.

EXAMPLE 45

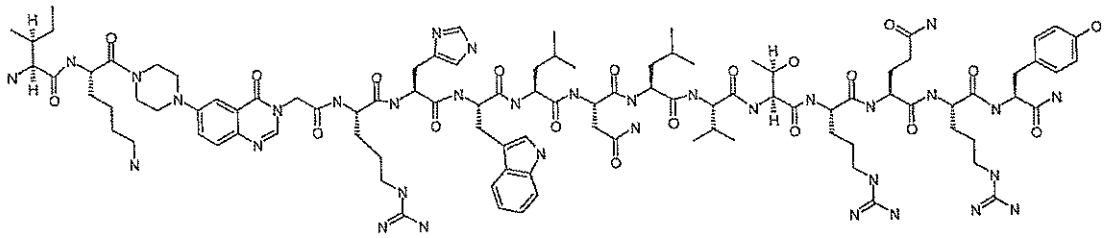
Preparation of Ac-Ile-Nle-Pqa-*Phe*-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 25)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Phe was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 92 mg (7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{103}H_{151}N_{29}O_{22}$ 2147.53 found 2148.00.

EXAMPLE 46

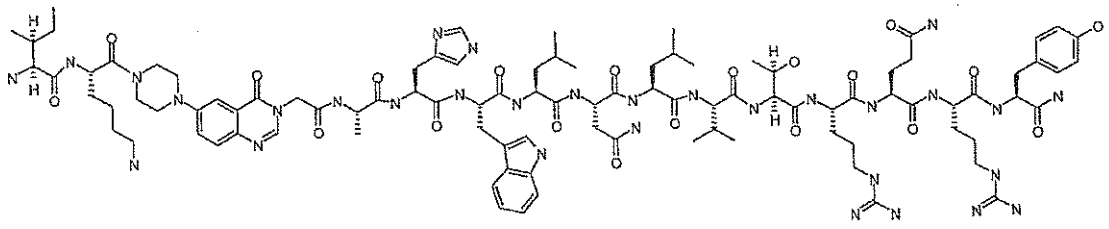
Preparation of H-Ile-Lys-Pqa-Arg-His-*Trp*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 26)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Trp was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 30 mg (6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{154}N_{34}O_{20}$ 2153.56 found 2152.20

EXAMPLE 47

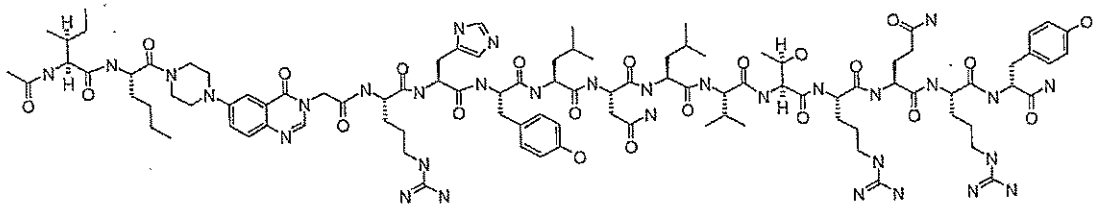
Preparation of H-Ile-Lys-Pqa-*Ala*-His-*Trp*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 27)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ala 25 and Trp 27 were inserted in the sequence) and purification by following the procedure in Example 3 to yield 50 mg (9%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{97}H_{147}N_{31}O_{20}$ 2067.46 found 2067.30.

EXAMPLE 48

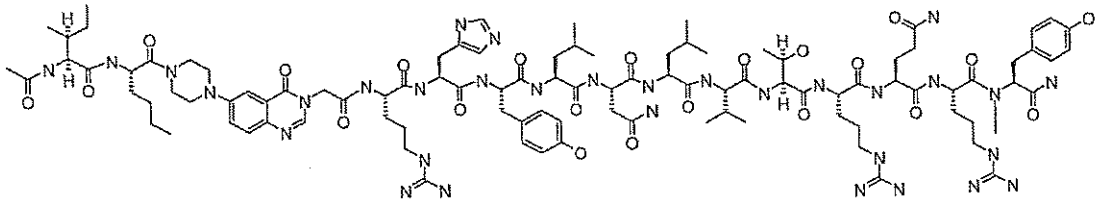
Preparation of Ac-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-*(D)Tyr*-NH_2



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((D)Tyr was inserted in position 36 of the sequence) and purification by following the procedure in Example 3 to yield 104 mg (19%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{154}N_{32}O_{22}$ 2156.54 found 2157.00.

EXAMPLE 49

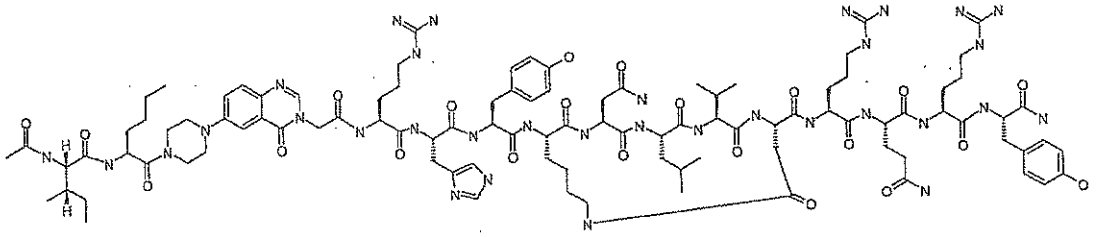
Preparation of Ac-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-*N-methylTyr*-NH_2 (SEQ ID NO: 15)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (N-methyl Tyr was inserted in position 36 of the sequence) and purification by following the procedure in Example 3 to yield 28 mg (5%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{101}H_{156}N_{32}O_{22}$ 2170.57 found 2170.50.

EXAMPLE 50

Preparation of *Cyclo Lys28-Asp32* Ac-Ile-Nle-Pqa-Arg-His-Tyr-*Lys*-Asn-Leu-Val-*Asp*-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 28)



The peptide was prepared through a combination of automated synthesis on an ABI 433 synthesizer using a standard protocol and manual synthesis on a 0.25 mM scale. Fragment Fmoc-Asn(Trt)-Leu-Val-Asp(2Pip)-Arg(Pmc)-Gln(Trt)-Arg(Pmc)-Tyr(OBut)- BHA Resin (SEQ ID NO: 29) was synthesized using the ABI protocol and then extended manually to obtain Fmoc-Ile-Nle-Pqa-Arg(Pmc)-His(Trt)- Tyr(OBut)-Lys-Asn(Trt)-Leu-Val-Asp-Arg(Pmc)-Gln(Trt)-rg(Pmc)-Tyr(OBut)- BHA Resin (SEQ ID NO: 30). After completion of the automated synthesis the peptide resin was transferred to a manual solid phase vessel and washed with methylene chloride and DMF multiple times. Fmoc-Lys(Mtt) 1.2 g (2.0 mM 8 eqv) was added as a solid and 15 mL DMF was added followed by 1.2 mL Dic (7 mM 28 eqv.). The coupling was allowed to proceed until a negative or near negative ninhydrin was obtained. If a negative ninhydrin was not obtained the resin was Acetylated with Ac_2O 6 mL, DIEA 1 mL and DMF 18 mL for 30 min and completion checked with ninhydrin. The resin was then washed 3 X DMF and 4 X CH_2Cl_2. The Lys(Mtt) and Asp(2Pip) were deprotected 10 times using 2% TFA in CH_2Cl_2. After deprotection, the peptide resin was washed 2 X CH_2Cl_2; 2 X 6% TFA/ CH_2Cl_2 ; 2 X 6% DIEA/DMF and 2 X DMF. The side chain was cyclized with HATU (240 mg 0.625 mM (2.5 eqv.) and DIEA (175µL 1.0 mM (4 eqv)) and monitored with ninhydrin until negative

After completion of the cyclization, the peptide resin was transferred to an ABI vessel for extension to Fmoc-Arg(Pmc)-His(Trt)- Tyr(OBut)-Lys-Asn(Trt)-Leu-Val-Asp-Arg(Pmc)-Gln(Trt)-Arg(Pmc)-Tyr(OBut)- BHA Resin (SEQ ID NO: 30). Fmoc-Pga-OH (160 mg, 313 mM (1.25 eqv)) and HOBt (5 mg, 313 mM (1.25 eqv)) were added as solids and 15 mL DMF was added followed by 1.2 mL Dic (7 mM 28 eqv.). The coupling was allowed to run over night (generally 18 hr.) at room temperature. After washing 4 times with DMF the coupling was monitored with ninhydrin and was generally negative. After standard deprotection and washes, Fmon-Nle-OH (1.2g, 3.0 mM (12 eqv)) was preactivated with 6.6 mL of 0.45 M HBTT/HOBt in DMF for 3 min and added to the peptide resin. Coupling

