MELANOCORTIN RECEPTOR-SPECIFIC PEPTIDES

Technical field

The present invention relates to melanocortin receptor-specific cyclic peptides which may be used in the treatment of melanocortin receptor-mediated diseases, indications, conditions and syndromes, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

Background

The following discussion refers to a number of publications by author(s) and year of publication, and that due to recent publication dates certain publications are not to be considered as prior art vis-a-vis the present invention. Discussion of such publications herein is given for more complete background and is not to be construed as an admission that such publications are prior art for patentability determination purposes.

A family of melanocortin receptor types and subtypes have been identified, including melanocortin-1 receptors (MC1-R) expressed on normal human melanocytes and melanoma cells, melanocortin-2 receptors (MC2-R) for ACTH (adrenocorticotropin) expressed in cells of the adrenal gland, melanocortin-3 and melanocortin-4 receptors (MC3-R and MC4-R) expressed primarily in cells in the hypothalamus, mid-brain and brainstem, and melanocortin-5 receptors (MC5-R), expressed in a wide distribution of peripheral tissues. MC1-R has been suggested to be associated with hair and skin pigmentation and inflammation, MC2-R is believed to mediate steroidogenesis, MC3-R has been suggested to be associated with energy homeostasis, food intake, and inflammation, MC4-R is believed to control feeding behavior, energy homeostasis, and sexual function (e.g. erectile function), and MC5-R has been suggested to be involved in the exocrine gland system.

Significant work has been done in determining the structure of melanocortin receptors, including both the nucleic acid sequences encoding for the receptors and the amino acid sequences constituting the receptors. MC4-R is a G protein-coupled, 7-transmembrane receptor that is believed to be expressed primarily in the brain.

MC4-R inactivation has been shown to result in obesity (Hadley, 1999, Ann N Y Acad Sci., 885:1-21). Agouti-related protein (AgRP) is an endogeneous compound that has been suggested to be a MC antagonist or an inverse agonist on MC4-R. The α-melanocyte stimulating hormone (α-MSH) is believed to be the principle endogenous MC4-R agonist.

Also peripherally located MC4-R receptors have been suggested to be involved in the control of energy homeostasis, and the role of MC4-R signalling in the vagus nerve and its relevance for treatment of obesity and diabetes is discussed by Gautron et al, The Journal of Comparative Neurology, 518:6-24 (2010).

Peptides specific for MC4-R, and secondarily peptides specific for MC3-R, are believed to be useful in regulation of mammalian energy homeostasis, including use as agents for attenuating food intake and body weight gain. MC4-R agonist peptides are believed to be useful for treating sexual dysfunction, including male erectile dysfunction, and for decreasing food intake and body weight gain, such as for treatment of obesity. Such peptides may also be employed for decreasing voluntary ethanol consumption, treatment of drug addictions, and the like. MC4-R agonist peptides, as well as MC3-R agonist peptides, may further be employed for treatment of circulatory shock, ischemia, hemorrhagic shock, inflammatory diseases and related diseases, indications, conditions and syndromes. MC4-R antagonist peptides, by contrast, are believed to be useful for weight gain aid, such as for use in treatment of cachexia, sarcopenia, wasting syndrome or disease, and anorexia. Such peptides may also be employed for treatment of depression and related disorders. (Wikberg et al, Nature Reviews, Drug Discovery, 7, 307, (2008); Adan et al, British J. Pharm., 149, 815-827 (2006); Nogueiras et al, J. Clin., Invest., 117(11): 3475-3488 (2007); Maaser et al, Ann. N.Y. Acad. Sci., 1072, 123-134 (2006); Giuliani et al, British J. Pharm., 150, 595-603 (2007); Balbani, Expert Opin. Ther. Patents, 17(3), 287-297 (2007); and Navarro et al, Alcohol. Clin. Exp. Res., 29(6), 949-957 (2005)).

Melanocortin receptor-specific peptides include cyclic α-MSH analog peptides such as Ac-Nle-*cyclo*(-Asp-His-D-Phe-Arg-Trp-Lys)-NH_2 (SEQ ID NO:1) (*See* U.S. Patent Nos.

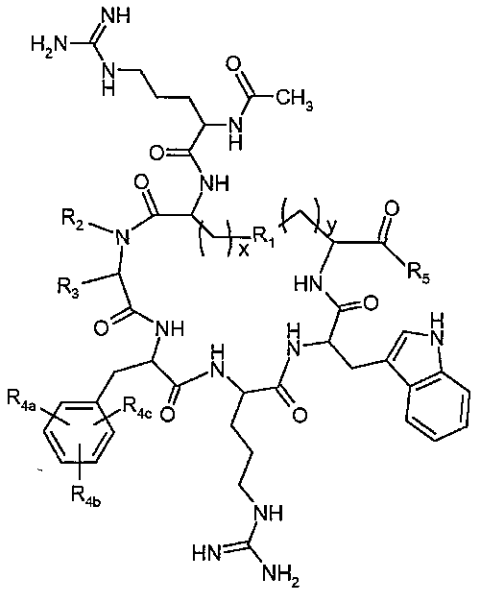
5,674,839 and 5,576,290) and Ac-Nle-*cyclo*(-Asp-His-D-Phe-Arg-Trp-Lys)-OH (SEQ ID NO:2) (*See* U.S. Patent Nos. 6,579,968 and 6,794,489). These and other melanocortin receptor-specific peptides generally contain the central tetrapeptide sequence of native α-MSH, His^6-Phe^7-Arg^8-Trp^9 (SEQ ID NO:3), or a mimetic or variation thereof, including the substitution of D-Phe for Phe^7. Other peptides or peptide-like compounds asserted to be specific for one or more melanocortin receptors are disclosed in U.S. Patent Nos. 5,731,408, 6,054,556, 6,350,430, 6,476,187, 6,600,015, 6,613,874, 6,693,165, 6,699,873, 6,887,846, 6,951,916, 7,008,925, and 7,176,279; in U.S. published patent application Publication Nos. 2001/0056179, 2002/0143141, 2003/0064921, 2003/0105024, 2003/0212002, 2004/0023859, 2005/0130901, 2005/0187164, 2005/0239711, 2006/0105951, 2006/0111281, 2006/0293223, 2007/0027091, 2007/0105759, 2007/0123453, 2007/0244054, and 2008/0039387; and in international patent applications nos. WO 98/27113, WO 99/21571, WO 00/05263, WO 99/54358, WO 00/35952, WO 00/58361, WO 01/30808, WO 01/52880, WO 01/74844, WO 01/85930, WO 01/90140, WO 02/18437, WO 02/26774, WO 03/006604, WO 2004/099246, WO 2004/046166, WO 2005/000338, WO 2005/000339, WO 2005/000877, WO 2005/030797, WO 2005/060985, WO2006/048449, WO 2006/048450, WO 2006/048451, WO 2006/048452, WO 2006/097526, WO 2007/008684, WO 2007/008704, and WO 2007/009894. Notwithstanding the intense scientific and pharmaceutical interest in melanocortin receptor-specific peptides, evidenced by numerous articles in the scientific literature and numerous patent applications and issued patents (Nozawa et al, Expert Opin. Ther. Patents 18(4):403-427 (2008); Bednarek et al, Expert Opin. Ther. Patents 14(3):327-336 (2004); Todorovic et al, Peptides, 26, 2026-2036 (2005); and Ujjainwalla et al, Current Topics in Med. Chem., 7, 1068-1084 (2007)), no melanocortin receptor-specific peptide has been approved as a drug for any therapeutic indication. Indeed, there are no reports of any melanocortin receptor-specific peptide for any therapeutic indication having advanced past Phase II clinical trials. There remains a significant and substantial need for melanocortin receptor-specific peptides for use in pharmaceutical applications. It is against this background that the present invention was made.

Summary of the invention

It is therefore an object of the present invention to provide novel compounds that are useful for the treatment of diseases, disorders and/or conditions responsive to modulation,

including activation, of MC4-R and/or MC3-R, in particular treatment of energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

In one aspect, the present invention relates to a cyclic peptide of the structural Formula (I):



(I)

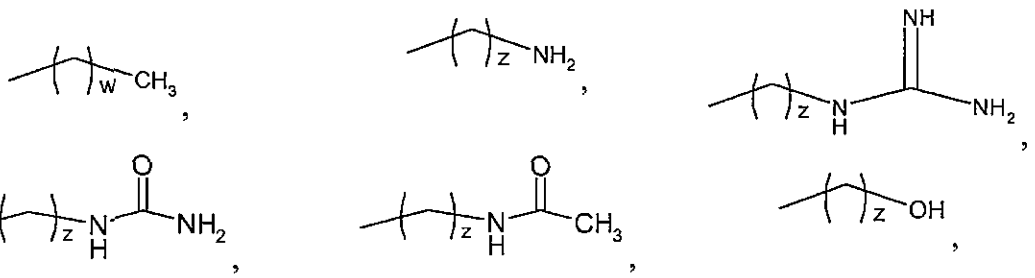
including all enantiomers, stereoisomers or diastereoisomers thereof, or a pharmaceutically acceptable salt of any of the foregoing,

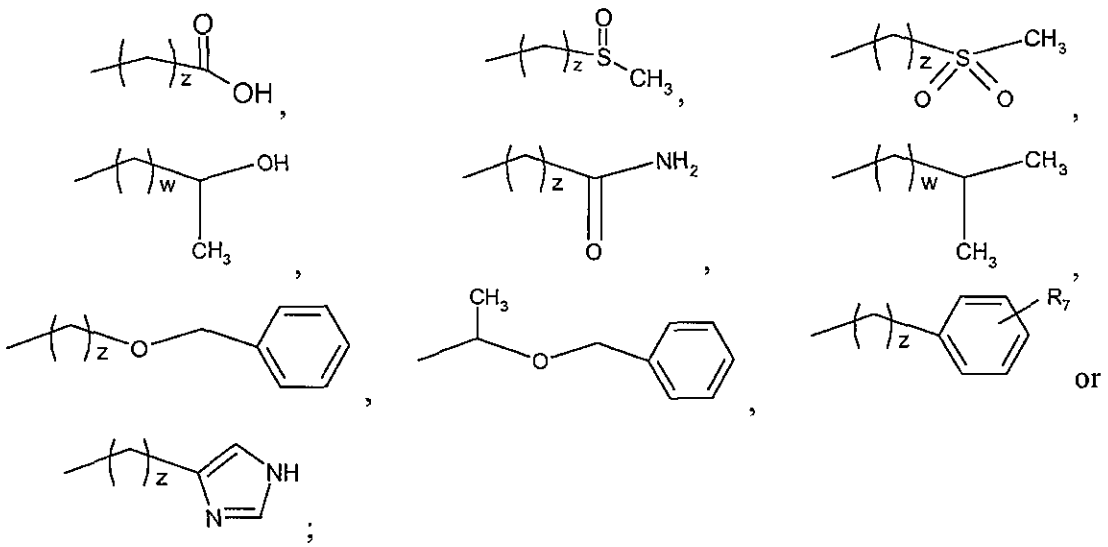
wherein:

R_1 is -NH-C(=O)- or -C(=O)-NH-;

R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH;

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from





R_{4a}, R_{4b} and R_{4c} are are each independently selected from hydrogen, halo, (C_1-C_{10})alkyl-halo, (C_1-C_{10})alkyl-dihalo, (C_1-C_{10})alkyl-trihalo, (C_1-C_{10})alkyl, (C_1-C_{10})alkoxy, (C_1-C_{10})alkylthio, aryl, aryloxy, nitro, nitrile, sulfonamide, amino, monosubstituted amino, disubstituted amino, hydroxy, carboxy, or alkoxy-carbonyl, on the proviso that at least one of R_{4a}, R_{4b} and R_{4c} is not hydrogen;

R_5 is -OH or -N(R_{6a})(R_{6b});

R_{6a} and R_{6b} are each independently H or a C_1 to C_4 linear, branched or cyclic alkyl chain;

R_7 is -H or -C(=O)-NH_2;

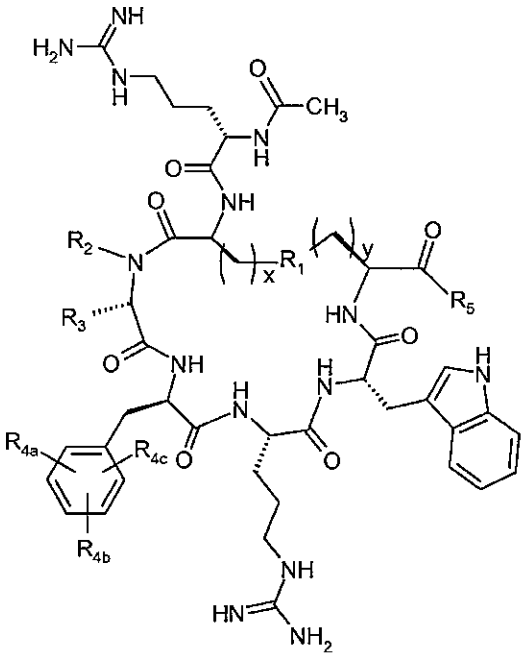
w is in each instance independently 0 to 5;

x is 1 to 5;

y is 1 to 5; and

z is in each instance independently 1 to 5.

In another aspect, the present invention relates to a cyclic peptide of Formula (I) which is of formula (II):



(II)

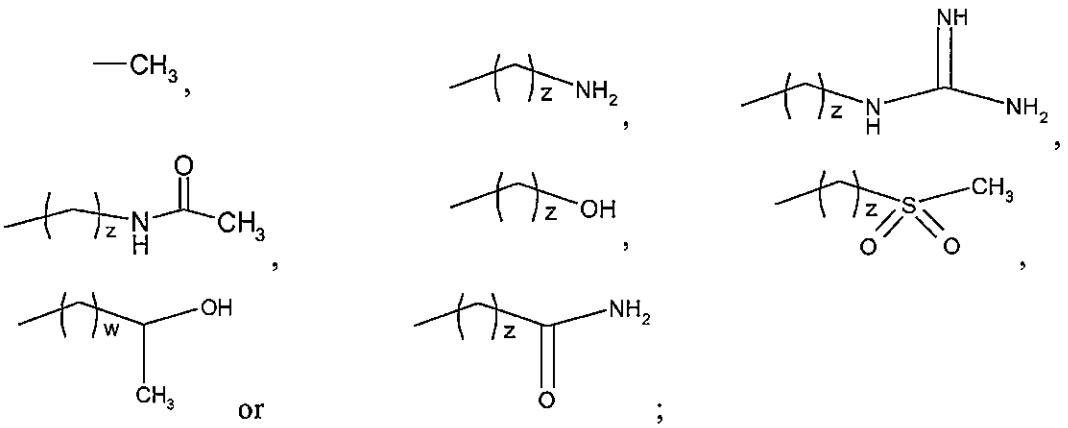
or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention relates to a cyclic peptide of Formula I, in particular of Formula (II), wherein R_1 is -C(=O)-NH-, x is 2 and y is 3.

In another aspect, the present invention relates to a cyclic peptide of Formula (I), in particular of Formula (II), wherein R_1 is -NH-C(=O)-, x is 3 and y is 2.

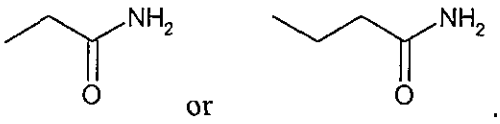
In another aspect, the present invention relates to a cyclic peptide of Formula (I), in particular of Formula (II), wherein R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is



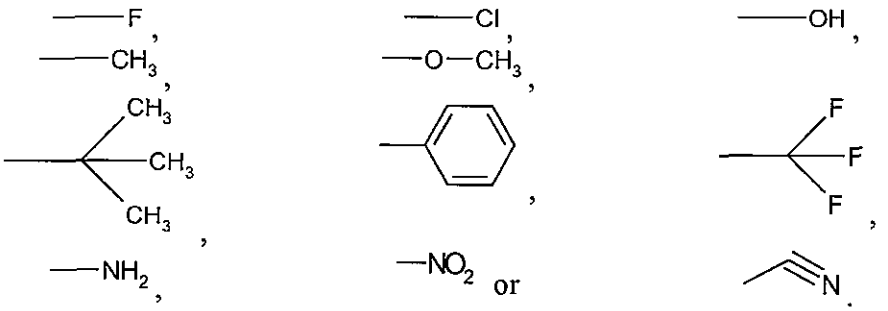
wherein w is in each instance independently selected from 0 to 5, and z is in each instance independently selected from 1 to 5.

In another aspect, the present invention relates to a cyclic peptide of Formula I, in particular of Formula (II) or (III), wherein R_2 is H, R_3 is selected from



In another aspect, the present invention relates to a cyclic peptide of Formula I, in particular of Formula (II), wherein R_2 is $-CH_2-$ and R_3 is $-(CH_2)_2-$, R_2 and R_3 together forming an unsubstituted pyrrolidine ring.