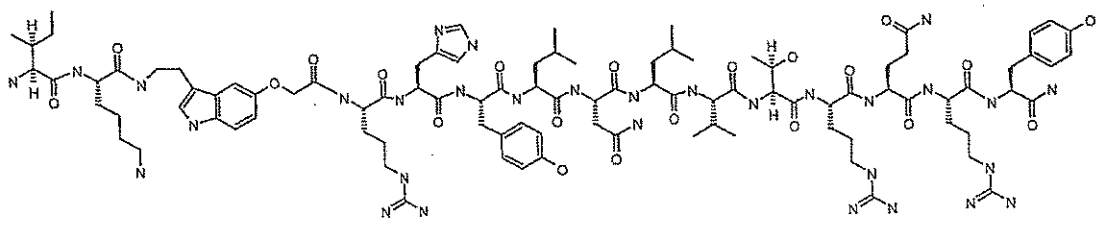
was allowed to proceed for 3 to 4 hrs, the reaction vessel drained and washed 4 times with DMF. This coupling was monitored with chlorinal (2% Acetaldehyde in DMAc and 2% tetrachloro-1,4-benzoquinone). If the coupling was judged to be incomplete, the resin was recoupled overnight. When the coupling was complete, the resin was deprotected and washed and Fmoc-Ile (1.2g, 3.0 mM (12 eqv)) was preactivated with 6.6 mL of 0.45 M HBTT/HOBt in DMF for 3 min and added to the peptide resin. Coupling was allowed to proceed for 3 to 4 hrs, the reaction vessel drained and washed 4 times with DMF. This coupling was monitored with ninhydrin and was generally negative. The resin was deprotected and washed and Acetylated with Ac_2O 6 mL, DIEA 1 mL and DMF 18 mL for 30 min and completion checked with until a negative or near negative ninhydrin was obtained. If a negative ninhydrin was not obtained the resin was acetylated with Ac_2O 6 mL, DIEA 1 mL and DMF 18 mL for 30 min and completion checked with ninhydrin.

After completion of the synthesis, the resin was washed 4 times with CH_2Cl_2 and dried under a stream of N_2. The peptide was deprotected and cleaved from the resin with 1.5 mL triisopropylsilane and 13.5 mL 97% TFA/3% H_2O for 180 minutes. The deprotection solution was added to 100 mL cold ET_2O, and washed with 1 mL TFA and 30 mL cold ET_2O to precipitate the peptide then centrifuged and dried in a desiccator under house vacuum.

The crude peptide was purified and lyophilized by following the procedure described in Example 3 to yield 49 mg (9%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{151}N_{33}O_{22}$ 2167.53 found 2167.80.

EXAMPLE 51

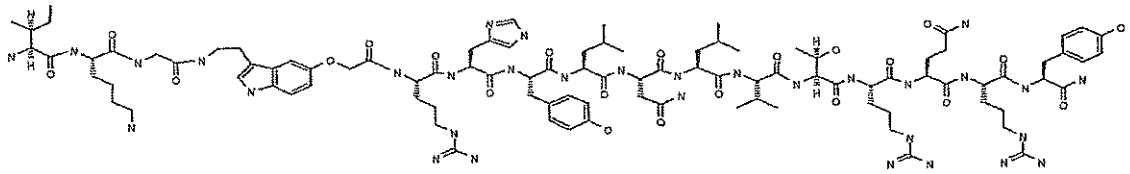
Preparation of H-Ile-Lys-*Cms*-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis and purification by following the procedure in Example 3 to yield 44 mg (8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{96}H_{151}N_{31}O_{21}$ 2075.47 found 2074.80.

EXAMPLE 52

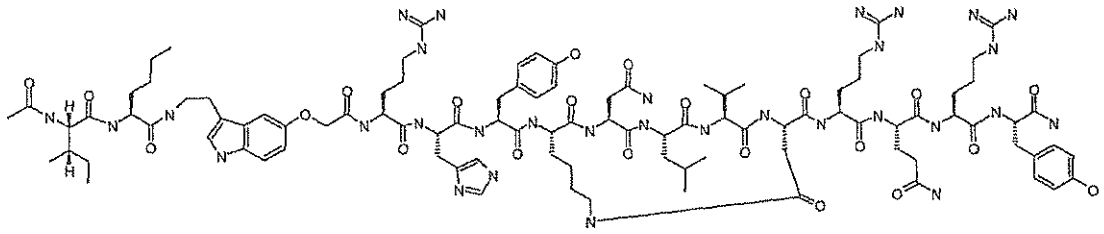
Preparation of H-Ile-Lys- ***Gly*****-Cms-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2** (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis and purification by following the procedure in Example 3 to yield 129 mg (24%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{154}N_{32}O_{22}$ 2132.52 found 2133.00.

EXAMPLE 53

Preparation of *Cyclo Lys28-Aspu32* Ac-Ile-Nle-Cms-Arg-His-Tyr-*Lys*-Asn-Leu-Val-*Asp*-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 28)

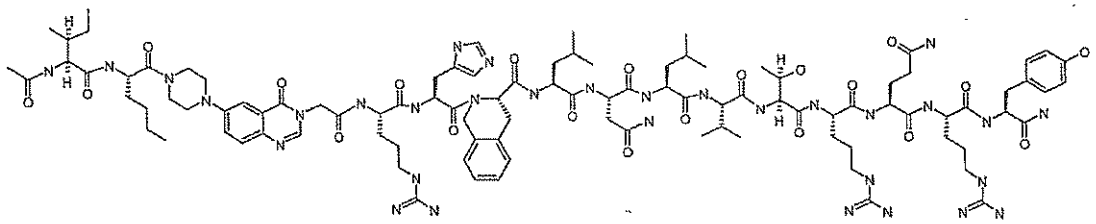


This peptide was prepared through a combination of automated synthesis on an ABI 433 and manual synthesis on a 0.25 mM scale according to the procedure disclosed in Example 50. Fmoc-Cms-OH (151 mg, 313 mM (1.25 eqv)) and HOBt (45 mg, 313 mM (1.25 eqv)) were added as solids and 15 mL DMF was added followed by 1.2 mL Dic (7 mM 28 eqv.). The coupling was allowed to run over night (generally 18 hr.) at room temperature.

The crude peptide was purified and lyophilized to yield 19 mg (9%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{149}N_{31}O_{22}$ 2113.47 found 2113.80.

EXAMPLE 54

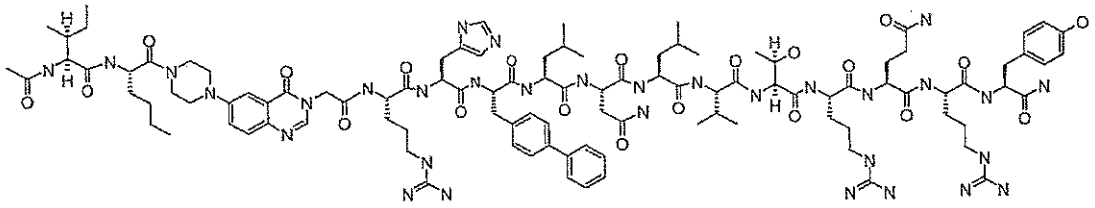
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*Tic*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 31)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Tic was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 87 mg (16%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{101}H_{154}N_{32}O_{21}$ 2152.56 found 2152.50.

EXAMPLE 55

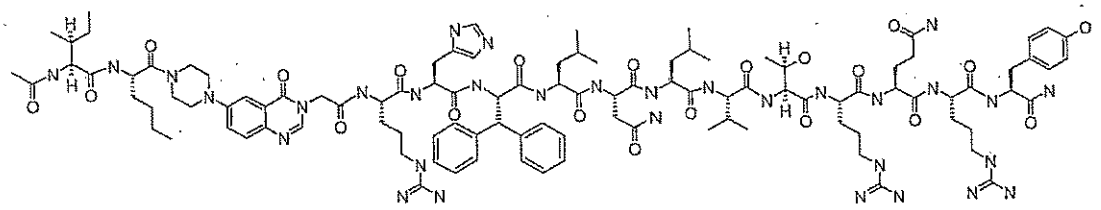
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*Bip*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 32)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Bip was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 90 mg (16%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{106}H_{158}N_{32}O_{21}$ 2216.64 found 2217.00.