In another aspect, the present invention relates to a cyclic peptide of Formula I, in particular Formula (II), wherein at least one of R_{4a}, R_{4b} and R_{4c} is selected from



In another aspect, the present invention relates to a cyclic peptide of Formula I, in particular Formula (II) or (III), wherein R_{4a} is in the 4 position and is $-C\equiv N$ and R_{4b} and R_{4c} are each H.

In another aspect, the present invention relates to a cyclic peptide of Formula (I), in particular Formula (II) or (III), wherein R_{4a} is in the 4 position and is -F and R_{4b} and R_{4c} are each H.

The peptides according to the invention are ligands of one or more of the melanocortin receptors, in particular ligands of the MC4-R, more particularly agonists (including full and partial agonists) of the MC4-R. The term "ligands" as used herein include peptides binding to the active site as well as peptides binding to one or more allosteric sites of any one of said receptors.

Thus, the peptides of the invention can be used as a medicament, in particular for the treatment of disorders, diseases, or conditions responsive to modulation of the MC3-R and/or MC4-R, and in particular disorders, diseases, or conditions responsive to the activation of the MC4-R. More particularly, the peptides of the invention are believed to attenuate food intake, body weight and/or body weight gain and are therefore believed to

be useful for treatments of energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity and overweight, and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome, in a patient in need thereof. The patient may be a human or non-human animal, in particular a human.

Peptides of the invention may have advantageous properties compared to peptides of the prior art, in particular enhanced potency and/or enhanced selectivity. These advantages may provide for corresponding useful properties in practice. For example, when used as pharmaceutical agents, peptides of the present invention may be used at a lower daily clinical dose, may have longer duration of action, and/or an improved side effect profile.

In another aspect of the invention, there is provided a method of treating disorders, diseases, or conditions responsive to modulation of the MC4-R and/or MC3-R, such as disorders, diseases, or conditions responsive to activation of the MC4-R, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity and overweight, and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome, by administering a therapeutically effective amount of a peptide of the invention to a patient in need thereof.

According to a further aspect of the invention, there is provided a method of reducing food intake, body weight and/or body weight gain by administering a pharmacologically effective amount of a peptide of the invention to an individual, such as a human, in need thereof.

According to a further aspect of the invention, there is provided a method of preventing body weight regain after weight loss by administering a pharmacologically effective amount of a peptide of the invention to an individual, such as a human, in need thereof.

In a further aspect, the invention provides the use of a peptide of Formula I in the preparation of a medicament for treatment of a disease, disorder and/or condition

responsive to activation of the MC4-R, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity and overweight, and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

In another aspect, the present invention provides a melanocortin receptor-specific peptide-based pharmaceutical composition for use in treatment of melanocortin receptor-mediated diseases, indications, conditions and syndromes, in particular diseases, disorders, conditions and/or syndromes responsive to modulation of MC4-R, such as activation of MC4-R, comprising a peptide of Formula I and a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a peptide-based melanocortin receptor-specific pharmaceutical composition comprising a peptide of Formula I and a pharmaceutically acceptable carrier, wherein the peptide is a selective MC4-R ligand, for use in treatment of energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

In another aspect, the present invention provides peptides which are specific for MC4-R and which are partial or full agonists at MC4-R. In particular, the present invention provides peptides which are specific for MC4-R and which are partial agonists at MC4-R.

In another aspect, the present invention provides a specific MC4-R cyclic peptide that is effective over a significant dose range.

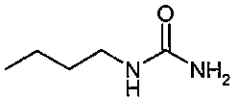
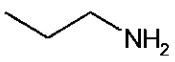
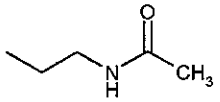
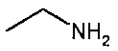
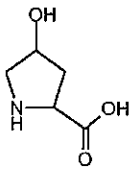
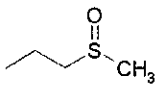
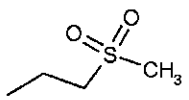
Other aspects and novel features, and the further scope of applicability of the present invention will be set forth in part in the detailed description to follow, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The aspects of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

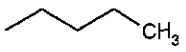
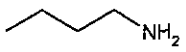
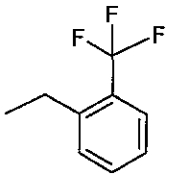
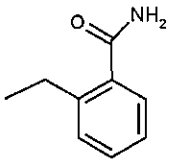
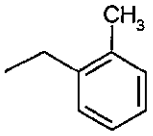
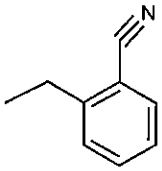
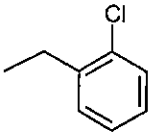
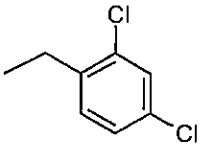
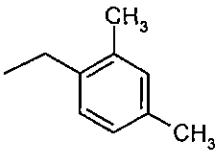
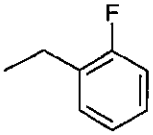
Detailed description of the invention

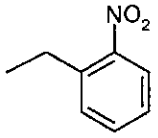
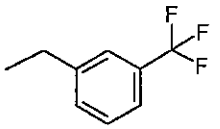
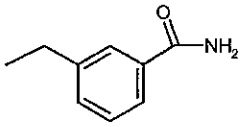
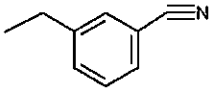
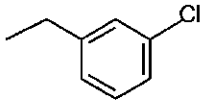
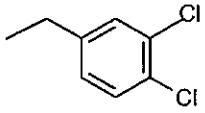
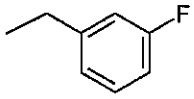
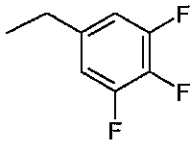
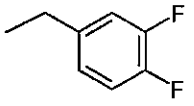
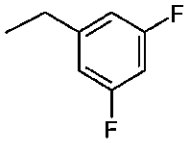
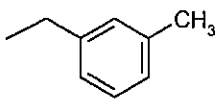
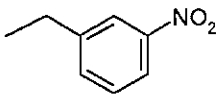
1.0 Definitions.

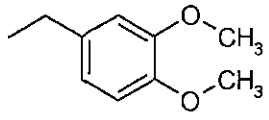
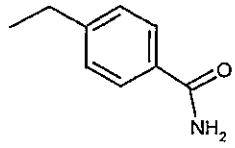
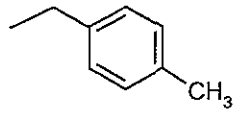
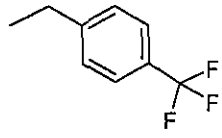
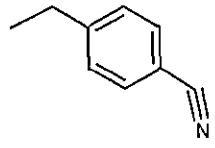
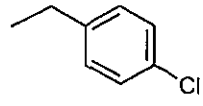
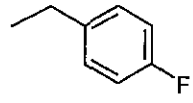
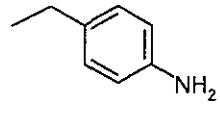
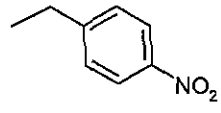
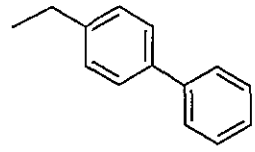
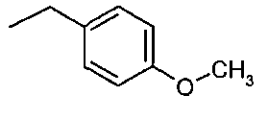
Before proceeding with the description of the invention, certain terms are defined as set forth herein.

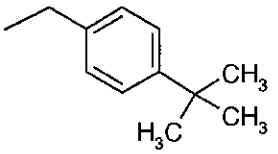
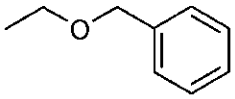
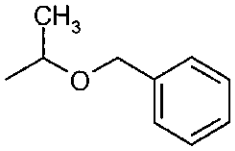
In the sequences given for the peptides according to the present invention, the amino acid residues have their conventional meaning as given in Chapter 2400 of the Manual of Patent Examining Procedure, 8th Ed. Thus, "Ala" is alanine, "Asn" is asparagine, "Asp" is aspartic acid, "Arg" is arginine, "Cys" is cysteine, "Gly" is glycine, "Gln" is glutamine, "Glu" is glutamic acid, "His" is histidine, "Ile" is isoleucine, "Leu" is leucine, "Lys" is lysine, "Met" is methionine, "Phe" is phenylalanine, , "Pro" is proline, "Ser" is serine, "Thr" is Threonine, "Trp" is tryptophan, "Tyr" is tyrosine, and "Val" is valine, and so on. It is to be understood that "D" isomers are designated by a "D-" before the three letter code or amino acid name, such that for example D-Phe is D-phenylalanine. Amino acid residues not encompassed by the foregoing have the following definitions:

Abbreviation	Common Name	Side Chain or Amino Acid Structure
Cit	citrulline	
Dab	diaminobutyric acid	
Dab(Acetyl)	2-amino, 4-acetylaminobutyric acid	
Dap	diaminoproprionic acid	
Hyp	hydroxyproline	
Met(O)	methionine sulfoxide	
Met(O_2)	methionine sulfone	

Abbreviation	Common Name	Side Chain or Amino Acid Structure
Nle	norleucine	
Nva	norvaline	
Orn	ornithine	
Phe(2-CF_3)	2-trifluoromethyl phenylalanine	
Phe(2-C(=O)-NH_2)	2-carbamoyl-phenylalanine	
Phe(2-Me)	2-methyl phenylalanine	
Phe(2-CN)	2-cyano phenylalanine	
Phe(2-Cl)	2-chloro phenylalanine	
Phe(2,4-diCl)	2, 4-dichloro phenylalanine	
Phe(2,4-diMe)	2, 4-dimethyl phenylalanine	
Phe(2-F)	2-flouro phenylalanine	

Abbreviation	Common Name	Side Chain or Amino Acid Structure
Phe(2-NO_2)	2-nitro phenylalanine	
Phe(3-CF_3)	3-triflouromethyl phenylalanine	
Phe(3-C(=O)-NH_2)	3-carbamoyl-phenylalanine	
Phe(3-CN)	3-cyano phenylalanine	
Phe(3-Cl)	3-chloro phenylalanine	
Phe(3,4-diCl)	3,4-dichloro phenylalanine	
Phe(3-F)	3-fluoro phenylalanine	
Phe(3,4,5-triF)	3,4,5-trifluoro phenylalanine	
Phe(3,4-diF)	3,4-difluoro phenylalanine	
Phe(3,5-diF)	3,5-difluoro phenylalanine	
Phe(3-Me)	3-methyl phenylalanine	
Phe(3-NO_2)	3-nitro phenylalanine	

Abbreviation	Common Name	Side Chain or Amino Acid Structure
Phe(3,4-diOMe)	3,4-dimethoxy phenylalanine	
Phe(4-C(=O)-NH_2)	4-carbamoyl-phenylalanine	
Phe(4-Me)	4-methyl phenylalanine	
Phe(4-CF_3)	4-trifluoromethyl phenylalanine	
Phe(4-CN)	4-cyano phenylalanine	
Phe(4-Cl)	4-chloro phenylalanine	
Phe(4-F)	4-fluoro phenylalanine	
Phe(4-NH_2)	4-amino phenylalanine	
Phe(4-NO_2)	4-nitro phenylalanine	
Phe(4-Ph)	4-phenyl phenylalanine	
Phe(4-OMe)	4-methoxy phenylalanine	

Abbreviation	Common Name	Side Chain or Amino Acid Structure
Phe(4-tBu)	4-*tert* butyl phenylalanine	
Ser(Bzl)	O-benzyl-serine	
Thr(OBzl)	O-benzyl-threonine	

The term "acyl" includes a group R(C=O)-, where R is an organic group, such as an alkyl, aryl, heteroaryl, carbocyclyl or heterocyclyl. Thus, when reference is made herein to a substituted acyl group, it means that said organic group (R) is substituted. An example is the acetyl group CH_3-C(=O)-, referred to herein as "Ac". A peptide or aliphatic moiety is "acylated" when an alkyl or substituted alkyl group as defined above is bonded through one or more carbonyl {-(C=O)-} groups. A peptide is most usually acylated at the N-terminus.

The term "alkane" includes linear or branched saturated hydrocarbons. Examples of linear alkane groups include methane, ethane, propane, and the like. Examples of branched or substituted alkane groups include methylbutane or dimethylbutane, methylpentane, dimethylpentane or trimethylpentane, and the like. In general, any alkyl group may be a substitutent of an alkane.

The term "alkene" includes unsaturated hydrocarbons that contain one or more double carbon-carbon bonds. Examples of such alkene groups include ethylene, propene, and the like.

The term "alkenyl" includes a linear monovalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms containing at least one double bond; examples thereof include ethenyl, 2-propenyl, and the like.

The term "alkyl" includes a straight or branched chain saturated aliphatic hydrocarbon group . C_{1-10} alkyl means an alkyl having from 1 to 10 carbon atoms. Non-

limiting examples of such alkyl radicals include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

The term "alkyne" includes a linear monovalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms containing at least one triple bond; examples thereof include ethyne, propyne, butyne, and the like.

The term "aryl" includes a monocyclic or bicyclic aromatic hydrocarbon radical of 6 to 12 ring atoms, and optionally substituted independently with one or more substituents selected from alkyl, haloalkyl, cycloalkyl, alkoxy, alkythio, halo, nitro, acyl, cyano, amino, monosubstituted amino, disubstituted amino, hydroxy, carboxy, or alkoxy-carbonyl. The term "aryl" also includes bicyclic aromatic ring systems wherein one ring is aromatic and one ring is non-aromatic (including saturated or partially saturated rings). In bicyclic aromatic ring systems, two or more ring carbons are common to two adjoining rings (the rings are "fused rings"). Examples of an aryl group include phenyl, biphenyl, indanyl, naphthyl, 1-naphthyl, and 2-naphthyl, derivatives thereof, and the like.

The term "aralkyl" includes a radical – R^aR^b where R^a is an alkylene (a bivalent alkyl) group and R^b is an aryl group as defined above. Examples of aralkyl groups include benzyl, phenylethyl, 3-(3-chlorophenyl)-2-methylpentyl, and the like.

The term "aliphatic" includes compounds with hydrocarbon chains, such as for example alkanes, alkenes, alkynes, and derivatives thereof.

As used herein, the term "amide" includes compounds that have a trivalent nitrogen attached to a carbonyl group, i.e. -C(=O)-NH_2 (i.e. primary amide), –C(=O)-NHR_c and –C(=O)-NR_cR_d, wherein each of R_c and R_d independently represents an organic group. When reference is made herein to a substituted amide group, it means that at least one of said organic groups (R_c and R_d) is substituted. Examples of amides include methylamide, ethylamide, propylamide, and the like.

The term "amine" includes -NH_2 (i.e. an amino group), -NHR_a and -NR_aR_b, wherein each of R_a and R_b independently represents an organic group. When reference is made herein to a substituted amine group, it means that at least one of said organic groups (R_a and R_b) is substituted.

The term "nitrile" includes the functional group -C≡N.

The term "halogen" (or "halo") is intended to include the halogen atoms fluorine, chlorine, bromine and iodine.

The term "alkyl-halo" includes an alkyl substituted with one halogen atom, such as -CH_2F. The term "alkyl-dihalo" includes an alkyl substituted with two halogen atoms, such as –CHF_2. The term "alkyl-trihalo" includes an alkyl substituted with three halogen atoms, such as -CF_3.

The term "alkylthio" includes -S-alkyl wherein alkyl is as defined above. Non-limiting examples of C_1-C_{10} alkylthio include methylthio, ethylthio, n-propylthio, iso-propylthio, and n-butylthio.

The term "nitro" is intended to include –NO_2.

The term "hydroxy" is intended to include –OH.

The term "alkoxy" includes -O-alkyl wherein alkyl is as defined above. C_1-C_{10} alkoxy includes an alkyl having from 1 to 10 carbon atoms. Non-limiting examples of C_1-C_{10} alkoxy include methoxy, ethoxy, *n*-propyloxy, *iso*-propyloxy, and 2-methyl-1-propyloxy.

The term "aryloxy" includes -O-aryl wherein aryl is as defined above.

The term "alkoxycarbonyl" includes –C(=O)-O-R, wherein R is an alkyl as defined above. Non-limiting examples of C_1-C_{10} alkoxycarbonyl include methoxycarbonyl, ethoxycarbonyl, isopropoxy-carbonyl and isopentoxycarbonyl.

The term "carboxy" includes –C(=O)OH.

The term "oxo" includes =O.