EXAMPLE 56

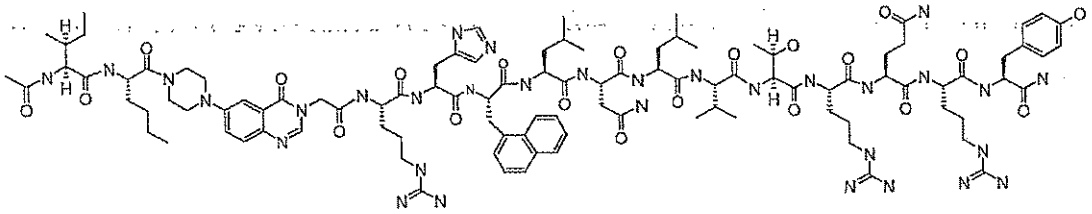
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*Dip*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 33)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Dip was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 84 mg (15%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{106}H_{158}N_{32}O_{21}$ 2216.64 found 2217.30.

EXAMPLE 57

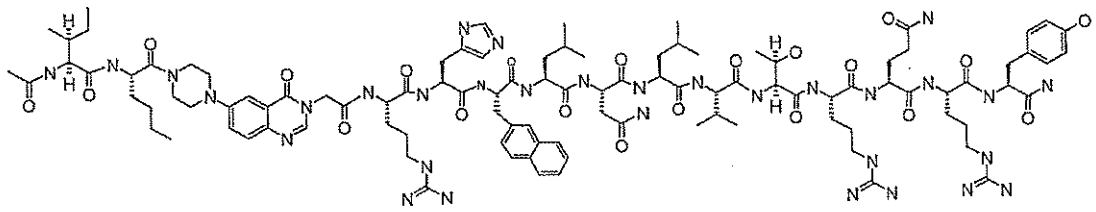
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*(1)Nal*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr- NH_2 (SEQ ID NO: 34)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((1) Nal was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 88 mg (16%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{104}H_{156}N_{32}O_{21}$ 2190.60 found 2190.61.

EXAMPLE 58

Preparation of Ac-Ile-Nle-Pqa-Arg-His-*(2)Nal*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 35)

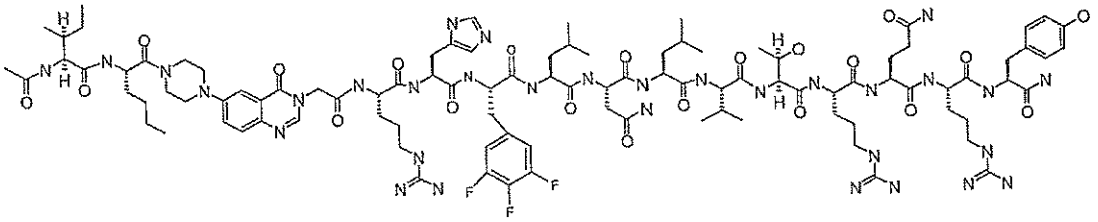


Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((2) Nal was inserted in position 27 of the sequence) and purification by

following the procedure in Example 3 to yield 40 mg (7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{104}H_{156}N_{32}O_{21}$ 2190.60 found 2191.20.

EXAMPLE 59

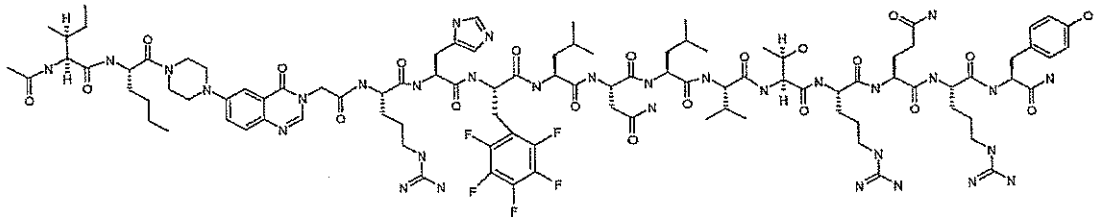
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*(3,4,5trifluorPhe)*-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 36)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (3,4,5, trifluoro Phe was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 101.4mg (18%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{151}F_3N_{32}O_{21}$ 2194.52 found 2194.20.

EXAMPLE 60

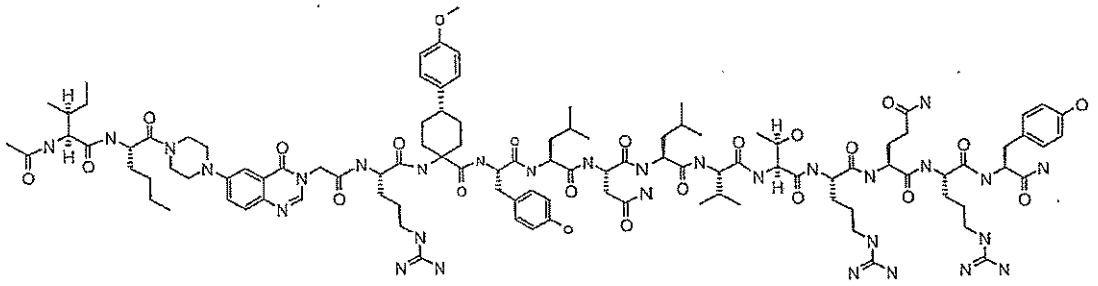
Preparation of Ac-Ile-Nle-Pqa-Arg-His-*(2,3,4,5,6 Penta fluoroPhe*)-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 37)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (2,3,4,5,6, pentafluoro Phe was inserted in position 27 of the sequence) and purification by following the procedure in Example 3 to yield 122.5 mg (22%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{149}F_5N_{32}O_{21}$ 2230.50 found 2230.50.

EXAMPLE 61

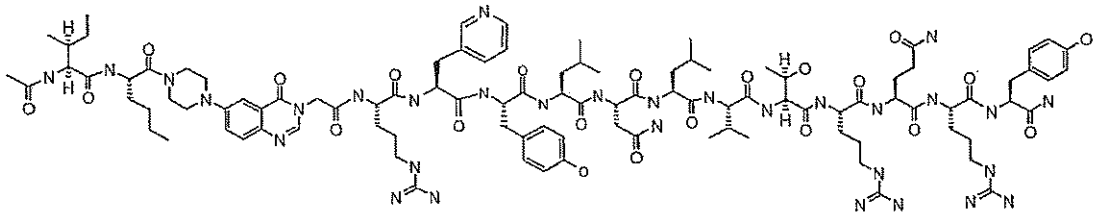
Preparation of Ac-Ile-Nle-Pqa-Arg-*(4-MeOApc)*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 38)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((4-MeO-Apc) was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 43 mg (8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{108}H_{164}N_{30}O_{23}$ 2250.70 found 2250.60.

EXAMPLE 62

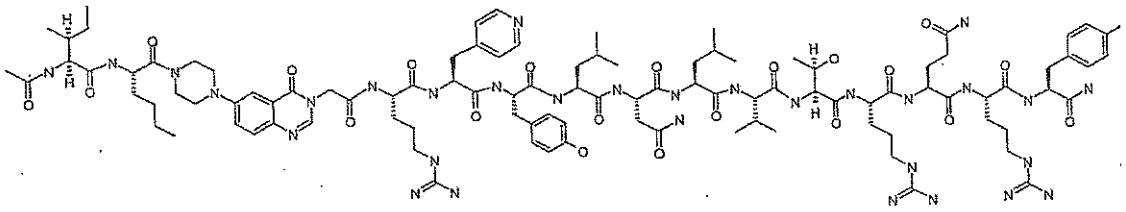
Preparation of Ac-Ile-Nle-Pqa-Arg-*(3-Pal)*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 39)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((3-Pal) was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 112 mg (21%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{102}H_{155}N_{31}O_{22}$ 2167.57 found 2167.20.

EXAMPLE 63

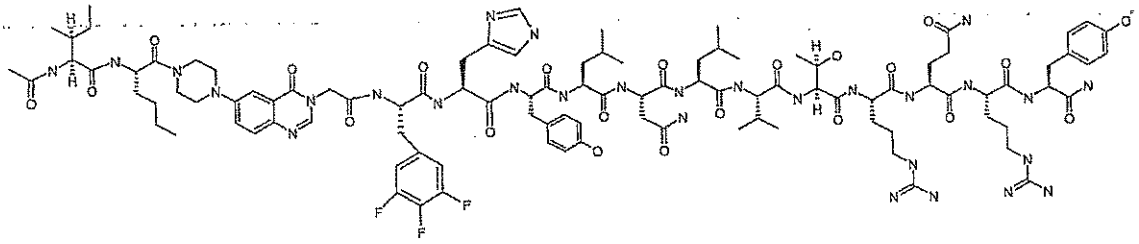
Preparation of Ac-Ile-Nle-Pqa-Arg-*(4-Pal)*-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 40)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((4-Pal) was inserted in position 26 of the sequence) and purification by following the procedure in Example 3 to yield 146 mg (27%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{102}H_{155}N_{31}O_{22}$ 2167.57 found 2167.20.