The term "sulfonamide" includes a sulfonyl group connected to an amine group, i.e. –$S(=O)_2NH_2$, –$S(=O)_2NHR_a$, and –$S(=O)_2NR_aR_b$, wherein each of R_a and R_b independently represents an organic group. When reference is made herein to a substituted sulfonamide group, it means that at least one of said organic groups (R_a and R_b) is substituted.

The term "composition", as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from

dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions utilized in the present invention encompass any composition made by admixing an active ingredient and one or more pharmaceutically acceptable carriers.

By a melanocortin receptor "agonist" is meant an endogenous substance, drug substance or compound, including a compound such as the peptides of the present invention, which can interact with a melanocortin receptor and initiate a pharmacological response, including but not limited to adenyl cyclase activation, characteristic of the melanocortin receptor.

By "α-MSH" is meant the peptide Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 (SEQ ID NO:4) and analogs and homologs thereof, including without limitation NDP-α-MSH.

By "NDP-α-MSH" is meant the peptide Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 (SEQ ID NO:5) and analogs and homologs thereof.

By "EC_{50}" is meant the molar concentration of an agonist, including a partial agonist, which produced 50% of the maximum possible response for that agonist. By way of example, a test compound which, at a concentration of 72 nM, produces 50% of the maximum possible response for that compound as determined in a cAMP assay in an MC4-R cell expression system has an EC_{50} of 72 nM. Unless otherwise specified, the molar concentration associated with an EC_{50} determination is in nanomoles per liter (nM).

By "Ki (nM)" is meant the equilibrium inhibitor dissociation constant representing the molar concentration of a competing compound that binds to half the binding sites of a receptor at equilibrium in the absence of radioligand or other competitors. In general, the numeric value of the Ki is inversely correlated to the affinity of the compound for the receptor, such that if the Ki is low, the affinity is high. Ki may be determined using the equation of Cheng and Prusoff (Cheng Y., Prusoff W. H., *Biochem. Pharmacol.* 22: 3099-3108, 1973):

Ki may be expressed in terms of specific receptors (e.g., MC1-R, MC3-R, MC4-R or MC5-R) and specific ligands (e.g. α-MSH or NDP-α-MSH).

By "inhibition" is meant the percent attenuation, or decrease in receptor binding, in a competitive inhibition assay compared to a known standard. Thus, by "inhibition at 1 μM (NDP-α-MSH)" is meant the percent decrease in binding of NDP-α-MSH by addition of a determined amount of the compound to be tested, such as 1 μM of a test compound, such as under the assay conditions hereafter described. By way of example, a test compound that does not inhibit binding of NDP-α-MSH has a 0% inhibition, and a test compound that completely inhibits binding of NDP-α-MSH has a 100% inhibition. Typically, as described hereafter, a radio assay is used for competitive inhibition testing, such as with I^{125}-labeled NDP-α-MSH, or a lanthanide chelate fluorescent assay, such as with Eu-NDP-α-MSH. However, other methods of testing competitive inhibition are known, including use of label or tag systems other than radioisotopes, and in general any method known in the art for testing competitive inhibition may be employed in this invention. It may thus be seen that "inhibition" is one measure to determine whether a test compound attenuates binding of α-MSH to melanocortin receptors.

By "binding affinity" is meant the ability of a compound or drug to bind to its biological target, expressed herein as Ki (nM).

By "intrinsic activity" is meant the maximal stimulation (functional activity) achievable by a compound in a specified melanocortin receptor expressing cell system, such as the maximal stimulation of adenylyl cyclase. The maximal stimulation achieved by α-MSH or NDP-α-MSH is designated as an intrinsic activity of 1.0 (or 100%) and a compound capable of stimulating half the maximal activity that of α-MSH or NDP-α-MSH is designated as having an intrinsic activity of 0.5 (or 50%). A compound of this invention that under assay conditions described herein has an intrinsic activity of 0.7 (70%) or higher is classified as an agonist, a compound with intrinsic activity between 0.1 (10%) and 0.7 (70%) is classified as a partial agonist, and a compound with intrinsic activity below 0.1 (10%) is classified as inactive or having no intrinsic activity. In one aspect, the cyclic peptides of the present invention may generally be characterized as a partial agonist at MC4-R with respect to α-MSH or NDP-α-MSH.

In general, "functional activity" is a measure of the signaling of a receptor, or measure of a change in receptor-associated signaling, such as a melanocortin receptor, and in particular MC4-R or hMC4-R, upon activation by a compound. Melanocortin receptors initiate signal transduction through activation of heterotrimeric G proteins. In one aspect,

melanocortin receptors signal through $G\alpha_S$, which catalyzes production of cAMP by adenylyl cyclase. Thus determination of stimulation of adenylyl cyclase, such as determination of maximal stimulation of adenylyl cyclase, is one measure of functional activity, and is the primary measure exemplified herein. However, it is to be understood that alternative measures of functional activity may be employed in the practice of this invention, and are specifically contemplated and included within the scope of this invention. Thus, in one example intracellular free calcium may be measured, such as reported by and using the methods disclosed in Mountjoy K.G. et al., Melanocortin receptor-medicated mobilization of intracellular free calcium in HEK293 cells. *Physiol Genomics* 5:11-19, 2001, or Kassack M.U. et al., Functional screening of G protein-coupled receptors by measuring intracellular calcium with a fluorescence microplate reader. *Biomol Screening* 7:233-246, 2002. It is also possible to measure activation by measurement of the production of inositol triphosphate or diacylglycerol from phosphatidylinositol 4,5-biphosphate, such as by use of radioassays. Yet another measure of functional activity is receptor internalization, resulting from activation of regulatory pathways, such as using the methods disclosed in Nickolls S.A. et al., Functional selectivity of melanocortin 4 receptor peptide and nonpeptide agonists: evidence for ligand specific conformational states. *J Pharm Exper Therapeutics* 313:1281-1288, 2005. Yet another measure of functional activity is the exchange, and exchange rate, of nucleotides associated with activation of a G protein receptor, such as the exchange of GDP (guanosine diphosphate) for GTP (guanosine triphosphase) on the G protein α subunit, which may be measured by any number of means, including a radioassay using guanosine 5′-(γ-[^{35}S]thio)-triphosphate, as disclosed in Manning D.R., Measures of efficacy using G proteins as endpoints: differential engagement of G proteins through single receptors. *Mol Pharmacol* 62:451-452, 2002. Various gene-based assays have been developed for measuring activation of G-coupled proteins, such as those disclosed in Chen W. et al., A colorimetric assay from measuring activation of Gs- and Gq-coupled signaling pathways. *Anal Biochem* 226:349-354, 1995; Kent T.C. et al., Development of a generic dual-reporter gene assay for screening G-protein-coupled receptors. *Biomol Screening*, 5:437-446, 2005; or Kotarsky K. et al., Improved receptor gene assays used to identify ligands acting on orphan seven-transmembrane receptors. *Pharmacology & Toxicology* 93:249-258, 2003. The colorimetric assay of Chen et al. has been adapted for use in measuring

melanocortin receptor activation, as disclosed in Hruby V.J. et al., Cyclic lactam α-melanocortin analogues of Ac-Nle^4-cyclo[Asp^5,D-Phe^7,Lys^{10}] α-melanocyte-stimulating hormone-(4-10)-NH_2 with bulky aromatic amino acids at position 7 shows high antagonist potency and selectivity at specific melanocortin receptors. *J Med Chem* 38:3454-3461, 1995. In general, functional activity may be measured by any method, including methods of determining activation and/or signaling of a G-coupled receptor, and further including methods which may be hereafter developed or reported. The terms "treat," "treating" and "treatment," as used herein, contemplate an action that occurs while a patient is suffering from the specified disease, disorder and/or condition, which reduces the severity of the disease, disorder and/or condition. Moreover, the terms "treat," "treating" and "treatment," as used herein are intended to embrace therapeutic (curative), prophylactic (preventing), controlling and palliative treatment of the indicated diseases, disorders and/or conditions.

As used herein, the term "pharmacologically effective amount" (including "therapeutically effective amount") means an amount of a peptide according to the invention that is sufficient to induce a desired therapeutic or biological effect.

As used herein, the term "therapeutically effective amount" means the amount of a peptide of the invention that will elicit a biological or medical response in the mammal that is being treated by a medical doctor or other clinician.

As used herein, the term "prophylactically effective" or "preventive" means the amount of a peptide of the invention that will prevent or inhibit affliction or mitigate affliction of a mammal with a medical condition that a medical doctor or other clinician is trying to prevent, inhibit, or mitigate before a patient begins to suffer from the specified disease or disorder.

The term "diabetes" includes Type 1 Diabetes, which is insulin-dependent diabetes mellitus as diagnosed according to criteria published in the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (*Diabetes Care*, Vol. 24, Supp. 1, January 2001) whereby fasting plasma glucose level is greater than or equal to 126 milligrams per deciliter and for which the primary cause is pancreatic beta cell destruction, Type 2 Diabetes, which is non-insulin-dependent diabetes mellitus as diagnosed according to criteria published in the Report of the Expert Committee on the

Diagnosis and Classification of Diabetes Mellitus whereby fasting plasma glucose level is greater than or equal to 126 milligrams per deciliter, and latent autoimmune diabetes mellitus of adults (LADA).

The term "metabolic syndrome" refers to metabolic disorders, particularly glucose and lipid regulatory disorders, including insulin resistance and defective secretion of insulin by pancreatic beta cells, and may further include conditions and states such as abdominal obesity, dyslipidemia, hypertension, glucose intolerance or a prothrombitic state, and which may further result in disorders such as hyperlipidemia, obesity, diabetes, insulin resistance, glucose intolerance, hyperglycemia, and hypertension.

2.0 Clinical Indications and Utility.

The compositions and methods disclosed herein can be used for both medical applications and animal husbandry or veterinary applications. Typically, the methods are used in humans, but may also be used in other mammals. The term "patient" is intended to denote a mammalian individual, and is so used throughout the specification and in the claims. The primary applications of the present invention involve human patients, but the present invention may be applied to laboratory, farm, zoo, wildlife, pet, sport or other animals. Clinical indications and specific utilities include the following:

2.1 Obesity and Related Metabolic Syndrome.

Peptides of Formula (I), and in particular Formula (II) or (III), have been found to be ligands of the MC4 receptor. In particular, peptides of Formula (I) are believed to be useful in treating diseases, disorders and/or conditions responsive to modulation of the MC4-R function, more particularly activation of the MC4-R, i.e. diseases, disorders and/or conditions which would benefit from agonism (including full or partial agonism) at the MC4-R, including energy homeostasis and metabolism related (such as diabetes, in particular type 2 diabetes; dyslipidemia; fatty liver; hypercholesterolemia; hypertriglyceridemia; hyperuricacidemia; impaired glucose tolerance; impaired fasting glucos; insulin resistance syndrome; and metabolic syndrome), food intake related (such as hyperphagia; binge eating; bulimia; and compulsive eating) and/or energy balance and body weight related diseases, disorders and/or conditions, more particularly such diseases, disorders and conditions characterized by excess body weight and/or excess food intake.

Peptides of Formula (I), and in particular Formula (II) or (III), are particularly believed to be useful for treatment of body weight related diseases, disorders and/or conditions characterized by excess body weight, including obesity and overweight (by promotion of weight loss, maintenance of weight loss, and/or prevention of weight gain, including medication-induced weight gain or weight gain subsequent to cessation of smoking), and diseases, disorders and/or conditions associated with obesity and/or overweight, such as insulin resistance; impaired glucose tolerance; type 2 diabetes; metabolic syndrome; dyslipidemia (including hyperlipidemia); hypertension; heart disorders (e.g. coronary heart disease, myocardial infarction); cardiovascular disorders; non-alcoholic fatty liver disease (including non-alcoholic steatohepatitis); joint disorders (including secondary osteoarthritis); gastroesophageal reflux; sleep apnea; atherosclerosis; stroke; macro and micro vascular diseases; steatosis (e.g. in the liver); gall stones; and gallbladder disorders.

Peptides of Formula (I), and in particular Formula (II) or (III), are particularly believed to be useful for treatment of obesity and type 2 diabetes, more specifically obesity.

It will be understood that there are medically accepted definitions of obesity and overweight. A patient may be identified by, for example, measuring body mass index (BMI), which is calculated by dividing weight in kilograms by height in meters squared, and comparing the result with the definitions. The recommended classifications for BMI in humans, adopted by the Expert Panel on the Identification, Evaluation and Treatment of Overweight and Obesity in Adults, and endorsed by leading organizations of health professionals, are as follows: underweight < 18.5 kg/m^2, normal weight 18.5-24.9 kg/m^2, overweight 25-29.9 kg/m_2, obesity (class 1) 30-34.9 kg/m^2, obesity (class 2) 35-39.9 kg/m^2, extreme obesity (class 3) $\geq$ 40 kg/m^2 (Practical Guide to the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, The North American Association for the Study of Obesity (NAASO) and the National Heart, Lung and Blood Institute (NHLBI) 2000). Modifications of this classification may be used for specific ethnic groups and for children. Another alternative for assessing overweight and obesity is by measuring waist circumference. There are several proposed classifications and differences in the cutoffs based on ethnic group. For instance, according to the

classification from the International Diabetes Federation, men having waist circumferences above 94 cm (cut off for europids) and women having waist circumferences above 80 cm (cut off for europids) are at higher risk of diabetes, dyslipidemia, hypertension and cardiovascular diseases because of excess abdominal fat. Another classification is based on the recommendation from the Adult Treatment Panel III where the recommended cut-offs are 102 cm for men and 88 cm for women. However, the peptides of Formula (I) may also be used for reduction of self-diagnosed overweight and for decreasing the risk of becoming obese due to life style, genetic considerations, heredity and/or other factors.

It is believed that peptides of Formula (I), and in particular Formula (II) or (III), upon administration to an animal, including man, will reduce food intake, body weight and/or body weight gain in that animal.

Without being bound by any theory, it is believed that peptides of Formula (I), and in particular Formula (II) or (III), act by modulating appetite and/or satiety, increasing metabolic rate, reducing intake of and/or craving for fat and/or carbohydrates.

Without being bound by any theory, it is also believed that peptides of Formula (I), and in particular Formula (II) or (III), act by enhancing glucose tolerance and/or decreasing insulin resistance. It is therefore believed that peptides of Formula (I) can be useful also for treatment of type 2 diabetes in underweight and normal weight individuals as well as in overweight and obese individuals.

Peptides of the invention might also be useful for (i) prevention of organ or tissue damage caused by hypoperfusion due to vessel occlusion (e.g. caused by thrombosis), haemorrhage, trauma, surgery, haemorrhagic shock, cardiogenic shock, toxic shock or septic shock or (iii) treatment of male and female sexual dysfunctions, such as male erectile dysfunction or female sexual arousal dysfunction.

According to a further aspect of the invention, there is provided a peptide of Formula (I), and in particular Formula (II) or (III), as previously defined for use as a medicament.

In another aspect, the invention provides the use of a peptide of Formula (I), and in particular Formula (II) or (III), for treatment of diseases, disorders and/or conditions responsive to modulation of the MC4-R, such as diseases, disorders and/or conditions responsive to activation of the MC4-R, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related

diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

In a further aspect, the invention provides the use of a peptide of Formula (I), and in particular Formula (II) or (III), in the preparation of a medicament for treatment of diseases, disorders and/or conditions responsive to modulation of the MC4-R, such as activation of the MC4-R, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

Peptides of the invention are advantageously more selective (i.e. higher affinity and/or higher specificity) for MC4-R and/or MC3-R than for MC1-R, MC2-R and MC5-R, in particular MC1-R. In particular, peptides of the invention are advantageously more selective for MC4-R than for any of MC3-R and MC1-R. Peptides of the invention are suitably at least 3-fold, in particular 10-fold, more particularly 30-fold, more selective for MC4-R than for any of MC1-R, MC2-R, MC3-R and MC5-R. Some peptides of the invention are even more than 100-fold, such as even about 150-fold, more selective for MC4-R than MC1-R as determined in the receptor binding assay described in 7.1. It is noted that the selectivity profile may affect in vivo safety and side effects obtained upon administration of the peptides.

Upon treatment of energy homeostasis and metabolism related, food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, it is desirable to reduce or eliminate unwanted side-effects that may result from MC4-R activation, such as sexual side-effects, including penile erection, and blood pressure effects.

Peptides of the invention are believed to be useful for treatment of energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome, without causing substantial adverse cardiovascular effects, including a substantial increase in blood pressure.

Peptides of the invention are believed to be useful for treatment of energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome, without causing substantial unwanted sexual effects resulting from MC4-R activation, such as penile erection.

It is believed that peptides of Formula I, and in particular Formula (II) or (III), possess a satisfactory pharmacological profile and promising biopharmaceutical properties, such as toxicological profile, metabolism and pharmacokinetic properties, solubility, and permeability. It will be understood that determination of appropriate biopharmaceutical properties is within the knowledge of a person skilled in the art.