EXAMPLE 64

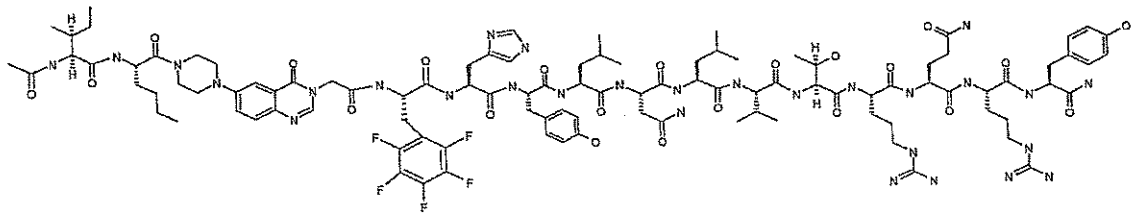
Preparation of Ac-Ile-Nle-Pqa -*(3,4,5 Trifluoro Phe)*-His- Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 41)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((3,4,5 trifluoro Phe) was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 55 mg (10%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{103}H_{148}F_3N_{29}O_{22}$ 2201.50 found 2201.40.

EXAMPLE 65

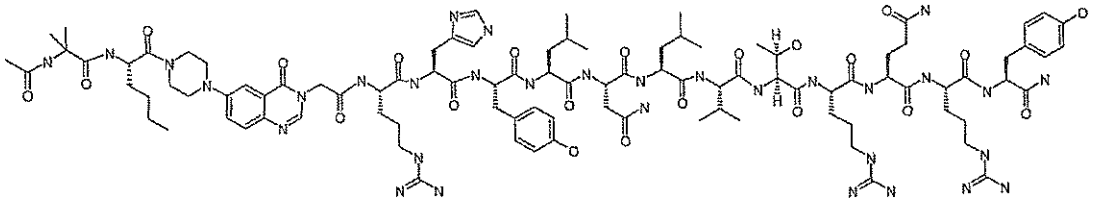
Preparation of Ac-Ile-Nle-Pqa-*(2,3,4,5,6 Penta fluoro Phe)*-His Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 42)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((2,3,4,5,6 Pentafluoro Phe) was inserted in position 25 of the sequence) and purification by following the procedure in Example 3 to yield 65 mg (12%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{103}H_{146}F_5N_{29}O_{22}$ 2237.49 found 2237.70.

EXAMPLE 66

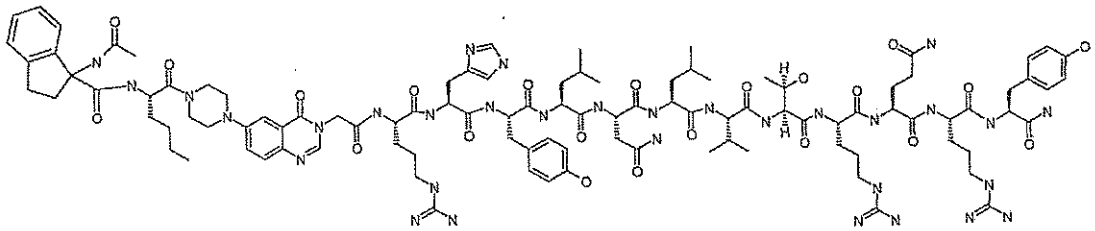
Preparation of Ac-*Aib*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((Aib) was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 32 mg (6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{150}N_{32}O_{22}$ 2128.49 found 2128.00.

EXAMPLE 67

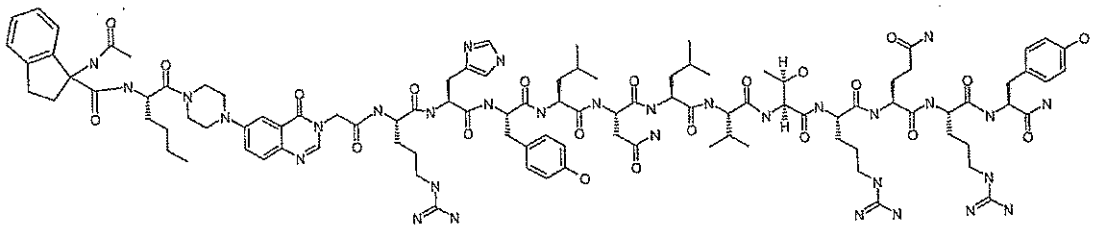
Preparation of Ac-*1,1-Aic*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((1,1 Aic) was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield a racemic mixture separated as Pk A: 31mg (6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{104}H_{152}N_{32}O_{22}$ 2202.57 found 2202.60.

EXAMPLE 68

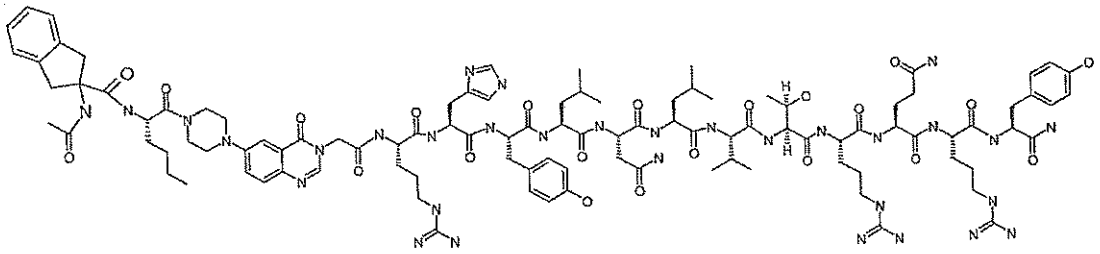
Preparation of Ac-*1,1-Aic*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((1,1 Aic) was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield a racemic mixture separated as **Pk. B**; 46 mg (8%) of white amorphous powder. (ES)+-LCMS m/e calcd for Pk B. $C_{104}H_{152}N_{32}O_{22}$ 2202.57 found 2202.60.

EXAMPLE 69

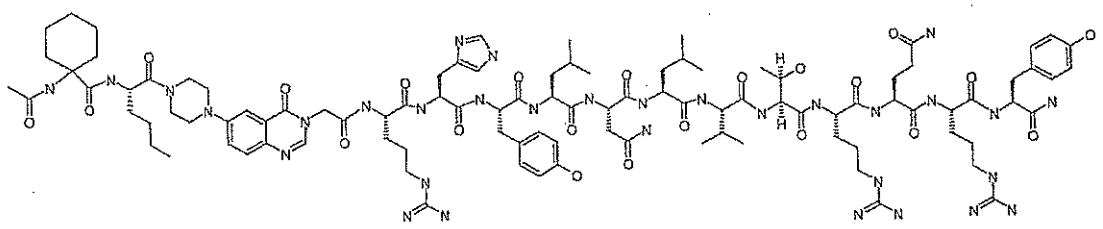
Preparation of Ac-*2,2-Aic*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis ((2,2 Aic) was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 103 mg (19%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{104}H_{152}N_{32}O_{22}$ 2202.57 found 2202.30.

EXAMPLE 70

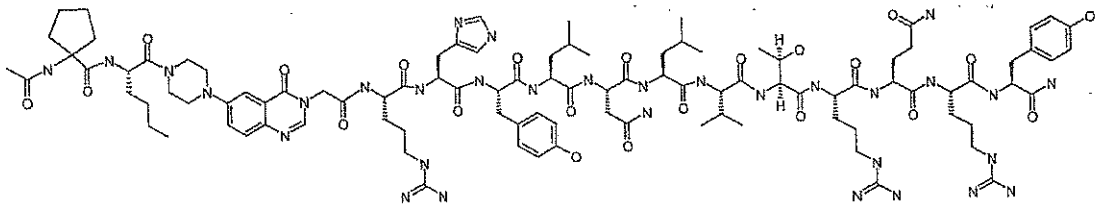
Preparation of Ac-*Ach*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Ach was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 81 mg (15%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{101}H_{154}N_{32}O_{22}$ 2168.55 found 2168.10.

EXAMPLE 71

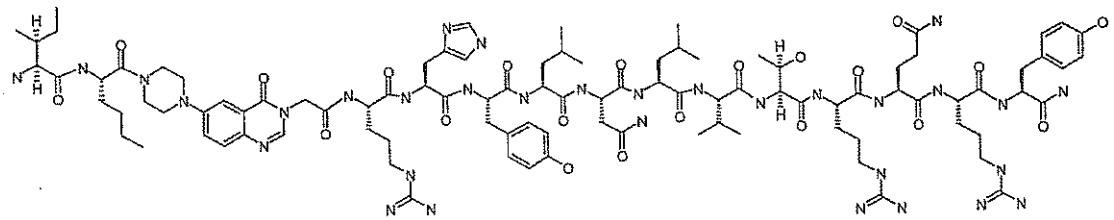
Preparation of Ac-*Acp*-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis (Acp was inserted in position 3 of the sequence) and purification by following the procedure in Example 3 to yield 95 mg (18%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{100}H_{152}N_{32}O_{22}$ 2154.53 found 2154.30.