3.0 Combination Therapy for Certain Indications.

The peptides, compositions and methods of the present invention may be used for treatment of any of the foregoing diseases, indications, conditions or syndromes, or any disease, indication, condition or syndrome which is melanocortin receptor mediated, by administration in combination with one or more other pharmaceutically active compounds. Such combination administration may be by means of a single dosage form which includes both a peptide of the present invention and one more other pharmaceutically active compound, such single dosage form including a tablet, capsule, spray, inhalation powder, injectable liquid or the like. Alternatively, combination administration may be by means of administration of two different dosage forms, with one dosage form containing a peptide of the present invention, and the other dosage form including another pharmaceutically active compound. In this instance, the dosage forms may be the same or different. Without meaning to limit combination therapies, the following exemplifies certain combination therapies which may be employed.

3.1 Combination Therapy for Obesity and Related Metabolic Syndrome.

One or more peptides of the invention may be combined with one or more other pharmacologically active agent(s) that is (are) useful in the treatment of various weight and feeding-related disorders, such as obesity and/or overweight, in particular other anti-obesity drugs that affect energy expenditure, glycolysis, gluconeogenesis, glucogenolysis, lipolysis, lipogenesis, fat absorption, fat storage, fat excretion, hunger and/or satiety and/or craving mechanisms, appetite/motivation, food intake, or gastrointestinal motility. Drugs

that reduce energy intake include, in part, various pharmacological agents, referred to as anorectic drugs, which are used as adjuncts to behavioral therapy in weight reduction programs.

Generally, a total dosage of the below-described obesity control agents or medications, when used in combination with one or more peptides of the present invention can range from 0.1 to 3,000 mg/day, preferably from about 1 to 1,000 mg/day and more preferably from about 1 to 200 mg/day in single or 2-4 divided doses. The exact dose, however, is determined by the attending clinician and is dependent on such factors as the potency of the compound administered, the age, weight, condition and response of the patient.

One or more peptides of the invention may be combined with one or more other pharmacologically active agent(s) that is (are) useful in the treatment of diabetes, such as other anti-diabetic drugs.

One or more peptides of the invention may in addition or alternatively further be combined with one or more other pharmacologically active agent(s) that is (are) useful in the treatment of diseaeses, disorders and/or conditions associated with obesity and/or overweight, such as insulin resistance; impaired glucose tolerance; type 2 diabetes; metabolic syndrome; dyslipidemia (including hyperlipidemia); hypertension; heart disorders (e.g. coronary heart disease, myocardial infarction); cardiovascular disorders; non-alcoholic fatty liver disease (including non-alcoholic steatohepatitis); joint disorders (including secondary osteoarthritis); gastroesophageal reflux; sleep apnea; atherosclerosis; stroke; macro and micro vascular diseases; steatosis (e.g. in the liver); gall stones; and gallbladder disorders.

According to a further aspect of the invention there is provided a combination treatment comprising the administration of a pharmacologically effective amount of a peptide according to the invention, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable diluent or carrier, with the simultaneous, sequential or separate administration one or more of the following agents selected from:

- insulin and insulin analogues;

- insulin secretagogues, including sulphonylureas (e.g. glipizide) and prandial glucose regulators (sometimes called "short-acting secretagogues"), such as meglitinides (e.g. repaglinide and nateglinide);
- agents that improve incretin action, for example dipeptidyl peptidase IV (DPP-4) inhibitors (e.g. vildagliptin, saxagliptin, and sitagliptin), and glucagon-like peptide-1 (GLP-1) agonists (e.g. exenatide);
- insulin sensitising agents including peroxisome proliferator activated receptor gamma (PPARγ) agonists, such as thiazolidinediones (e.g. pioglitazone and rosiglitazone), and agents with any combination of PPAR alpha, gamma and delta activity;
- agents that modulate hepatic glucose balance, for example biguanides (e.g. metformin), fructose 1,6-bisphosphatase inhibitors, glycogen phopsphorylase inhibitors, glycogen synthase kinase inhibitors, and glucokinase activators;
- agents designed to reduce/slow the absorption of glucose from the intestine, such as alpha-glucosidase inhibitors (e.g. miglitol and acarbose);
- agents which antagonise the actions of or reduce secretion of glucagon, such as amylin analogues (e.g. pramlintide);
- agents that prevent the reabsorption of glucose by the kidney, such as sodium-dependent glucose transporter 2 (SGLT-2) inhibitors (e.g. dapagliflozin);
- agents designed to treat the complications of prolonged hyperglycaemia, such as aldose reductase inhibitors (e.g. epalrestat and ranirestat); and agents used to treat complications related to micro-angiopathies;
- anti-dyslipidemia agents, such as HMG-CoA reductase inhibitors (statins, e.g. rosuvastatin) and other cholesterol-lowering agents; PPARα agonists (fibrates, e.g. gemfibrozil and fenofibrate); bile acid sequestrants (e.g.cholestyramine); cholesterol absorption inhibitors (e.g. plant sterols (i.e. phytosterols), synthetic inhibitors); cholesteryl ester transfer protein (CETP) inhibitors; inhibitors of the ileal bile acid transport system (IBAT inhibitors); bile acid binding resins; nicotinic acid (niacin) and analogues thereof; anti-oxidants, such as probucol; and omega-3 fatty acids;

- antihypertensive agents, including adrenergic receptor antagonists, such as beta blockers (e.g. atenolol), alpha blockers (e.g. doxazosin), and mixed alpha/beta blockers (e.g. labetalol); adrenergic receptor agonists, including alpha-2 agonists (e.g. clonidine); angiotensin converting enzyme (ACE) inhibitors (e.g. lisinopril), calcium channel blockers, such as dihydropyridines (e.g. nifedipine), phenylalkylamines (e.g. verapamil), and benzothiazepines (e.g. diltiazem); angiotensin II receptor antagonists (e.g. candesartan); aldosterone receptor antagonists (e.g. eplerenone); centrally acting adrenergic drugs, such as central alpha agonists (e.g. clonidine); and diuretic agents (e.g. furosemide);
- haemostasis modulators, including antithrombotics, such as activators of fibrinolysis; thrombin antagonists; factor VIIa inhibitors; anticoagulants, such as vitamin K antagonists (e.g. warfarin), heparin and low molecular weight analogues thereof, factor Xa inhibitors, and direct thrombin inhibitors (e.g. argatroban); antiplatelet agents, such as cyclooxygenase inhibitors (e.g. aspirin), adenosine diphosphate (ADP) receptor inhibitors (e.g. clopidogrel), phosphodiesterase inhibitors (e.g. cilostazol), glycoprotein IIB/IIA inhibitors (e.g. tirofiban), and adenosine reuptake inhibitors (e.g. dipyridamole);
- anti-obesity agents, such as appetite suppressant (e.g. ephedrine), including noradrenergic agents (e.g. phentermine) and serotonergic agents (e.g. sibutramine), pancreatic lipase inhibitors (e.g. orlistat), microsomal transfer protein (MTP) modulators, diacyl glycerolacyltransferase (DGAT) inhibitors, and cannabinoid (CB1) receptor antagonists (e.g. rimonabant);
- feeding behavior modifying agents, such as orexin receptor modulators and melanin-concentrating hormone (MCH) modulators;
- glucagon like peptide-1 (GLP-1) receptor modulators;
- neuropeptideY (NPY)/NPY receptor modulators;
- pyruvate dehydrogenase kinase (PDK) modulators;
- serotonin receptor modulators;
- leptin/leptin receptor modulators;
- ghrelin/ghrelin receptor modulators; or

- monoamine transmission-modulating agents, such as selective serotonin reuptake inhibitors (SSRI) (e.g. fluoxetine), noradrenaline reuptake inhibitors (NARI), noradrenaline-serotonin reuptake inhibitors (SNRI), triple monoamine reuptake blockers (e.g. tesofensine), and monoamine oxidase inhibitors (MAOI) (e.g. toloxatone and amiflamine),

or a pharmaceutically acceptable salt, solvate, solvate of such a salt or a prodrug thereof, optionally together with a pharmaceutically acceptable carrier to a mammal, such as man, in need of such therapeutic treatment.

According to an additional further aspect of the present invention there is provided a combination treatment comprising the administration of a pharmacologically effective amount of a compound according to the invention, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, with the simultaneous, sequential or separate administration of very low calorie diets (VLCD) or low-calorie diets (LCD).

4.0 Methods of Making.

In general, the peptides of the present invention may be synthesized by solid-phase synthesis and purified according to methods known in the art. Any of a number of well-known procedures utilizing a variety of resins and reagents may be used to prepare the peptides of the present invention.

The cyclic peptides of the present invention may be readily synthesized by known conventional procedures 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. In a preferred conventional procedure, the cyclic peptides of the present invention may be synthesized by solid-phase synthesis and purified according to methods known in the art. Any of a number of well-known procedures utilizing a variety of resins and reagents may be used to prepare the peptides of the present invention.

The process for synthesizing the cyclic peptides may be carried out by a procedure whereby each amino acid in the desired sequence is added one at a time in succession to another amino acid or residue thereof or by a procedure whereby peptide fragments with the desired amino acid sequence are first synthesized conventionally and then condensed to provide the desired peptide. The resulting peptide is then cyclized to yield a cyclic peptide of the invention.

Solid phase peptide synthesis methods are well known and practiced in the art. In such methods the synthesis of peptides of the invention 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. These methods are disclosed in numerous references, including Merrifield, R.B., Solid phase synthesis (Nobel lecture). *Angew Chem* 24:799-810 (1985) and Barany et al., The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross, E. and Meienhofer, J., Eds. Academic Press 1-284 (1980).

In chemical syntheses of peptides, reactive side chain groups of the various amino acid residues are protected with suitable protecting groups, which prevent a chemical reaction from occurring at that site until the protecting group is removed. Also common is the protection of the alpha amino group of an amino acid residue or fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha amino protecting group to allow a subsequent reaction to take place at that site. Specific protecting groups have been disclosed and are known in solid phase synthesis methods and solution phase synthesis methods.

Alpha amino groups may be protected by a suitable protecting group, including a urethane-type protecting group, such as benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-biphenyl-isopropoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc) and p-methoxybenzyloxycarbonyl (Moz) and aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropoxycarbonyl, and allyloxycarbonyl (Alloc). Fmoc are preferred for alpha amino protection.

Guanidino groups may be protected by a suitable protecting group, such as nitro, p-toluenesulfonyl (Tos), Z, pentamethylchromanesulfonyl (Pmc), adamantyloxycarbonyl,

pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and Boc. Pbf and Pmc are preferred protecting groups for Arg.

The peptides of the invention described herein were prepared using solid phase synthesis, such as by means of a Symphony Multiplex Peptide Synthesizer (Rainin Instrument Company/Protein Technologies Inc) automated peptide synthesizer, using programming modules as provided by the manufacturer and following the protocols set forth in the manufacturer's manual.

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 starting material is prepared by attaching an alpha amino-protected amino acid by an amide linkage to a 4-(2', 4'-dimethoxylphenyl-aminomethyl-phenoxy (Rink Amide) resin, a 4-(2', 4'-dimethoxylphenyl-aminomethyl)-phenoxyacetamido-norleucyl-MBHA resin, an amino-xanthen-3-yloxy-merifiel resin (Sieber Amide) resin, or by an ester linkage to a p-benzyloxybenzyl alcohol (Wang) resin, a 2-chlorotrityl chloride resin or by other means well known in the art. Fmoc-Linker-BHA resin supports are commercially available and generally used when feasible. The resins are carried through repetitive cycles as necessary to add amino acids sequentially. The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, piperazine, diethylamine, or morpholine (20-40% v/v) in N,N-dimethylformamide (DMF) may be used for this purpose.

Following 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. After the peptide is synthesized, if desired, the orthogonally protected side chain protecting groups may be removed using methods well known in the art for further derivatization of the peptide.

Typically, orthogonal protecting groups are used as appropriate. For example, the peptides of the invention contain multiple amino acids with an amino group-containing side chain. In one aspect, an Allyl-Alloc protection scheme is employed with the amino acids forming a lactam bridge through their side chains, and orthogonal protecting groups, cleavable under different reactive conditions, use for other amino acids with amino group-containing side chains. Thus, for example, Fmoc-Orn(Alloc)-OH and Fmoc-Glu(OAll)-OH amino acids (Glu(OAll) refers to glutamic acid 5-allyl ester) can be employed for the

positions forming a lactam bridge upon cyclization, while other amino acids with amino group-containing side chains have a different and orthogonal protecting group, such as with Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Pbf)-OH, Fmoc-Dab(Pbf)-OH or the like. Other protecting groups may be similarly employed; by way of example and not limitation, Mtt/OPp (4-methyltrityl/ 2-phenylisopropyl) can be employed with the side chains forming a lactam bridge upon cyclization, with orthogonal protecting groups being utilized for other positions that are not cleavable using conditions suitable for cleavage of Mtt/OPp.

Reactive groups in a peptide can be selectively modified, either during solid phase synthesis or after removal from the resin. For example, peptides can be modified to obtain N-terminus modifications, such as acetylation, while on resin, or may be removed from the resin by use of a cleaving reagent and then modified. Similarly, methods for modifying side chains of amino acids are well known to those skilled in the art of peptide synthesis. The choice of modifications made to reactive groups present on the peptide will be determined, in part, by the characteristics that are desired in the peptide.

In the peptides of the present invention, in one embodiment the N-terminus group is modified by introduction of an N-acetyl group. In one aspect, a method is employed wherein after removal of the protecting group at the N-terminal, the resin-bound peptide is reacted with acetic anhydride in DMF in the presence of an organic base, such aspyridine. Other methods of N-terminus acetylation are known in the art, including solution phase acetylation, and may be employed.

The peptide can, in one embodiment, be cyclized prior to cleavage from the peptide resin. For cyclization through reactive side chain moieties, the desired side chains are deprotected, and the peptide suspended in a suitable solvent and a cyclic coupling agent added. Suitable solvents include, for example DMF, dichloromethane (DCM) or 1-methyl-2-pyrrolidone (NMP). Suitable cyclic coupling reagents include, for example, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate (BOP), benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphoniumhexafluorophosphate (PyBOP), 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TATU), 2-(2-oxo-1(2H)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU) or N,N'-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCCI/HOBt). Coupling is

conventionally initiated by use of a suitable base, such as N,N-diisopropylethylamine (DIPEA), sym-collidine or N-methylmorpholine (NMM).

The cyclized peptides can then be cleaved from solid phase, using any suitable reagent, such as ethylamine in DCM or various combinations of agents, such as trifluoroacetic acid (TFA), tri-isopropylsilane (TIS), dimethoxybenezene (DMB), water and the like. The resulting crude peptide is dried and remaining amino acid side chain protecting groups, if any, are cleaved using any suitable reagent, such as TFA in the presence of water, TIS, 2-mercaptopethane (ME), and/or 1,2-ethanedithiol (EDT). The final product is precipitated by adding cold ether and collected by filtration. Final purification is by reverse phase high performance liquid chromatography (RP-HPLC), using a suitable column, such as a C_{18} column, or other methods of separation or purification, such as methods based on the size or charge of the peptide, can also be employed. Once purified, the peptide can be characterized by any number of methods, such as high performance liquid chromatography (HPLC), amino acid analysis, mass spectrometry, and the like.

For peptides of the present invention which have a C-terminus substituted amide derivative or N-alkyl group, synthesis may proceed by solid phase synthesis commenced from the C-terminal end of the peptide by coupling a protected alpha amino acid to a suitable resin. Such methods for preparing substituted amide derivatives on solid-phase have been described in the art. See, for example, Barn D.R. et al., Synthesis of an array of amides by aluminum chloride assisted cleavage on resin bound esters. *Tetrahedron Letters*, 37:3213-3216 (1996); DeGrado W.F. and Kaiser E.T., Solid-phase synthesis of protected peptides on a polymer bound oxime: Preparation of segments comprising the sequences of a cytotoxic 26-peptide analogue. *J. Org. Chem.*, 47:3258-3261 (1982). 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 an by amide linkage to a 4-(2', 4'-dimethoxylphenyl-aminomethyl-phenoxy (Rink Amide) resin by well known means. The peptide chain is grown with the desired sequence of amino acids. Before cleavage, the peptide is cyclized on the solid phase. Peptides employing a p-benzyloxybenzyl alcohol (Wang) resin may be cleaved from resin by aluminum chloride in DCM, and peptides employing a Rink Amide resin may be cleaved by mixture of TFA, TIS and water.

While synthesis has been described primarily with reference to solid phase Fmoc chemistry, it is to be understood that other chemistries and synthetic methods may be employed to make the cyclic peptides of the invention, such as by way of example and not limitation, methods employing Boc chemistry, solution chemistry, and other chemistries and synthetic methods.

5.1 Salt Form of Cyclic Peptides of the Present Invention.

It shall be understood that as used herein all references to peptides according to the invention, including a specific chemical formula or name, are intended to include all pharmaceutically acceptable salts, solvates, hydrates, polymorphs, prodrugs, metabolites, stereoisomers, and tautomeric isomers thereof.

Cyclic peptides of the present invention may be in the form of any pharmaceutically acceptable salt. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids (see "Handbook of Pharmaceutical Salts: Properties, Selection and Use", P. H. Stahl, P. G. Wermuth, IUPAC, Wiley-VCH, 2002). Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, lithium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like. When cyclic peptide of the present invention is basic, acid addition salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, carboxylic, citric, ethanesulfonic, formic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, malonic, mucic, nitric, pamoic, pantothenic, phosphoric, propionic, succinic, sulfuric,

tartaric, p-toluenesulfonic acid, trifluoroacetic acid, and the like. Acid addition salts of peptides of the present invention are prepared in a suitable solvent for the peptide and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic (TFA), citric, tartaric, maleic, succinic or methanesulfonic acid. The acetate, ammonium acetate and TFA acid salt forms are especially useful.

Where peptides of the present invention include an acidic moiety, suitable pharmaceutically acceptable salts may include alkali metal salts, such as sodium or potassium salts, or alkaline earth metal salts, such as calcium or magnesium salts.It is also to be understood that certain peptides of the Formula (I) can exist in solvated forms, including solvates of the free peptide or solvates of a salt of the compound, as well as unsolvated forms. The term "solvate" is used herein to describe a molecular complex comprising the compound of the invention and one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term "hydrate" is employed when said solvent is water.It is to be understood that all polymorphs, including mixtures of different polymorphs, are included within the scope of the claimed peptides.

5.2 Pharmaceutical Compositions.

The invention provides a pharmaceutical composition that includes one or more cyclic peptides of the present invention and a pharmaceutically acceptable carrier. When formulated with a pharmaceutically acceptable carrier, the compound of the invention may be present in the pharmaceutical composition in a concentration from 0.1 to 99.5%, such as from 0.5 to 95%, by weight of the total composition. The choice of carrier is within the knowledge of a person skilled in the art and depends on, for instance, the mode of administration, the dosage form, and the physical properties of the active compound, such as solubility and stability. The term "carrier" as used herein relates to a therapeutically inactive ingredient. The dosage form may be a solid, semi-solid or liquid system. The formulation may be an immediate and/or modified release, including delayed-, sustained-, pulsed-, controlled-, targeted and programmed release formulation.

The carrier may be a liquid formulation, and is preferably a buffered, isotonic, aqueous solution. Pharmaceutically acceptable carriers also include excipients, such as diluents, carriers and the like, and additives, such as stabilizing agents, preservatives, solubilizing agents, buffers and the like, as hereafter described.

The cyclic peptide compositions of the present invention may be formulated or compounded into pharmaceutical compositions that include at least one cyclic peptide of the present invention together with one or more pharmaceutically acceptable carriers, including excipients, such as diluents, carriers and the like, and additives, such as stabilizing agents, preservatives, solubilizing agents, buffers and the like, as may be desired. Formulation excipients may include polyvinylpyrrolidone, gelatin, hydroxy propyl cellulose (HPC), acacia, polyethylene glycol, mannitol, sodium chloride and sodium citrate. For injection or other liquid administration formulations, water containing at least one or more buffering constituents is preferred, and stabilizing agents, preservatives and solubilizing agents may also be employed. For solid administration formulations, any of a variety of thickening, filler, bulking and carrier additives may be employed, such as starches, sugars, cellulose derivatives, fatty acids and the like. For topical administration formulations, any of a variety of creams, ointments, gels, lotions and the like may be employed. For most pharmaceutical formulations, non-active ingredients will constitute the greater part, by weight or volume, of the preparation. For pharmaceutical formulations, it is also contemplated that any of a variety of measured-release, slow-release or sustained-release formulations and additives may be employed, so that the dosage may be formulated so as to provide delivery of a peptide of the present invention over a period of time.

In general, the actual quantity of cyclic peptides of the present invention administered to a patient will vary between fairly wide ranges depending on the mode of administration, the formulation used, and the response desired.

In practical use, the cyclic peptides of the invention can be combined as the active ingredient in an admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, for example, oral, parenteral (including intravenous), urethral, vaginal, nasal, buccal, sublingual, or the like. In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating

agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets.

Because of their ease of administration, tablets and capsules represent an advantageous oral dosage unit form. If desired, tablets may be coated by standard aqueous or non-aqueous techniques. The amount of active peptide in such therapeutically useful compositions is such that an effective dosage will be obtained. In another dosage unit form, sublingual constructs may be employed, such as sheets, wafers, tablets or the like.

The tablets, pills, capsules, and the like may also contain a binder such as povidone, gum tragacanth, acacia, corn starch or gelatin; diluents; fillers such as microcrystalline cellulose; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch or alginic acid; preservatives; colorants; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as fatty oil. Various other materials may be utilized as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

If formulated for oral delivery, the peptide is preferably formulated and made such that it is encased in an enteric protectant, more preferably such that it is not released until the tablet or capsule has transited the stomach, and optionally has further transited a portion of the small intestine. In the context of this application it will be understood that the term enteric coating or material refers to a coating or material that will pass through the stomach essentially intact but will disintegrate after passing through the stomach to release the active drug substance. Materials that may be used includes cellulose acetate phthalate, hydroxypropylmethyl-ethylcellulose succinate, hydroxypropylmethylcellulose phthalate, polyvinyl acetate phthalate, and methacrylic acid-methyl methacrylate copolymer. The enteric coating employed promotes dissolution of the dosage form primarily at a site outside the stomach, and may be selected such that the enteric coating dissolves at a pH of approximately at least 5.5, more preferable at a pH of from about 6.0 to about 8.0.

Any of a variety of permeation enhancers may be employed, to increase uptake in the intestines upon dissolution of the enteric coating. In one aspect, permeation enhancers

increase either paracellular or transcellular transport systems. Representative, non-limiting examples of such permeation enhancers include calcium chelators, bile salts (such as sodium cholate), and fatty acids. In some embodiments, peptides or polypeptides that act as substrates for intestinal proteases are further added.Cyclic peptides may also be administered parenterally. Solutions or suspensions of these active peptides may for instance be prepared in water mixed with for instance hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. These preparations may optionally contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that it may be administered by syringe. The form must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a polyol, for example glycerol, propylene glycol or liquid polyethylene glycol, suitable mixtures thereof, and vegetable oils.

The cyclic peptides of the present invention may be therapeutically applied by means of nasal administration. By "nasal administration" is meant any form of intranasal administration of any of the cyclic peptides of the present invention. The peptides may be in an aqueous solution, such as a solution including saline, citrate or other common excipients or preservatives. The peptides may also be in a dry or powder formulation.

If in an aqueous solution, the cyclic peptides may be appropriately buffered by means of saline, acetate, phosphate, citrate, acetate or other buffering agents, which may be at any physiologically acceptable pH, such as from about pH 4 to about pH 7. A combination of buffering agents may also be employed, such as phosphate buffered saline, a saline and acetate buffer, and the like. In the case of saline, a 0.9% saline solution may be employed. In the case of acetate, phosphate, citrate, and the like, a 50 mM solution may be employed. In addition to buffering agents, a suitable preservative may be employed, to prevent or limit bacteria and other microbial growth. One such preservative that may be employed is 0.05% benzalkonium chloride.

In an alternative embodiment, cyclic peptides of the present invention may be administered directly into the lung. Intrapulmonary administration may be performed by means of a metered dose inhaler, a device allowing self-administration of a metered bolus of a peptide of the present invention when actuated by a patient during inspiration. Any of a variety of different techniques may be used to make dry powder microparticles, including but not limited to micro-milling, spray drying and a quick freeze aerosol followed by lyophilization.

The cyclic peptides of the present invention may be therapeutically administered by means of an injection of a sustained release formulation. In general, any of a number of injectable and bioerodible polymers may be employed in a sustained release injectable formulation. Alternatively other sustained release formulations may be employed, including formulations permitting subcutaneous injection, which other formulations may include one or more of nano/microspheres, liposomes, emulsions (such as water-in-oil emulsions), gels, insoluble salts or suspensions in oil The formulation may be such that an injection is required on a daily, weekly, monthly or other periodic basis, depending on the concentration and amount of cyclic peptide, the sustained release rate of the materials employed, and other factors known to those of skill in the art.

5.3 Routes of Administration.

If a composition including one or more peptides of the present invention is administered by injection, the injection may be intravenous, subcutaneous, intramuscular, intraperitoneal or other means known in the art.

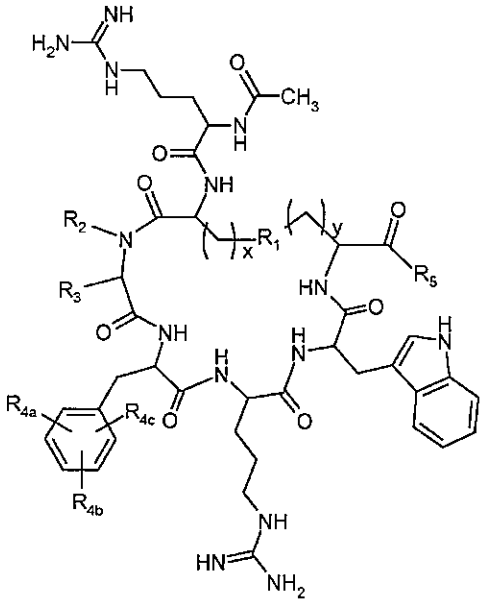
The peptides of the present invention may be formulated by any means known in the art, including but not limited to formulation as tablets, capsules, caplets, suspensions, powders, lyophilized preparations, suppositories, ocular drops, skin patches, oral soluble formulations, sprays, aerosols and the like, and may be mixed and formulated with buffers, binders, excipients, stabilizers, anti-oxidants and other agents known in the art. In general, any route of administration by which the peptides of invention are introduced across an epidermal layer of cells may be employed. Administration means may thus include administration through mucous membranes, buccal administration, oral administration, dermal administration, inhalation administration, nasal administration, urethral administration, vaginal administration, and the like.

5.4 Therapeutically Effective Amount.

In general, the actual quantity of cyclic peptide of the present invention administered to a patient will vary between fairly wide ranges depending upon the mode of administration, the patient (including weight, sex, health condition and diet), the formulation used, and the response desired. The dosage for treatment is administration, by any of the foregoing means or any other means known in the art, of an amount sufficient to bring about the desired therapeutic effect. The cyclic peptides of the present invention are highly active. For example, the cyclic peptide can be administered (as a single dose or in divided daily doses) at about 0.1, 0.5, 1, 5, 50, 100, 500, 1000 or 5000 μg/kg body weight, depending on the specific peptide selected, the desired therapeutic response, the route of administration, the formulation and other factors known to those of skill in the art.

6.0 Peptides of the Present Invention.

In one aspect, the present invention relates to a cyclic peptide of the structural Formula (I):



(I)

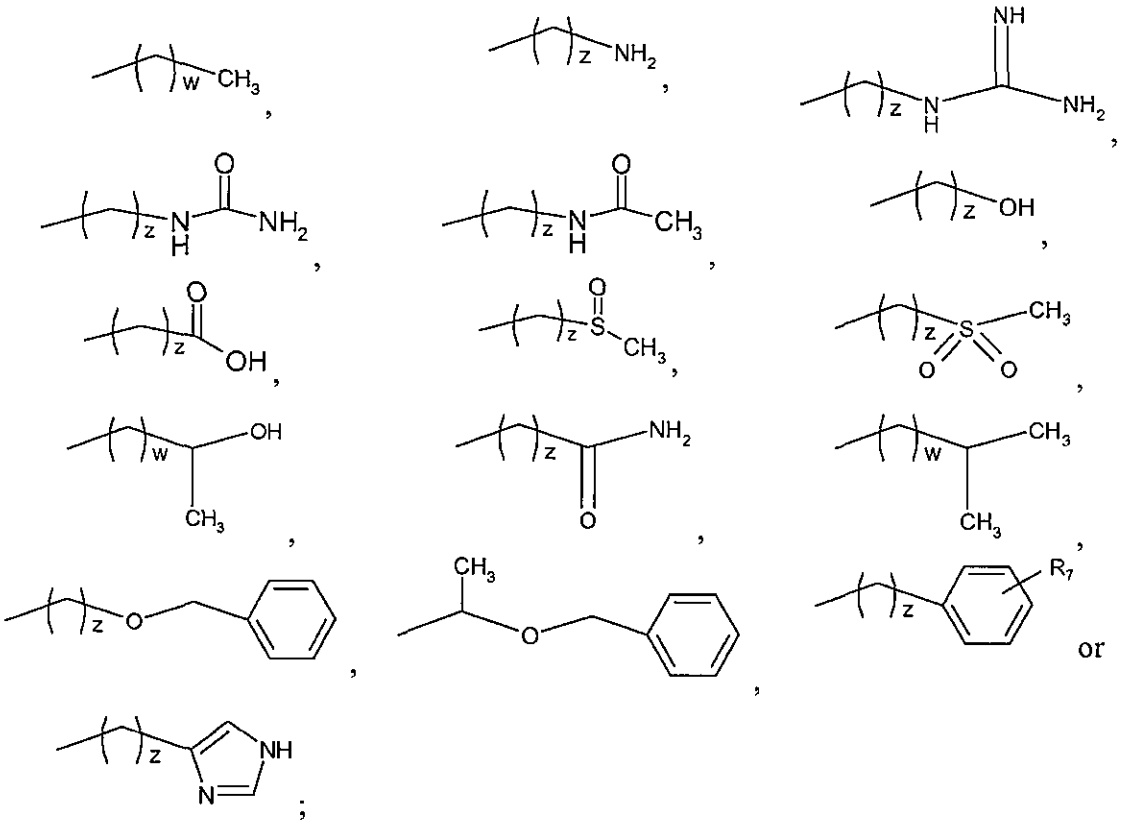
including all enantiomers, stereoisomers or diastereoisomers thereof, or a pharmaceutically acceptable salt of any of the foregoing,

wherein:

R_1 is -NH-C(=O)- or -C(=O)-NH-;

R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH;

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



or

;

R_{4a}, R_{4b} and R_{4c} are each independently selected from hydrogen, halo, (C_1-C_{10})alkyl-halo, (C_1-C_{10})alkyl-dihalo, (C_1-C_{10})alkyl-trihalo, (C_1-C_{10})alkyl, (C_1-C_{10})alkoxy, (C_1-C_{10})alkylthio, aryl, aryloxy, nitro, nitrile, sulfonamide, amino, monosubstituted amino, disubstituted amino, hydroxy, carboxy, or alkoxy-carbonyl, on the proviso that at least one of R_{4a}, R_{4b} and R_{4c} is not hydrogen;

R_5 is -OH or $-N(R_{6a})(R_{6b})$;

R_{6a} and R_{6b} are each independently H or a C_1 to C_4 linear, branched or cyclic alkyl chain;

R_7 is -H or $-C(=O)-NH_2$;

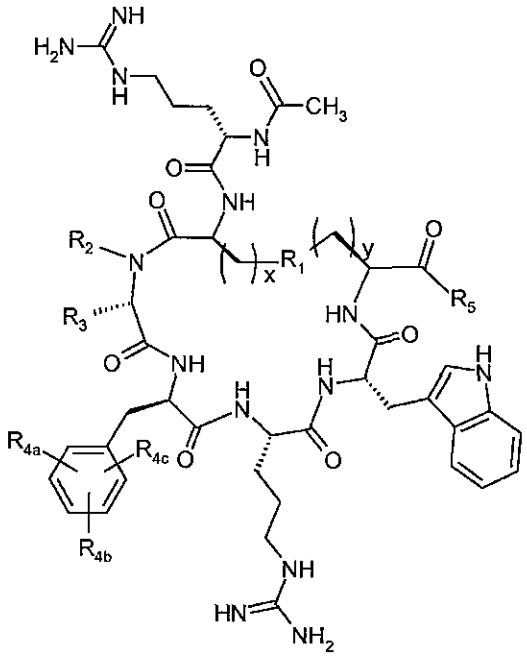
w is in each instance independently 0 to 5;

x is 1 to 5;

y is 1 to 5; and

z is in each instance independently 1 to 5.