EXAMPLE 72

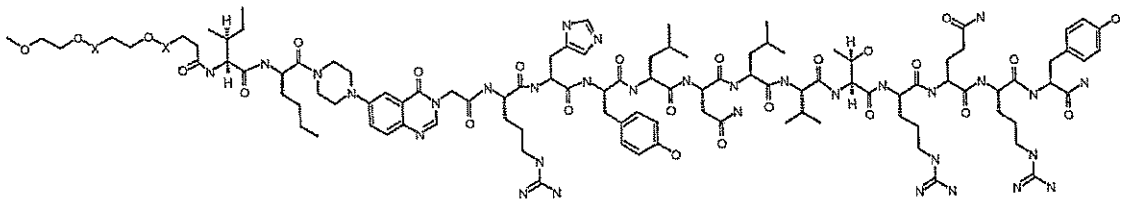
Preparation of *H*-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



Fmoc- Linker-BHA resin (450 mg, 0.25 mmol) from Example 1 was subjected to solid phase synthesis and purification by following the procedure in Example 3 to yield 80 mg (15%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{98}H_{152}N_{32}O_{21}$ 2114.51 found 2113.80.

EXAMPLE 73

Preparation of *PEG (10,000)*-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



In the structure above, the group -X-CH_2-CH_2-O-X-, according to common nomenclature, refers to the repeating glycol units of a polyethyleneglycol moiety, i.e. to a group -(CH_2-CH_2-O)$_n$-. "n" is chosen accordingly to achieve the desired weight of the desired polyethylene glycol unit. Fifteen mg of peptide from example 72 was weighed out and dissolved in 50 mM Borate, pH 7.4 buffer. 107 mg 10 kDa PEG-SPA-NHS (Nektar) was weighed to achieve a 2:1 PEG: peptide molar ratio and added to the dissolved peptide. The reaction mixture was agitated at room temperature for 2h before it was diluted 10-fold in 20 mM NaOAc, pH 4.5 buffer and purified by cation exchange chromatography on S-Sepharose (Amersham). Figure 1 is an HPLC chromatogram of 10 kDa PEG-PYY peptide. The reaction yielded 67.8% of 10 kDa peptide.

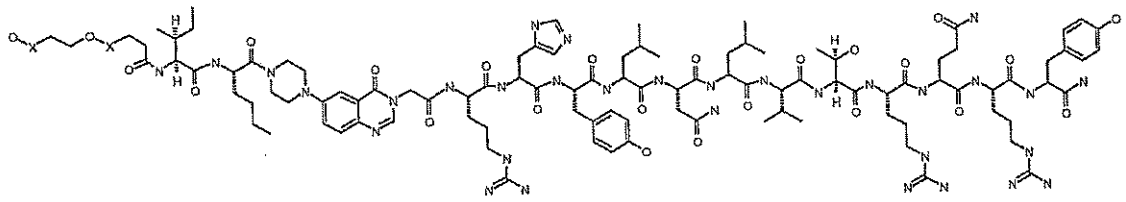
Mono-pegylated PYY peptide was eluted using a step NaCl gradient. Typically, the desired mono-pegylated peptide eluted with 125 mM NaCl. The eluted PEG-PYY-like peptide was concentrated in an Amicon ultrafiltration cell using a 10 kDa MW cutoff membrane. It was then diafiltered 10-fold once with PBS.

Concentrated peptide of example 73 was submitted for analysis, assayed and stored at -20C. Figure 2 is an HPLC chromatogram of purified 10 kDa PEG-PYY peptide. Purities of 10 kDa peptide was determined to be 97.6%. And Figure 3 is a graph representing a

MALDI-TOF of 10 kDa PEG-PYY peptide was performed to confirm the molecular weight.

EXAMPLE 74

Preparation of *PEG (30,000)*-Ile-Nle-Pqa-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ ID NO: 5)



In the structure above, the group -X-CH_2-CH_2-O-X-, according to common nomenclature, refers to the repeating glycol units of a polyethyleneglycol moiety, i.e. to a group -(CH_2-CH_2-O)$_n$-. "n" is chosen accordingly to achieve the desired weight of the desired polyethylene glycol unit. Fifteen mg of peptide from example 72 was weighed out and dissolved in 50 mM Borate, pH 7.4 buffer. Two hundred forty-four mg 30 kDa PEG-SPA-NHS was weighed to achieve a 2:1 PEG: peptide molar ratio and added to the dissolved peptide. The reaction mixture was agitated at room temperature for 2 h (Figure 4) before it was diluted 10-fold in 20 mM NaOAc, pH 4.5 buffer and purified by cation exchange chromatography on S-Sepharose (Amersham). Figure 4 is an HPLC chromatogram of 30 kDa PEG-PYY peptide. The reaction yielded 88.3% of 30 kDa peptide.

Mono-pegylated PYY peptide was eluted using a step NaCl gradient. Typically, the desired mono-pegylated PYY peptide eluted with 125 mM NaCl. The eluted PEG-PYY-like peptide was concentrated in an Amicon ultrafiltration cell using a 10 kDa MW cutoff membrane. It was then diafiltered 10-fold once with PBS. Concentrated peptide was submitted for analysis, assayed and stored at -20C. Figure 5 is an HPLC chromatogram of purified 30 kDa PEG-PYY peptide. Purity of 30 kDa peptide was determined to be 98.4%. And Figure 6 represents a MALDI-TOF of 30 kDa PEG-PYY peptides that was performed to confirm the Molecular weight.

EXAMPLE 75

Plasma Stability of Compound from Example 49

Human and mouse plasma stability of the peptide from Example 49 was measured. In this Example, 140 microgram of peptide from example 49 was incubated with 300 microliter of either human or mouse plasma for 0, 40, 90 and 150 minutes at 37.5°C. POSR incubation

each sample was diluted by 50 times in 0.1 % acetic acid in water. The diluted samples were then analyzed by LC/UV/MS and found to be stable.

EXAMPLE 76

Plasma Stability of Compound from Example 32

Human and mouse plasma stability of the peptide from Example 32 was measured. In this Example, 51 micrograms of peptide from example 32 was incubated with 300 microliter of either human or mouse plasma for 0, 40, 90 and 150 minutes at 37.5°C. POSR incubation each sample was diluted by 50 times in 0.1 % acetic acid in water. The diluted samples were then analyzed by LC/UV/MS and found to be stable.

EXAMPLE 77

cAMP agonist assay

In this example, the following materials were used: 384-well plate; Tropix cAMP-Screen Kit; cAMP ELISA System (Applied Biosystems, cat. #T1505; CS 20000); Forskolin (Calbiochem cat. # 344270); cells: HEK293/hNPY2R; growth medium: Dulbecco's modified eagle medium (D-MEM, Gibco); 10% Fetal bovine serum (FBS, Gibco), heat-inactivated; 1% Penicillin/Streptomycin (Pen 10000 unit/ml: Strep 10000 mg/ml, Gibco); 500 mg/ml G418 (Geneticin, Gibco cat. # 11811-031); and plating medium: DMEM/F12 w/o phenol red (Gibco); 10% FBS (Gibco, cat. # 10082-147), heat-inactivated; 1% Penicillin/Streptomycin (Gibco, cat. # 15140-122); 500 mg/ml G418 (Geneticin, Gibco, cat. # 11811-031).

On the first day, medium was discarded, and the monolayer cells were washed with 10 ml PBS per flask (T225). After decanting with PBS, 5 ml VERSENE (Gibco, cat#1504006) was used to dislodge the cells (5min @37C). The flask was gently tapped and the cell suspension was pooled. Each flask was rinsed with 10 ml plating medium and centrifuged at 1000rpm for 5 min. The suspension was pooled and counted.

The suspension was resuspended in plating medium at a density of 2.0 X 10^5 cells/ml for HEK293/hNPY2R. 50 microliters of cells (HEK293/hNPY2R – 10,000cells/well) were transferred into the 384-well plate using Multi-drop dispenser. The plates were incubated at 37°C overnight.

On the second day, the cells were checked for 75-85% confluence. The media and reagents were allowed to come to room temperature. Before the dilutions were prepared, the stock solution of stimulating compound in dimethyl sulphoxide (DMSO, Sigma, cat#D2650) was allowed to warm up to 32C for 5-10 min. The dilutions were prepared in DMEM/F12 with 0.5mM 3-Isobutyl-1-methylxanthine (IBMX, Calbiochem, cat#410957) and 0.5mg/ml BSA. The final DMSO concentration in the stimulation medium was 1.1% with Forskolin concentration of 5μM.

The cell medium was tapped off with a gentle inversion of the cell plate on a paper towel. 50μL of stimulation medium was placed per well (each concentration done in four replicates). The plates were incubated at room temperature for 30 min, and the cells were checked under a microscope for toxicity.

After 30 minutes of treatment, the stimulation media was discarded and 50μl/well of Assay Lysis Buffer (provided in the Tropix kit) was. The plates were incubated for 45 min@ 37°C.

20μL of the lysate was transferred from stimulation plates into the pre-coated antibody plates (384-well) from the Tropix kit. 10 μL of AP conjugate and 20μL of anti-cAMP antibody was added. The plates were incubated at room temperature while shaking for 1 hour. The plates were then washed 5 times with Wash Buffer, 70 μL per well for each wash. The plates were tapped to dry. 30 μL /well of CSPD/Saphire-II RTU substrate/enhancer solution was added and incubated for 45 min @ RT (shake). Signal for 1 sec/well in a Luminometer. (VICTOR-V) was measured.

EXAMPLE 78

In vivo reduction of food intake assay.

In this Example, compounds of Examples 5, 44, 73 and 74 were administered to four sets of mice in four separate experiments to measure effect on food intake. C57BL/6J male mice or DIO (diet-induced obese) male mice were used in the experiments, with eight mice per experimental group. The mice were maintained on a regular light cycle (6 AM - ON; 6

PM - OFF). The mice were fasted for 24 hours prior to testing with ad lib access to water. During the test, mice were housed one per cage.

The compounds of Examples 5 and 44 were administered intraperitoneally; and the compounds of Examples 73 and 74 were administered subcutaneously. Food intake was measured 1, 2, 4, 6, and 24 hours after dosing and the results presented in Figures 7 to 10. Food intake was measured for longer time intervals for the compounds of Examples 73 and 74, and shown in Figures 9 and 10. In each Figure, cumulative and time interval food consumptions were measured in individual mice. Mean food consumption and percent change from vehicle were calculated for each time interval. Data was analyzed by using two-tailed Student t-test. Figures 7 to 10 show that the compounds of the present invention are effective in reducing food intake when compared to controls.

EXAMPLE 79

CaFlux Assay

Hek-293 cells were stably transfected with the G protein chimera Gaqi9 and the hygromycin-B resistance gene were further transfected with the human NPY2 receptor and G418 antibiotic selection. Following selection in both hygromycin-B and G418, individual clones were assayed for their response to PYY. The transfected cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 50μg/ml hygromycin-B 2mM glutamine, 100U/ml penicillin, 100μg/ml streptomycin and 250μg/ml G418. Cells are harvested with trypsin-EDTA and counted using ViaCount reagent. The cell suspension volume is adjusted to $4.8x10^5$ cells /ml with complete growth media. Aliquots of 25μL are dispensed into 384 well Poly-D Lysine coated black/clear microplates (Falcon) and the microplates were placed in a 37°C CO_2 incubator overnight.

Loading Buffer (Calcium-3 Assay Kit, Molecular Devices) was prepared by dissolving the contents of one vial (Express Kit) into 1000 ml Hank's Balanced Salt Solution containing 20mM HEPES and 5mM probenecid. Aliquots of 25μL of diluted dye was dispensed into the cell plates and the plates are then incubated for 1 hour at 37°C.

During the incubation, test compounds were prepared at 3.5X the desired concentration in HBSS(20mM HEPES)/0.05%BSA/1%DMSO and transferred to a 384 well plate for use on FLIPR.

After incubation, both the cell and compound plates were brought to the FLIPR and 20µL of the diluted compounds were transferred to the cell plates by the FLIPR. During the assay, fluorescence readings were taken simultaneously from all 384 wells of the cell plate every 1.5 seconds. Five readings were taken to establish a stable baseline, and then 20µL of sample was rapidly (30µL/sec) and simultaneously added to each well of the cell plate. The fluorescence was continuously monitored before, during and after sample addition for a total elapsed time of 100 seconds. Responses (increase in peak fluorescence) in each well following addition was determined. The initial fluorescence reading from each well, prior to ligand stimulation, was used as a zero baseline value for the data from that well. The responses are expressed as % of maximal response of the positive control.

The compounds of the present invention exhibited selective Neuropeptide -2 receptor activity in vitro, as demonstrated in the cAMP assay (Example 77) and CaFlux Assay (FLIPR) (Example 78). Summary of the in vitro results, IC50 and EC50 for Examples 3 to 74, are illustrated in Table 1 below:

TABLE 1

Example	Sequence	Y2R EC50 (nM)	Y2R IC50 (nM)	Y1R EC50 (nM)	Y4R EC50 (nM)
		FLIPR	cAMP	FLIPR	FLIPR
3	YPIKPEAPGEDASPEELNRYY ASLRHYLNLVTRQRY (1-36)	0.76	0.042	62	123
4	IKPEAPGEDASPEELNRYYAS LRHYLNLVTRQRY (3-36)	2.93	0.032	557	>5000
5	IK-Pqa -RHYLNLVTRQRY	3.2	0.032	373	>5000
6	Ac-IK-Pqa -RHYLNLVTRQRY	0.58	0.026	184	>5000
7	IK-Pqa -RHYLNLVTRARY	176	29	>5000	>5000
8	IK-Pqa -RHYLNLVARQRY	18.6	5.27	>5000	>5000
9	IK-Pqa -RHYLNLATRQRY	9.65	0.21	>5000	>5000
10	IK-Pqa -RHYLNAVTRQRY	4.85	0.25	>5000	>5000
11	IK-Pqa -RHYLALVTRQRY	38.3	3.11	>5000	>5000
12	IK-Pqa -RHYANLVTRQRY	6.25	0.24	>5000	>5000

13	IK-Pqa -RHALNLVTRQRY	10.8	1.39	>5000	>5000
14	IK-Pqa -RAYLNLVTRQRY	12.2	0.062	>5000	>5000
15	IK-Pqa -AHYLNLVTRQRY	25	0.639	3400	>5000
16	IA-Pqa -RHYLNLVTRQRY	1.68	0.079	1000	>5000
17	Ac-IA-Pqa -RHYLNLVTRQRY	2.47	0.247	>5000	3017
18	AK-Pqa -RHYLNLVTRQRY	0.62	0.172	>5000	>5000
19	IK-Pqa -RHYLNLVTRQR(D)Y	3.5	1.09	3300	>5000
20	IK-Pqa -RHYLNLVTRQ(D)RY	110	25	>5000	>5000
21	IK-Pqa -RHYLNLVT(D)RQRY	324	65	>5000	>5000
22	IK-Pqa -RHYLNL(D)VTRQRY	322	21	>5000	>5000
23	IK-Pqa -RHYLN(D)LVTRQRY	66	22	>5000	>5000
24	IK-Pqa -RHYL(D)NLVTRQRY	48	0.92	3800	>5000
25	IK-Pqa -RHY(D)LNLVTRQRY	5.26	0.37	3800	>5000
26	IK-Pqa -RH(D)YLNLVTRQRY	2.98	0.26	654	>5000
27	IK-Pqa -R(D)HYLNLVTRQRY	12.3	3.18	431	>5000
28	IK-Pqa -(D)RHYLNLVTRQRY	5.2	0.201	1100	>5000
29	I(D)K-Pqa -RHYLNLVTRQRY	0.43	0.078	>5000	>5000
30	(D)IK-Pqa -RHYLNLVTRQRY	0.22	0.099	>5000	>5000
31	IK-Pqa -RHYLNLVTRQR(N-methyl)Y	11.8	2.6	>5000	>5000
32	IK-Pqa -RHYLNLVTRQ(N-methyl)RY	18.9	0.417	>5000	>5000
33	IK-Pqa -RHYLNLVT(N-methyl)RQRY	30.6	13.5	>5000	>5000
34	IK-Pqa -RHYLNLV(N-methyl)TRQRY	78.6	89	>5000	>5000
35	IK-Pqa -RHYLNL(N-methyl)VTRQRY	44.4	42	>5000	>5000
36	IK-Pqa -RHYLN(N-methyl)LVTRQRY	103	12	>5000	>5000
37	IK-Pqa -RHY(N-methyl)LNLVTRQRY	782	168	>5000	>5000
38	IK-Pqa -RH(N-methyl)YLNLVTRQRY	1.2	11.7	>5000	>5000
39	IK-Pqa -R(N-methyl)HYLNLVTRQRY	41.9	1.488	3800	>5000
40	IK-Pqa -(N-methyl)RHYLNLVTRQRY	4.4	0.218	1600	>5000
41	I(N-methyl)K-Pqa -RHYLNLVTRQRY	0.614	0.19	834	>5000
42	(N-methyl)IK-Pqa -RHYLNLVTRQRY	0.799	0.12	327	>5000
43	INle-Pqa -RHYLNLVTRQRY	0.89	0.096	430	>5000
44	Ac-INle-Pqa -RHYLNLVTRQRY	6.1	0.338	760	>5000