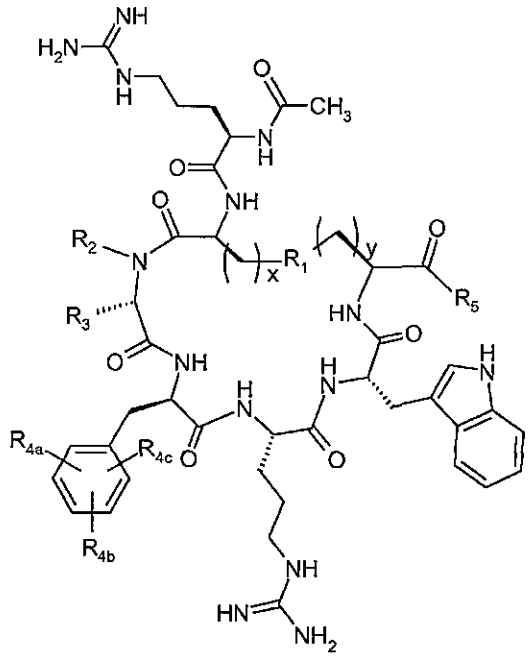
In another aspect, the present invention relates to a cyclic peptide of Formula (I) which is of formula (II):



(II)

or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention relates to a cyclic peptide of Formula (I) which is of Formula (III):



(III)

or a pharmaceutically acceptable salt thereof.

In one aspect, the invention provides a cyclic heptapeptide which contains a core sequence derived from His-Phe-Arg-Trp within the cyclic portion, and where the amino

acid in the first position is Arg and is outside the cyclic portion. Without being bound by any theory, the Arg residue is believed to largely contribute to the low activity at MC1-R.

Further, the core sequence in the peptides of Formula I includes a substituted D-Phe. Without being bound by any theory, it is believed that the intrinsic activity may be lowered while substantially maintaining potency by using substituted D-Phe in peptides in accordance with the invention. The peptide called Ref Ex 1 (including unsubstituted D-Phe) in Table 1 can be compared with one or more of Examples 1-5, 9-11, 17, 26 and 27. It may be noted that Table 1 includes mean values.

Table 1

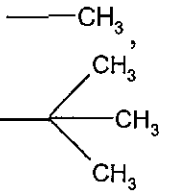
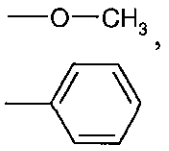
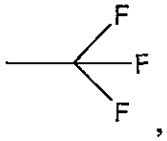
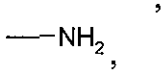
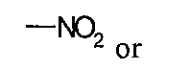
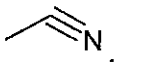
			0.1 ng/mL Doxycycline	
		K_i (nM)	EC_{50} (nM)	Intrinsic activity (%)
Ref Ex 1	Ac-Arg-cyclo(Glu-Dab-D-Phe-Arg-Trp-Orn)-NH_2	0.65	0.33	94
Ref Ex 2	Ac-Arg-cyclo(Glu-Dab-D-Phe-Arg-Trp-Orn)-OH	8	3.0	92

In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein one or more of R_{4a}, R_{4b} and R_{4c} are each independently selected from hydrogen, halo, (C_1-C_{10})alkyl-halo, (C_1-C_{10})alkyl-dihalo, (C_1-C_{10})alkyl-trihalo, (C_1-C_{10})alkyl, (C_1-C_{10})alkoxy, phenyl, nitro, nitrile, amino or hydroxy, on the proviso that at least one of R_{4a}, R_{4b} and R_{4c} is not hydrogen.

In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein one or more of R_{4a}, R_{4b} and R_{4c} are each independently selected from hydrogen, halo, (C_1-C_4)alkyl-halo, (C_1-C_4)alkyl-dihalo, (C_1-C_4)alkyl-trihalo, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, phenyl, nitro, nitrile, amino or hydroxy, on the proviso that at least one of R_{4a}, R_{4b} and R_{4c} is not hydrogen.

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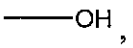
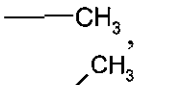
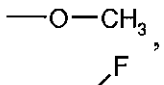
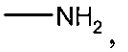
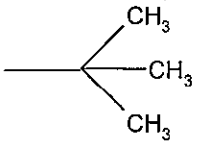
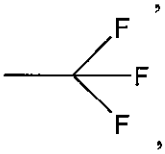
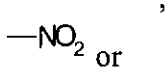
fluoro, chloro, ——OH,

In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein one or more of R_{4a}, R_{4b} and R_{4c} are each independently selected from hydrogen, halo, (C_1-C_4)alkyl-halo, (C_1-C_4)alkyl-dihalo, (C_1-C_4)alkyl-trihalo, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, nitro, nitrile, amino or hydroxy, on the proviso that at least one of R_{4a}, R_{4b} and R_{4c} is not hydrogen.

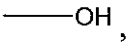
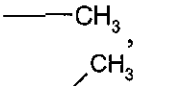
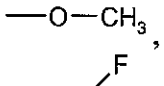
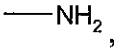
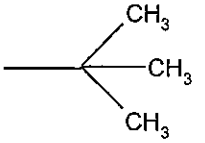
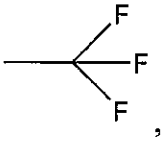
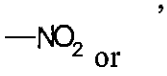
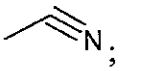
In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein one or more of R_{4a}, R_{4b} and R_{4c} are each independently selected from

fluoro, chloro,


In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein R_{4a}, is selected from

fluoro, chloro,

and R_{4b} and R_{4c} are each H.

In a further embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein R_{4a} is selected from -C≡N or

–F, and R_{4b} and R_{4c} are each H.

In a particular embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein R_{4a} is in the 4 position and is -C≡N and R_{4b} and R_{4c} are each H.

In another particular embodiment of the invention, there are provided peptides according to Formula (I), and in particular Formula (II) or (III), wherein R_{4a} is in the 4 position and is -F and R_{4b} and R_{4c} are each H.

The peptides encompassed within Formula (I) contain one or more asymmetric elements such as stereogenic centers, stereogenic axes and the like, so that the peptides encompassed within Formula (I) can exist in different stereoisomeric forms. For both specific and generically described peptides, including the peptides encompassed within Formula (I), all forms of isomers at all chiral or other isomeric centers, including enantiomers and diastereomers, are intended to be covered herein. The peptides of the invention each include multiple chiral centers, and may be used as a racemic mixture or an enantiomerically enriched mixture, in addition to use of the peptides of the invention in enantiopure preparations. Typically, the peptides of the invention will be synthesized with the use of chirally pure reagents, such as specified L- or D-amino acids, using reagents, conditions and methods such that enantiomeric purity is maintained, but it is possible and contemplated that racemic mixtures may be made. Such racemic mixtures may optionally be separated using well-known techniques and an individual enantiomer may be used alone. In cases and under specific conditions of temperature, solvents and pH wherein peptides may exist in tautomeric forms, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form. Thus a single enantiomer of a peptide of Formula (I), which is an optically active form, can be obtained by asymmetric synthesis, synthesis from optically pure precursors, or by resolution of the racemates.

The peptides of formulas (II) and (III) are specific stereoisomeric forms of the peptides of Formula (I), but the invention should not be construed as being limited to the stereoisomeric forms encompassed by formulas (II) and (III).

The invention is further intended to include prodrugs of the present peptides, which on administration undergo chemical conversion by metabolic processes before becoming active pharmacological peptides. In general, such prodrugs will be functional derivatives of

the present peptides, which are readily convertible in vivo into a peptide of Formula (I). Prodrugs are any covalently bonded compounds, which release the active parent peptide drug of Formula (I) in vivo. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985. Typical examples of prodrugs have biologically labile protecting groups on a functional moiety, such as for example by esterification of hydroxyl, carboxyl or amino functions. Thus by way of example and not limitation, a prodrug includes peptides of Formula (I) wherein an ester prodrug form is employed, such as, for example, lower alkyl esters of the R group of Formula (I), such as where R is –OH, which lower alkyl esters may include from 1-8 carbons in an alkyl radical or aralkyl esters which have 6-12 carbons in an aralkyl radical. Broadly speaking, prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated or dephosphorylated to produce an active parent peptide drug of Formula (I) in vivo.

Certain modifications of peptides of Formula I may be made in order to enhance the half-life of the peptide (see G. Pasuta and F. M. Veronese (2007) "Polymer-drug conjugation, recent achievements and general strategies" *Progress in Polymer Science* 32 (8-9): 933-961).

The subject invention also includes peptides which are identical to those recited in Formula (I), but for the fact that one or more atoms depicted in Formula (I) are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into peptides of the invention include isotopes of hydrogen, carbon, nitrogen and oxygen, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O and ^{17}O, respectively. Peptides of the present invention and pharmaceutically acceptable salts or solvates of said peptides which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labeled peptides of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, may have use in a variety of assays, such as in drug and/or substrate tissue distribution assays. Substitution with heavier isotopes, such as substitution of one or more hydrogen atoms with deuterium (^{2}H), can provide pharmacological advantages in some instances, including increased

metabolic stability. Isotopically labeled peptides of Formula (I) can generally be prepared by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

In one embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_1 is -C(=O)-NH-, x is 2 and y is 3.

In another embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_1 is -C(=O)-NH-, x is 1 and y is 4.

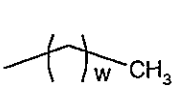
In another embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_1 is -NH-C(=O)-, x is 3 and y is 2.

In still another embodiment of the peptides according to the Formula (I), and in particular Formula (II) or (III), R_1 is -NH-C(=O)-, x is 4 and y is 1.

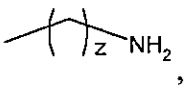
In a particular embodiment of the peptides according to the Formula (I), and in particular Formula (II) or (III), R_1 is -C(=O)-NH-, x is 2 and y is 3, or alternatively R_1 is -NH-C(=O)-, x is 3 and y is 2. More specifically, R_1 is -C(=O)-NH-, x is 2 and y is 3. It has surprisingly and unexpectedly been found that peptides according to the invention having a lactam bridge wherein the amide bond is positioned by means of the side chain of Glu (at the N-terminus end of the cyclic portion) and the side chain of Orn (at the C-terminus end of the cyclic portion) generally provides improved potency as determined by EC_{50}, in particular in the herein described low density hMC4-R system, compared to peptides wherein the amide bond is positioned by means of the side chains of Asp and Lys, but which are otherwise identical. This discovery is contrary to assertions in the prior art that the location and direction of an amide bond in the lactam bridge of melanocortin receptor specific peptides is of little importance for activity and does not interact with receptors. See, for example, Bednarek MA et al., Potent and selective peptide agonists of α-melanotropin action at human melanocortin receptor 4: their synthesis and biological evaluation *in vitro*. *Biochem. Biophys. Res. Comm.* 286:641-645 (2001).

In an embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH;

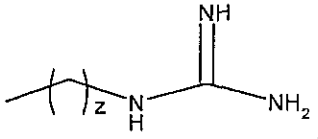
R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



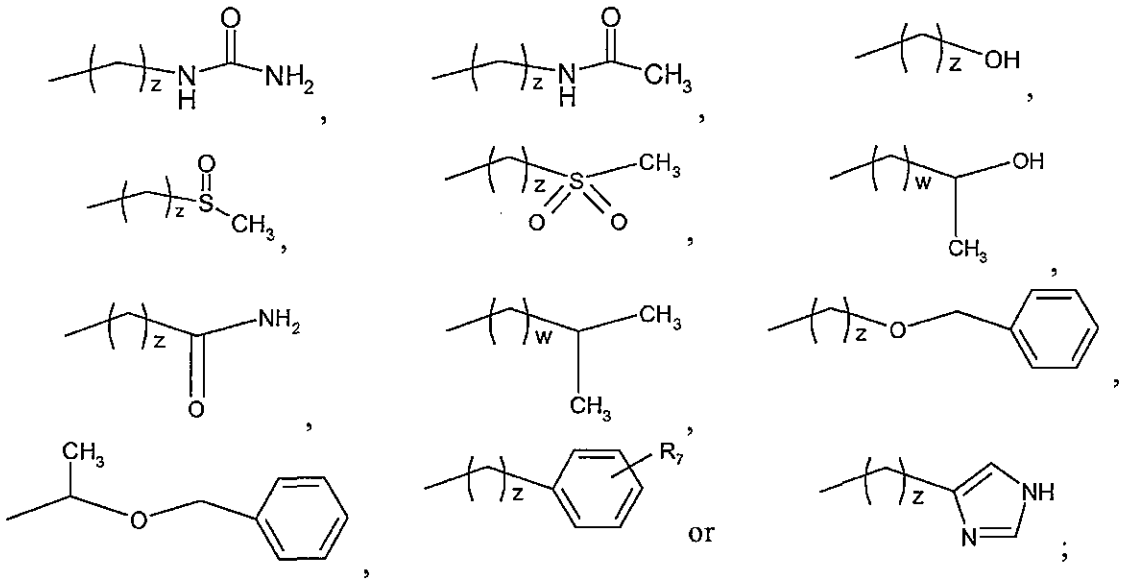
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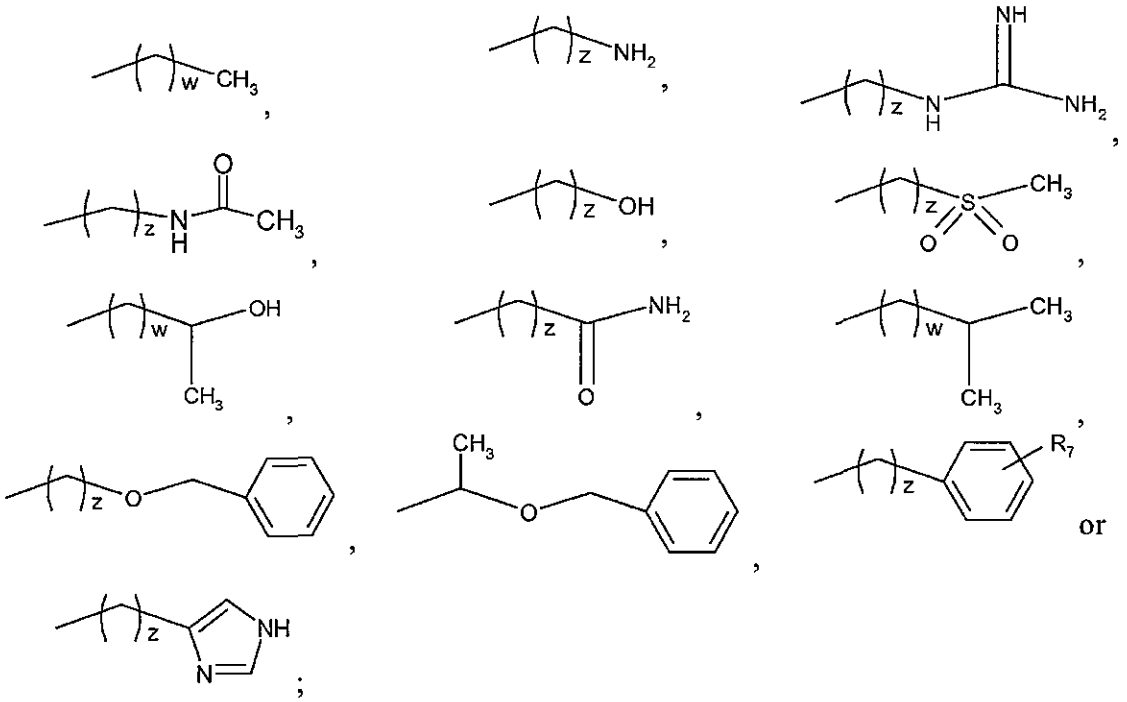


wherein w is in each instance independently selected from 0 to 5, and

z is in each instance independently selected from 1 to 5.

In an embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH;

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



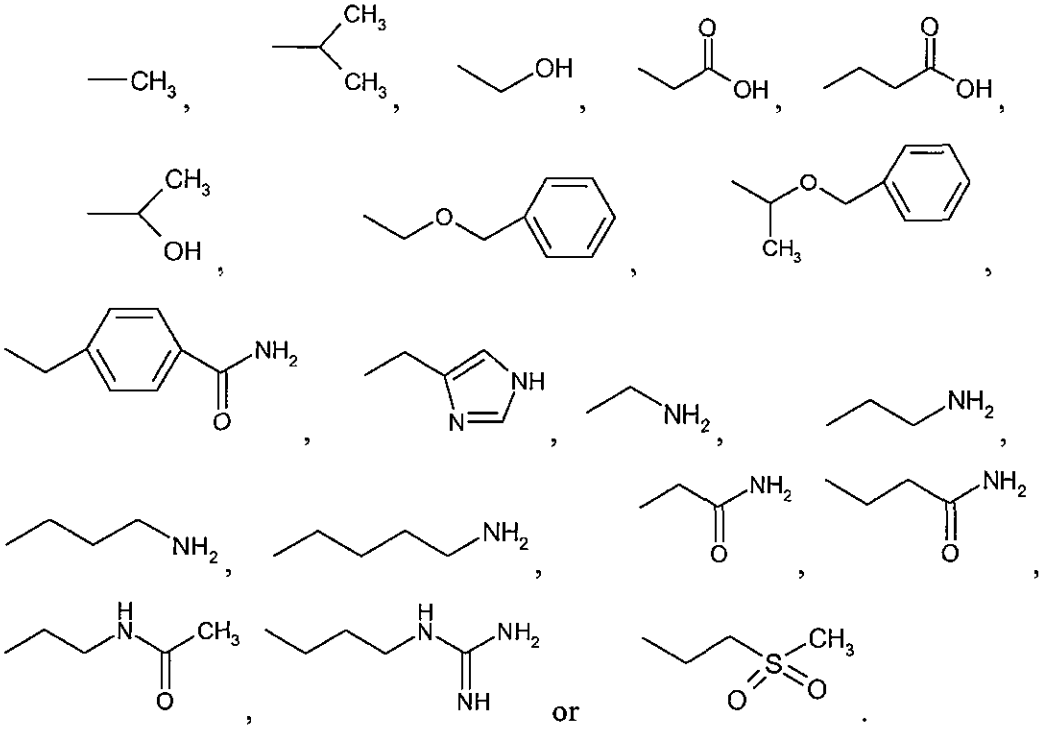
wherein w is in each instance independently selected from 0 to 5, and

z is in each instance independently selected from 1 to 5.

In an embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_2 is H.

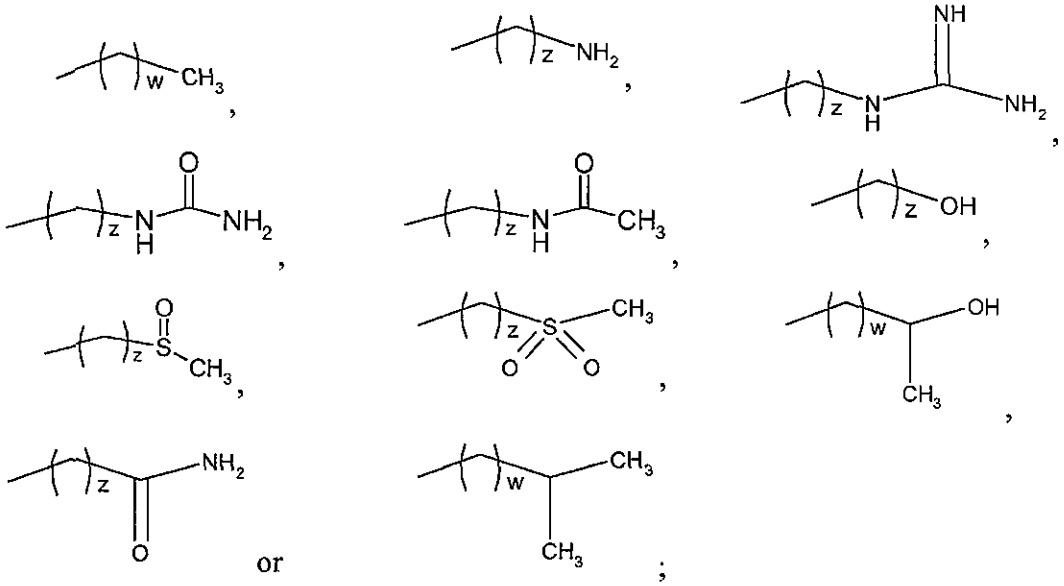
In an embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_2 is H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



Without being bound by any theory, replacing His by a non-aromatic amino acid, in particular a non-polar, small aliphatic amino acid, such as Pro or Ala, or a polar uncharged or charged amino acid, such as Lys, Asp, Gln and Asn, is believed to contribute to attenuation of blood pressure effects. Thus, the His position in the core sequence may for instance be replaced by Dab, Dab(Acetyl), Ser, Met(O), Met(O_2), Thr, Hyp, Gln, Pro, Ala, Asn, Cit, Orn, Dap, Lys, or Arg, in particular Pro, Asn or Gln.

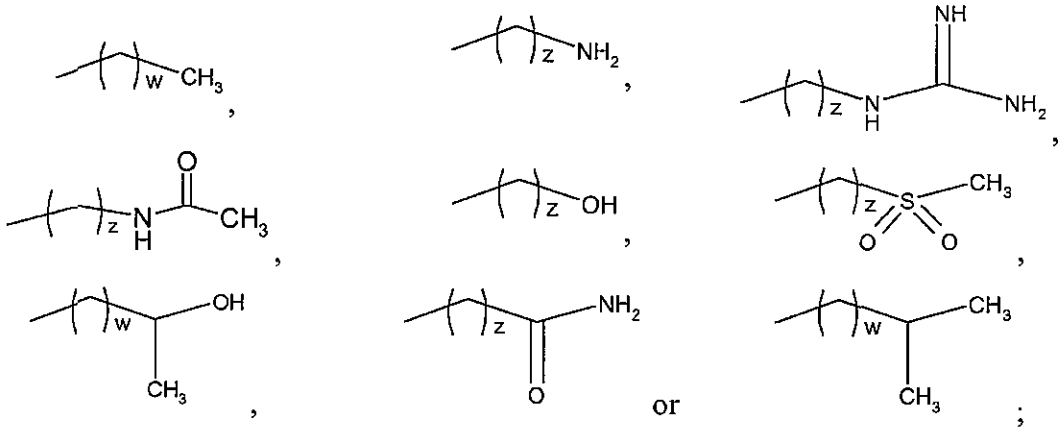
Thus, in a further embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



wherein w is in each instance independently selected from 0 to 5, and

z is in each instance independently selected from 1 to 5.

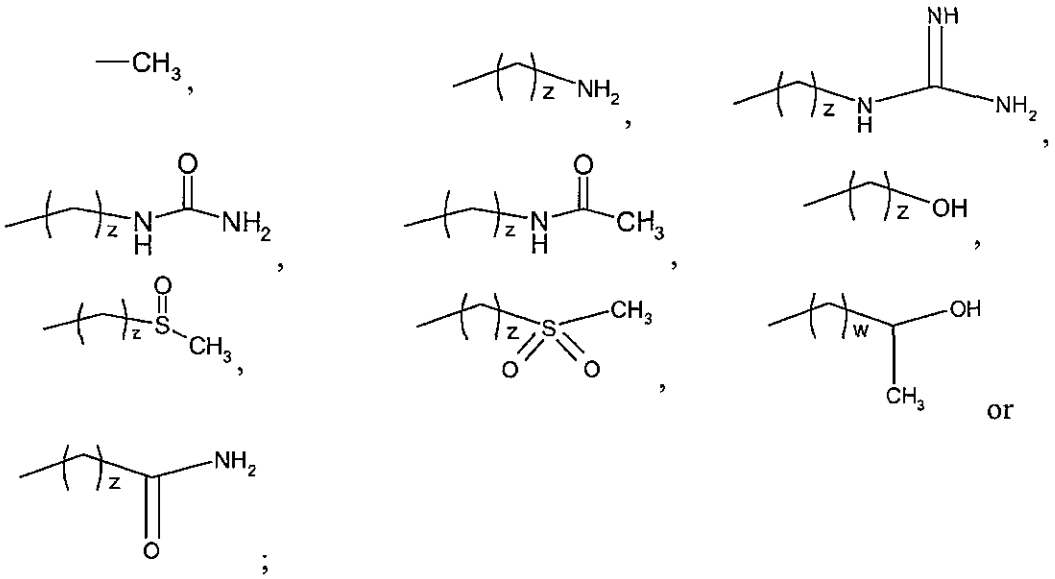
In a further embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



wherein w is in each instance independently selected from 0 to 5, and

z is in each instance independently selected from 1 to 5.

In a particular embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from

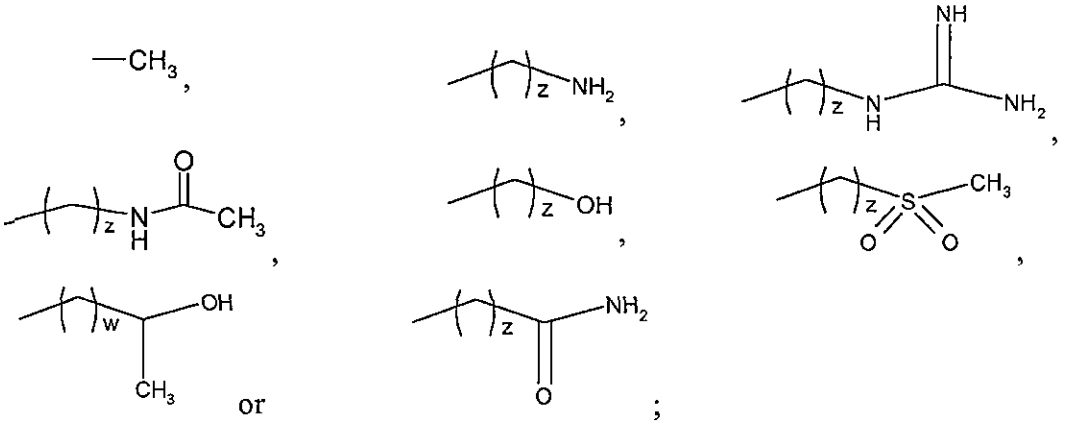


wherein w is in each instance independently selected from 0 to 5, in particular 0 to 2, such as 0, and

z is in each instance independently selected from 1 to 5, in particular 1 to 4.

In a further particular embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from

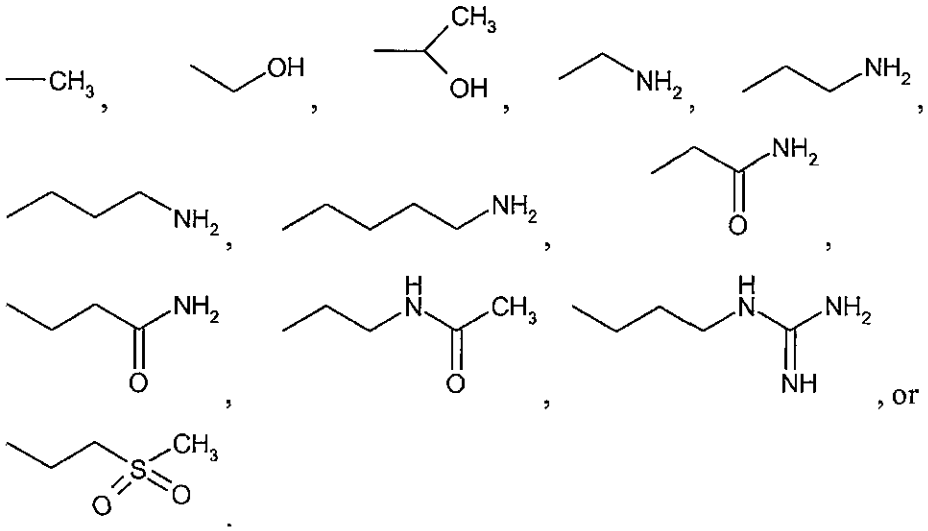


wherein w is in each instance independently selected from 0 to 5, in particular 0 to 2, such as 0, and

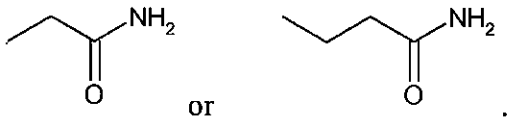
z is in each instance independently selected from 1 to 5, in particular 1 to 4.

In further embodiments of the peptides according to Formula (I), and in particular Formula (II) or (III), R_2 is H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH; and

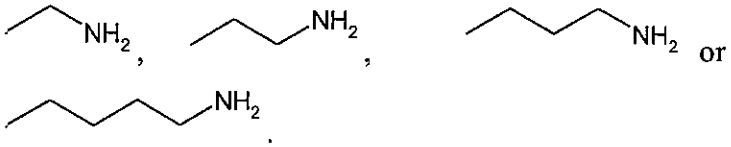
R_3 is selected from



In another embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is H and R_3 is selected from



In another embodiment of the invention, there are provided peptides of Formula (I), in particular of Formula (II) or (III), wherein R_2 is H and R_3 is selected from



In another embodiment of the invention, there are provided peptides of Formula (I), and in particular Formula (II) or (III), wherein R_2 is $-CH_2-$ and R_3 is $-(CH_2)_2-$, R_2 and R_3 together forming an unsubstituted pyrrolidine ring.

In still another embodiment of the invention, there are provided peptides of Formula (I), and in particular Formula (II) or (III), wherein R_2 is $-CH_2-$ and R_3 is $-(CH_2)_2-$, R_2 and R_3 together forming an pyrrolidine ring substituted with –OH. In a further embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_5 is $–NH_2$ or –OH.

In a specific embodiment of the peptides according to Formula (I), and in particular Formula (II) or (III), R_5 is $-NH_2$.

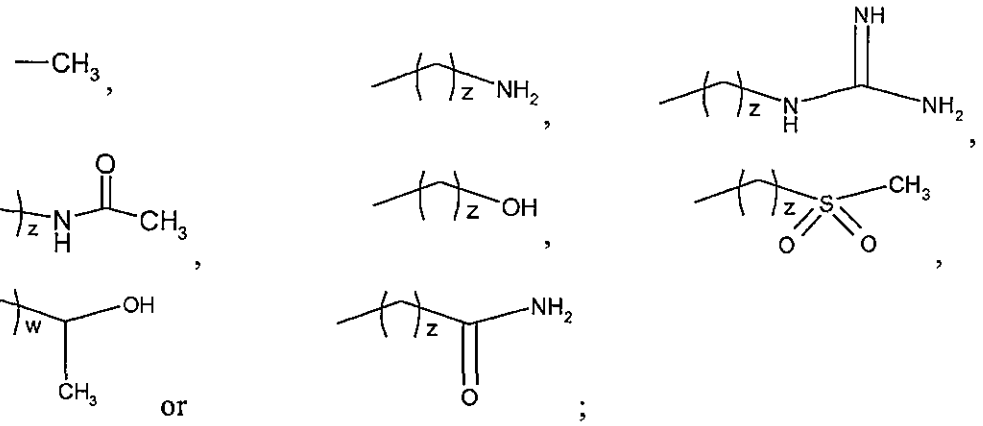
One or more of the above embodiments may be combined to provide further specific embodiments of the peptides according to the invention.

Thus, in a particular embodiment of the invention, there are provided peptides according to Formula (I), in particular Formula (II) or (III), wherein

R_1 is -C(=O)-NH-, x is 2 and y is 3;,

R_2 is -H or $-CH_2-$, and if R_2 is $-CH_2-$ forms with R_3 a pyrrolidine ring, the pyrrolidine ring optionally substituted with -OH;

R_3 is $-(CH_2)_2-$ if R_2 is $-CH_2-$, and otherwise R_3 is selected from



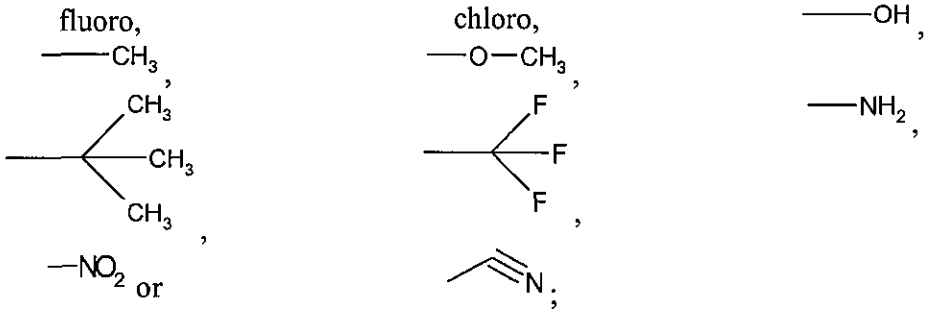
or ;

w is in each instance independently selected from 0 to 5;

z is in each instance independently selected from 1 to 5;

one or more of R_{4a}, R_{4b} and R_{4c} are each independently selected from

fluoro, chloro,



and

R_5 is $-NH_2$ or $-OH$.