45	Ac-INle-Pqa - FHYLNLVTRQRY	19.3	2.74	>5000	>5000
46	IK-Pqa -RHWLNLVTRQRY	6.1	0.103	252	>5000
47	IK-Pqa -AHWLNLVTRQRY	17.05	0.547	1100	>5000
48	Ac-INle-Pqa - RHYLNLVTRQR(D)Y	107	2.2	2020	>5000
49	Ac-INle-Pqa - RHYLNLVTRQR(N-methyl)Y	113	3.6	4200	>5000
50	Ac-INle-Pqa - RHYLys(28)NLVAsp(32)RQRY (cyclo Lys-Asp)	92.4	33.48	>5000	>5000
51	IK-Cms -RHYLNLVTRQRY	4.91	0.1	>5000	>5000
52	IKG-Cms -RHYLNLVTRQRY	4.16	0.085	>5000	>5000
53	Ac-INle-Cms- RHYLys(28)NLVAsp(32)RQRY (cyclo Lys-Asp)	146	32.25	>5000	>5000
54	Ac-INle-Pqa - RHTicLNLVTRQRY	163	45	>5000	>5000
55	Ac-INle-Pqa - RHBipLNLVTRQRY	24.7	3.72	561	>5000
56	Ac-INle-Pqa - RHDipLNLVTRQRY	33.5	1.21	1516	>5000
57	Ac-INle-Pqa - RH(1)NalLNLVTRQRY	13	1.36	701	>5000
58	Ac-INle-Pqa - RH(2)NalLNLVTRQRY	12.3	2.68	870	>5000
59	Ac-INle-Pqa -RH(3.4,5 Trifluoro Phe)LNLVTRQRY	13.8	1.34	1089	>5000
60	Ac-INle-Pqa -RH(2,3.4,5,6 Pentafluoro Phe)LNLVTRQRY	14.2	2.13	832	>5000
61	Ac-INle-Pqa -R(4-MeOApc)YLNLVTRQRY	11.9	7.8	>5000	>5000
62	Ac-INle-Pqa -R(3-Pal)YLNLVTRQRY	5.6	1.78	2022	>5000
63	Ac-INle-Pqa -R(4-Pal)YLNLVTRQRY	4.97	0.099	>5000	>5000
64	Ac-INle-Pqa -(3,4,5 Trifluro Phe)HYLNLVTRQRY	46	1.74	>5000	>5000
65	Ac-INle-Pqa -(2,3,4,5 ,6 Pentafluro Phe)HYLNLVTRQRY	134	9.37	>5000	>5000
66	Ac-Aib-Nle-Pqa-RHYLNLVTRQRT	8.9	0.38	3329	>5000
67	Ac1-1-Aic-Nle-Pqa - RHYLNLVTRQRT	8.8	0.64	1654	>5000
68	Ac1-1-Aic-Nle-Pqa - RHYLNLVTRQRT	6	1	2646	>5000

69	Ac-2-2Aic-Nle-Pqa - RHYLNLVTRQRT	8.2	0.45	>5000	>5000
70	Ac-Ach-Nle-Pqa- RHYLNLVTRQRT	7.1	0.57	>5000	>5000
71	Ac-Acp-Nle-Pqa - RHYLNLVTRQRT	8.7	0.303	>5000	>5000
72	H- INle-Pqa - RHYLNLVTRQRY	0.89	0.096	450	>5000
73	(PEG-10,000) INle-Pqa - RHYLNLVTRQRY	7.4	45		
74	(PEG-30,000) INle-Pqa - RHYLNLVTRQRY	15.4	79		

It is to be understood that the invention is not limited to the particular embodiments of the invention described above, as variations of the particular embodiments may be made and still fall within the scope of the appended claims.

Example A

Film coated tablets containing the following ingredients can be manufactured in a conventional manner:

Ingredients	*Per tablet*	
Kernel:		
Compound of formula (I)	10.0 mg	200.0 mg
Microcrystalline cellulose	23.5 mg	43.5 mg
Lactose hydrous	60.0 mg	70.0 mg
Povidone K30	12.5 mg	15.0 mg
Sodium starch glycolate	12.5 mg	17.0 mg
Magnesium stearate	1.5 mg	4.5 mg
(Kernel Weight)	120.0 mg	350.0 mg
Film Coat:		
Hydroxypropyl methyl cellulose	3.5 mg	7.0 mg
Polyethylene glycol 6000	0.8 mg	1.6 mg
Talc	1.3 mg	2.6 mg
Iron oxyde (yellow)	0.8 mg	1.6 mg
Titan dioxide	0.8 mg	1.6 mg

The active ingredient is sieved and mixed with microcristalline cellulose and the mixture is granulated with a solution of polyvinylpyrrolidon in water. The granulate is mixed with sodium starch glycolate and magesiumstearate and compressed to yield kernels of 120 or 350 mg respectively. The kernels are lacquered with an aqueous solution / suspension of the above mentioned film coat.

Example B

Capsules containing the following ingredients can be manufactured in a conventional manner:

Ingredients	Per capsule
Compound of formula (I)	25.0 mg
Lactose	150.0 mg
Maize starch	20.0 mg
Talc	5.0 mg

The components are sieved and mixed and filled into capsules of size 2.

Example C

Injection solutions can have the following composition:

Compound of formula (I)	3.0 mg
Polyethylene Glycol 400	150.0 mg
Acetic Acid	q.s. ad pH 5.0
Water for injection solutions	ad 1.0 ml

The active ingredient is dissolved in a mixture of Polyethylene Glycol 400 and water for injection (part). The pH is adjusted to 5.0 by Acetic Acid. The volume is adjusted to 1.0 ml by addition of the residual amount of water. The solution is filtered, filled into vials using an appropriate overage and sterilized.

Example D

Soft gelatin capsules containing the following ingredients can be manufactured in a conventional manner:

Capsule contents	
Compound of formula (I)	5.0 mg
Yellow wax	8.0 mg
Hydrogenated Soya bean oil	8.0 mg
Partially hydrogenated plant oils	34.0 mg
Soya bean oil	110.0 mg
Weight of capsule contents	165.0 mg
Gelatin capsule	
Gelatin	75.0 mg
Glycerol 85 %	32.0 mg
Karion 83	8.0 mg (dry matter)
Titan dioxide	0.4 mg
Iron oxide yellow	1.1 mg

The active ingredient is dissolved in a warm melting of the other ingredients and the mixture is filled into soft gelatin capsules of appropriate size. The filled soft gelatin capsules are treated according to the usual procedures.

Example E

Sachets containing the following ingredients can be manufactured in a conventional manner:

Compound of formula (I)	50.0 mg
Lactose, fine powder	1015.0 mg
Microcristalline cellulose (AVICEL PH 102)	1400.0 mg
Sodium carboxymethyl cellulose	14.0 mg
Polyvinylpyrrolidon K 30	10.0 mg
Magnesiumstearate	10.0 mg
Flavoring additives	1.0 mg

The active ingredient is mixed with lactose, microcristalline cellulose and sodium carboxymethyl cellulose and granulated with a mixture of polyvinylpyrrolidon in water. The granulate is mixed with magnesiumstearate and the flavouring additives and filled into sachets.

Claims

1. A neuropeptide-2 receptor agonist of the formula (I):

$$Y\text{-}R_1\text{-}R_2\text{-}X\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}R_7\text{-}R_8\text{-}R_9\text{-}R_{10}\text{-}R_{11}\text{-}R_{12}\text{-}R_{13}\text{-}R_{14}\text{-}NH_2 \quad (I)$$

wherein:

X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine,

Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,

R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp,

R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly),

R_3 is Arg, Ala, (D)Arg, N-methylArg, Phe, 3,4,5- TrifluoroPhe or 2,3,4,5,6- Pentafluoro Phe,

R_4 is His, Ala, (D)His, N-methyl His, 4-MeOApc, 3-Pal or 4-Pal,

R_5 is Tyr, Ala, (D) Tyr , N- methyl Tyr, Trp, Tic, Bip, Dip, (1)Nal, (2)Nal, 3,4,5-TrifluroPhe or 2,3,4,5,6- Pentafluoro Phe,

R_6 is Leu, Ala, (D)Leu or N-methyl Leu,

R_7 is Asn, Ala or (D)Asn,

R_8 is Leu, Ala, (D)Leu or N-methyl Leu,

R_9 is Val, Ala, (D) Val or N-methyl Val,

R_{10} is Thr, Ala or N-methyl Thr,

R_{11} is Arg, (D) Arg or N-methyl Arg,

R_{12} is Gln or Ala,

R_{13} is Arg, (D)Arg or N-methyl Arg, and

R_{14} is Tyr, (D) Tyr or N- methyl Tyr,

or a pharmaceutically acceptable salt thereof.

2. The neuropeptide-2 receptor agonist according to claim 1, wherein X is Pqa.

3. The neuropeptide-2 receptor agonist according to claim 1, wherein X is Cms.

4. The neuropeptide-2 receptor agonist according to claim 1, wherein Y is a (C_1-C_6) alkyl moiety.

5. A neuropeptide-2 receptor agonist of the formula (II):

Y-R_1-R_2-X-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (II)

wherein:
X is a moiety selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine,
Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,
R_1 is Ile, Ala, (D) Ile, N-methyl Ile, Aib, 1-1Aic, 2-2 Aic, Ach or Acp, and
R_2 is Lys, Ala, (D) Lys, NMelys, Nle or (Lys-Gly),
or a pharmaceutically acceptable salt thereof.

6. The neuropeptide-2 receptor agonist according to claim 5, wherein X is Pqa.

7. The neuropeptide-2 receptor agonist according to claim 5, wherein X is Cms.

8. A neuropeptide-2 receptor agonist of the formula (III):

Y-Ile-Lys-X-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (III)

wherein:
X is selected from the group consisting of N-piperazin-1-yl-4(3H)-quinozolinone-3-acetic acid (Pqa), N-(5-O-carboxymethyl)-serotonin (Cms), 4-(2-aminomethyl)-6-

dibenzofuranpropanoic acid, 4-(1-piperidin-4-yl)-butanoic acid and 4-(2-aminoethyl)-1-carboxymethyl piperazine, and

Y is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted lower alkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted alkoxy or a poly(ethylene) glycol moiety,

or a pharmaceutically acceptable salt thereof.

9. The neuropeptide-2 receptor agonist according to claim 8, wherein X is Pqa.

10. The neuropeptide-2 receptor agonist according to claim 8, wherein X is Cms.

11. A neuropeptide-2 receptor agonist according to any of claims 1 to 10, selected from the group consisting of

IK-Pqa -RHYLNLVTRQRY,
Ac-IK-Pqa -RHYLNLVTRQRY,
IK-Pqa -RHYLNLVTRARY,
IK-Pqa -RHYLNLVARQRY,
IK-Pqa -RHYLNLATRQRY,
IK-Pqa -RHYLNAVTRQRY,
IK-Pqa -RHYLALVTRQRY,
IK-Pqa -RHYANLVTRQRY,
IK-Pqa -RHALNLVTRQRY,
IK-Pqa -RAYLNLVTRQRY,
IK-Pqa -AHYLNLVTRQRY,
IA-Pqa -RHYLNLVTRQRY,
Ac-IA-Pqa -RHYLNLVTRQRY,
AK-Pqa -RHYLNLVTRQRY,
IK-Pqa -RHYLNLVTRQR(D)Y,
IK-Pqa -RHYLNLVTRQ(D)RY,
IK-Pqa -RHYLNLVT(D)RQRY,
IK-Pqa -RHYLNL(D)VTRQRY,
IK-Pqa -RHYLN(D)LVTRQRY,
IK-Pqa -RHYL(D)NLVTRQRY,

IK-Pqa -RHY(D)LNLVTRQRY,

IK-Pqa -RH(D)YLNLVTRQRY,

IK-Pqa -R(D)HYLNLVTRQRY,

IK-Pqa -(D)RHYLNLVTRQRY,

I(D)K-Pqa -RHYLNLVTRQRY,

(D)IK-Pqa -RHYLNLVTRQRY,

IK-Pqa -RHYLNLVTRQR(N-methyl)Y,

IK-Pqa -RHYLNLVTRQ(N-methyl)RY,

IK-Pqa -RHYLNLVT(N-methyl)RQRY,

IK-Pqa -RHYLNLV(N-methyl)TRQRY,

IK-Pqa -RHYLNL(N-methyl)VTRQRY,

IK-Pqa -RHYLN(N-methyl)LVTRQRY,

IK-Pqa -RHY(N-methyl)LNLVTRQRY,

IK-Pqa -RH(N-methyl)YLNLVTRQRY,

IK-Pqa -R(N-methyl)HYLNLVTRQRY,

IK-Pqa -(N-methyl)RHYLNLVTRQRY,

I(N-methyl)K-Pqa -RHYLNLVTRQRY,

(N-methyl)IK-Pqa -RHYLNLVTRQRY,

INle-Pqa -RHYLNLVTRQRY,

Ac-INle-Pqa -RHYLNLVTRQRY,

Ac-INle-Pqa -FHYLNLVTRQRY,

IK-Pqa -RHWLNLVTRQRY,

IK-Pqa -AHWLNLVTRQRY,

Ac-INle-Pqa -RHYLNLVTRQR(D)Y,

Ac-INle-Pqa -RHYLNLVTRQR(N-methyl)Y,

Ac-INle-Pqa -RHYLys(28)NLVAsp(32)RQRY (cyclo Lys-Asp),

IK-Cms -RHYLNLVTRQRY,

IKG-Cms -RHYLNLVTRQRY,

Ac-INle-Cms-RHYLys(28)NLVAsp(32)RQRY(cyclo Lys-Asp),

Ac-INle-Pqa -RHTicLNLVTRQRY,

Ac-INle-Pqa -RHBipLNLVTRQRY,

Ac-INle-Pqa -RHDipLNLVTRQRY,

Ac-INle-Pqa -RH(1)NalLNLVTRQRY,

Ac-INle-Pqa -RH(2)NalLNLVTRQRY,
Ac-INle-Pqa -RH(3.4,5 Trifluoro Phe)LNLVTRQRY,
Ac-INle-Pqa -RH(2,3.4,5,6 Pentafluoro Phe)LNLVTRQRY,
Ac-INle-Pqa -R(4-MeOApc)YLNLVTRQRY,
Ac-INle-Pqa -R(3-Pal)YLNLVTRQRY,
Ac-INle-Pqa -R(4-Pal)YLNLVTRQRY,
Ac-INle-Pqa -(3,4,5 Trifluro Phe)HYLNLVTRQRY,
Ac-INle-Pqa -(2,3,4,5 ,6 Pentafluro Phe)HYLNLVTRQRY,
Ac-Aib-Nle-Pqa-RHYLNLVTRQRT,
Ac1-1-Aic-Nle-Pqa -RHYLNLVTRQRT,
Ac1-1-Aic-Nle-Pqa -RHYLNLVTRQRT,
Ac-2-2Aic-Nle-Pqa -RHYLNLVTRQRT,
Ac-Ach-Nle-Pqa-RHYLNLVTRQRT,
Ac-Acp-Nle-Pqa -RHYLNLVTRQRT,
H- INle-Pqa -RHYLNLVTRQRY,
(PEG-10,000) INle-Pqa -RHYLNLVTRQRY, and
(PEG-30,000) INle-Pqa -RHYLNLVTRQRY.

12. A neuropeptide-2 receptor agonist according to any of claims 1 to 11, selected from the group consisting of
IK-Pqa -RHYLNLVTRQRY,
Ac-IK-Pqa -RHYLNLVTRQRY,
IK-Pqa -RHYLNLVTRQR(N-methyl)Y,
IK-Pqa -RHYLNLVTRQ(N-methyl)RY,
INle-Pqa -RHYLNLVTRQRY,
Ac-INle-Pqa -RHYLNLVTRQRY, and
(PEG-30,000) INle-Pqa -RHYLNLVTRQRY.

13. Pharmaceutical compositions comprising a compound according to any of claims 1 - 12 and a pharmaceutically acceptable carrier and/or adjuvant.

14. Compounds according to any of claims 1 - 12 for use as therapeutic active substances.

15. Compounds according to any of claims 1 - 12 for use as therapeutic active substances for the treatment and/or prophylaxis of diseases which are modulated by neuropeptide -2 receptor agonists.

16. The use of compounds according to any of claims 1 - 12 for the preparation of medicaments for the therapeutic and/or prophylactic treatment of diseases which are modulated by neuropeptide -2 receptor agonists.

17. The use of compounds according to any of claims 1 - 12 for the preparation of medicaments for the therapeutic and/or prophylactic treatment of obesity.

SEQUENCE LISTING

<110> F. HOFMANN-LA ROCHE AG

<120> NEUROPEPTIDE-2 RECEPTOR (Y-2R) AGONISTS AND USES THEREOF

<130> 22849

<140> 60/644,840
<141> 2005-01-18

<160> 42

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Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr
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Arg Gln Arg Tyr
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Arg Tyr

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