Some specific peptides of the invention are one or more of the following:

Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:6);

Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:7);

Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:8);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2,4-diCl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:9);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diCl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:10);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:11);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:12);
Ac-Arg-*cyclo*(Glu-Dab-D-Tyr-Arg-Trp-Orn)-NH_2 (SEQ ID NO:13);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:14);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:15);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:16);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:17);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:18);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:19);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:20);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-OMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:21);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,5-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:22);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:23);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:24);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:25);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2,4-diMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:26);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-NO_2)-Arg-Trp-Orn-NH_2 (SEQ ID NO:27);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-NO_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:28);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-NO_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:29);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diOMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:30);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:31);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:32);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-NH_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:33);
Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-tBu)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:35);
Ac-Arg-*cyclo*(Glu-Ser-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:36);
Ac-Arg-*cyclo*(Glu-Thr-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:37);
Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:38);
Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:39);
Ac-Arg-*cyclo*(Glu-Ser-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:40);

Ac-Arg-*cyclo*(Glu-Thr-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:41);

Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:42);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:43);

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:44);

Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:45);

Ac-Arg-*cyclo*(Glu-Ala-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:46);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:48);

Ac-Arg-*cyclo*(Glu-Thr-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:49);

Ac-Arg-*cyclo*(Glu-Ser-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:50);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:51);

Ac-Arg-*cyclo*(Glu-Orn-D-Phe(2-Cl)-Arg-Trp-Orn)-OH (SEQ ID NO:54);

Ac-Arg-*cyclo*(Glu-Orn-D-Phe(3-Cl)-Arg-Trp-Orn)-OH (SEQ ID NO:60);

Ac-Arg-*cyclo*(Glu-Dab(Acetyl)-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:61);

Ac-Arg-*cyclo*(Glu-Ser-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:63);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:64);

Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:65);

Ac-Arg-*cyclo*(Glu-Dap-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:66);

Ac-Arg-*cyclo*(Glu-Dap-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:67);

Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:69);

Ac-Arg-*cyclo*(Glu-Ser-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:70);

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:71);

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:72);

Ac-Arg-*cyclo*(Glu-Met(O_2)-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:73);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:74);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:78);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(3,5-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:81);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:89);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:90);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:91);

Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-OMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:92);

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:93);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:97);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:100);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:102);

Ac-Arg-*cyclo*(Glu-Arg-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:104);

Ac-Arg-*cyclo*(Glu-Lys-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:105);

Ac-Arg-*cyclo*(Glu-Orn-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:106)

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-OH (SEQ ID NO:107);

Ac-Arg-*cyclo*(Glu-Asp-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:111);

Ac-Arg-*cyclo*(Glu-Asp-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:112);

Ac-Arg-*cyclo*(Glu-Glu-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:113);

Ac-Arg-*cyclo*(Glu-Glu-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:114);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:115);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:119);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:121); or a pharmaceutically acceptable salt of any of the foregoing.

Particularly, the invention relates to:

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:48);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:74);

Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:90), or a pharmaceutically acceptable salt of any of the foregoing.

Particularly, the invention also relates to:

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:51);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:91);

Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:97), or a pharmaceutically acceptable salt of any of the foregoing.

Particularly, the invention also relates to:

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:44);

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:72),

Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-OH (SEQ ID NO:107); or a pharmaceutically acceptable salt of any of the foregoing.

Some further specific peptides of the invention are:

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(4-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:75);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:76);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:77);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:79);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,4-diF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:80);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,5-diF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:82);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(4-CN)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:96);

Ac-Arg-*cyclo*(Orn-Gln-D-Phe(4-CN)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:99);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,4,5-triF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:101);

Ac-Arg-*cyclo*(Orn-Gln-D-Phe(3,4,5-triF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:103);

Ac-Arg-*cyclo*(Orn-Pro-D-Phe(2-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:110);

Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:116);

Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:117);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:118);

Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-CF_3)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:120);

Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-CF_3)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:122); or a pharmaceutically acceptable salt of any of the foregoing.

6.1 Specific peptides.

Peptides of the following structures were synthesized by the general methods described above, and except where indicated MC1-R Ki and MC4-R Ki values for each peptide were determined in competitive binding assays using [I^{125}]-NDP-α-MSH as described in 7.1 below. All peptides were prepared in the TFA acid salt form, except for the peptides of Examples 43, 46 and 67, which were prepared in the acetate salt form.

The syntheses of some specific peptides of the invention are illustrated below. These peptides were prepared using solid phase peptide synthesis by means of a Symphony Multiplex Peptide Synthesizer (Rainin Instrument Company/Protein Technologies Inc) automated peptide synthesizer.

Step 1: Coupling of Orn

The Sierber resin 9-Fmoc-Aminoxanthen-3-yloxy-polystyrene resin (0.39 mol/g,ChemPep Inc.,#151902) was swelled in 3 x 5 mL of DMF for 10 min. Thereafter, Fmoc was deprotected using 2 x 5 mL of 20% piperidine in DMF for 10 min. The resin was then washed in 6 x 5 mL DMF for 30 sec. 5 mL of 200 mM Fmoc-Orn(Alloc)-OH in DMF and 5 mL 200 mM HBTU containing 400 mM NMM in DMF was added and after 30 min the resin was washed with 3 x 5 mL DMF for 30 sec.

Step 2: Coupling of next 6 amino acids (AA)

The resin from step 1 was first swelled in 3 x 5 mL of DMF for 30 sec, Fmoc was deprotected using 2 x 5 mL of 20% piperidine in DMF for 10 min and then washed with 6 x 5 mL DMF for 30 sec. 5 mL of 200 mM Fmoc-AA-OH solution and 5 mL 200 mM HBTU containing 400 mM NMM in DMF was added and after 30 min the resin was washed with 3 x 5 mL DMF for 30 sec.

This step was repeated for each amino acid (AA).

Step 3: Acetylation

Fmoc was deprotected using 2 x 5 mL of 20% piperidine in DMF for 10 min and the resin was then washed with 3 x 5 mL DMF for 30 sec. Thereafter, 5 mL of 50% Ac_2O/DMF solution was added and after 30 min the peptide resin was washed with 3 x 5 mL DMF for 30 sec and 6 x 5 mL DCM for 30 sec.

Step 4: Allyl/Alloc Deprotection

The peptide resin (0.6 mmol) was mixed with phenylsilane (Oakwood Chemical, #S13600) (20 eq.) in 20 mL of DCM and bubbled with nitrogen for 5 min.

Tetrakis(triphenylphosphine)-palladium(0) (Strem Chemicals, Inc., #46-2150) (0.2 eq.) was added and the mixture was agitated with nitrogen for 1 hour. The procedure was repeated one time for 1 hour and an additional time for 30 min with fresh reagents. The treated peptide resin was then washed with DCM x 3 and DMF x 3.

Step 5: Lactam Formation

The lactam ring was formed on the peptide resin using TBTU (2 eq.) and ethyldiisopropylamine (DIEA) (4 eq.) in 20 mL DMF for 1 hour. A second coupling may be needed if a positive Kaiser Ninhydrin test is observed.

Step 6: Peptide Cleavage

The peptide resin (0.6 mmol) was mixed with 20 mL of 5% sodium diethyldithio-carbamate trihydrate ($NaCS_2NEt_2$, Aldrich, #228680) in DMF for 20 min and then washed with DMF x 3, DCM x 3 and diethyl ether x 2.

The resin (0.6 mmol) was then stirred in a 25 mL of TFA/TIS/H2O (90 : 5.0 : 5.0 v/v/v) for 2.5 hours. The resin was filtered. The filtrate was concentrated to about 10 mL in volume and about 140 mL of cold diethyl ether (pre-cooled to about 0 °C) was added.

The mixture was vortexed, and then placed in the refrigerator (about -4 °C) for 1 h, centrifuged for 5 min at 2800 rpm, and the ether layer was decanted.

The peptide was washed with 90 mL of cold diethyl ether (pre-cooled to about 0 °C), vortexed, centrifuged for 5 min at 2800 rpm, and ether layer decanted.

The resulting solid was dissolved in 50% AcOH/H_2O and stored at room temperature overnight.

The crude peptide solution was concentrated to afford solid crude peptide for HPLC purification.

After HPLC purification, the peptide TFA salt was converted to peptide acetate salt using ion exchange (x 100 eq.). The anion exchange resin used was Dowex SBR LC NG, OH-form (Supelco, Cat# 14036-U).

Example 46: Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:51)

The procedure described above was followed in the preparation of the title peptide.

The amino acids added in step 2 were, in the order of being coupled, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-D-Phe(4-CN)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OAll)-OH, and Fmoc-Arg(Pbf)-OH.

The resulting peptide was purified by HPLC (column: Atlantis dC18 OBD™ 19 x 100 mm (5µ, Waters part # 186001367) using 10% MeOH/H_2O containing 0.1% TFA (solvent A) and 90% MeOH/H_2O containing 0.1% TFA (solvent B). A gradient of 0%-5% of solvent B for 5 min and 5%-35% of solvent B for 30 min was used.

The peptide yield was 10%.

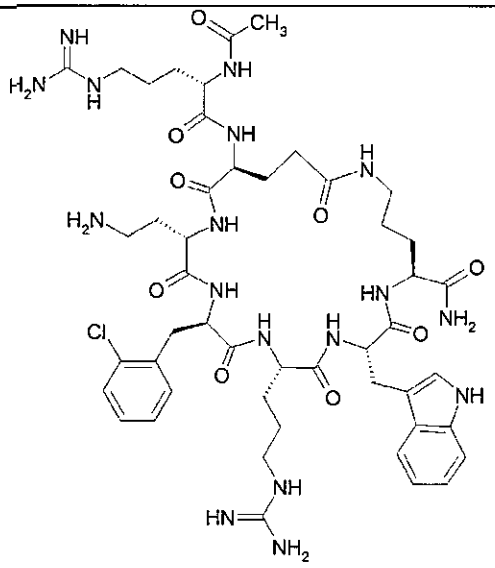
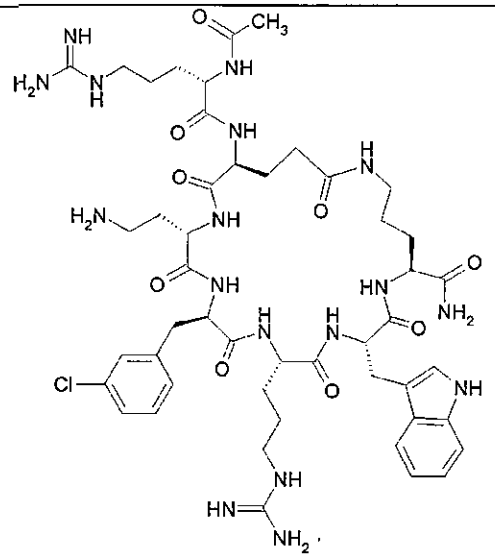
Example 67: Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:72)

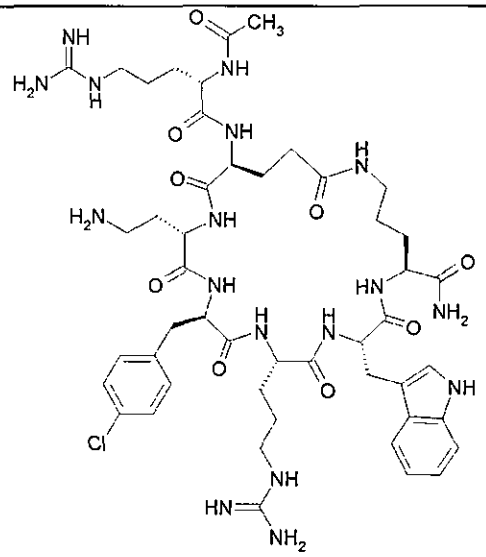
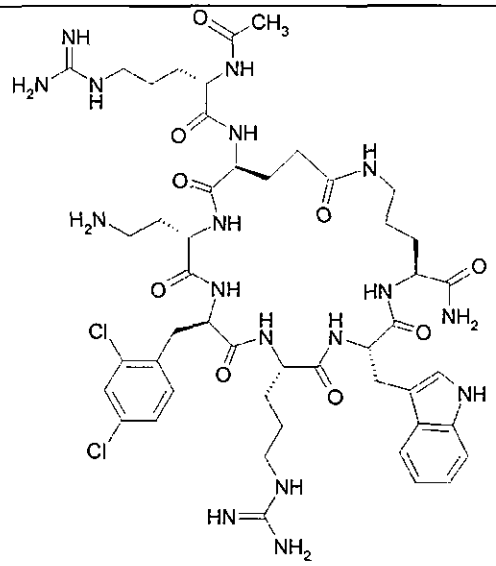
The procedure described above was followed in the preparation of the title peptide except for that 15 mL of 5% sodium diethyldithio-carbamate trihydrate in DMF, 16 mL of TFA/TIS/H_2O and 90 mL + 60 mL of diethyl ether were used in step 6. Moreover, the filtrate was concentrated to 5 mL.

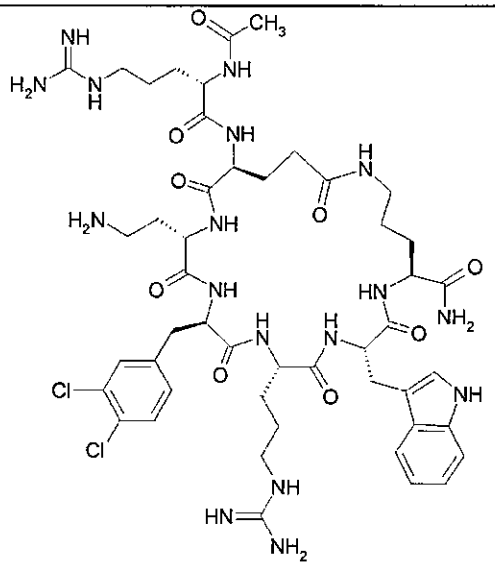
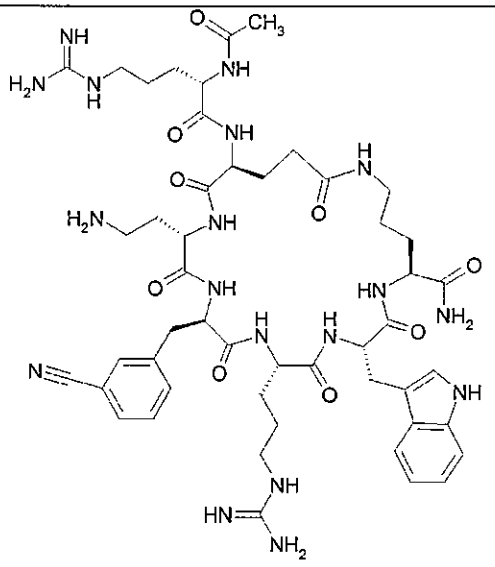
The amino acids added in step 2 were, in the order of being coupled, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-D-Phe(4-F)-OH, Fmoc-Pro-OH, Fmoc-Glu(OAll)-OH, and Fmoc-Arg(Pbf)-OH.

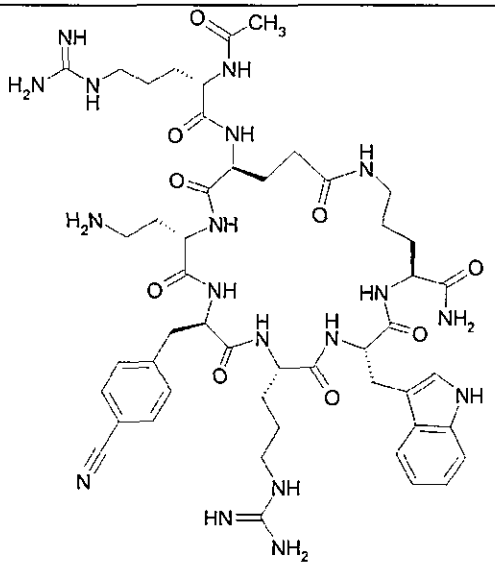
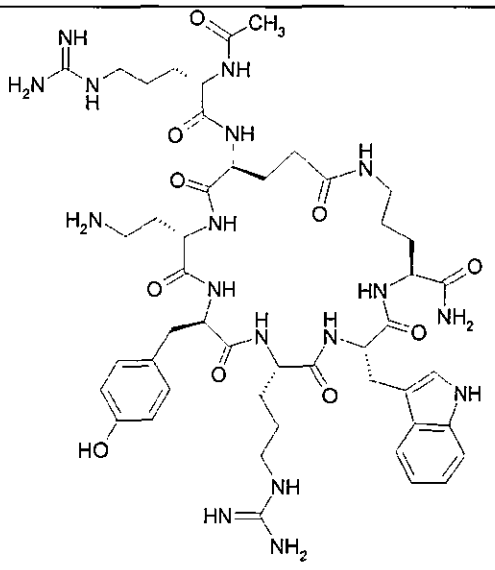
The resulting peptide was purified by HPLC (column: Atlantis dC18 OBD™ 19 x 100 mm (5µ, Waters part # 186001367) using 10% MeOH/H_2O containing 0.1% TFA (solvent A) and 90% MeOH/H_2O containing 0.1% TFA (solvent B). A gradient of 5%-10% of solvent B for 5 min and 10%-40% of solvent B for 30 min was used.

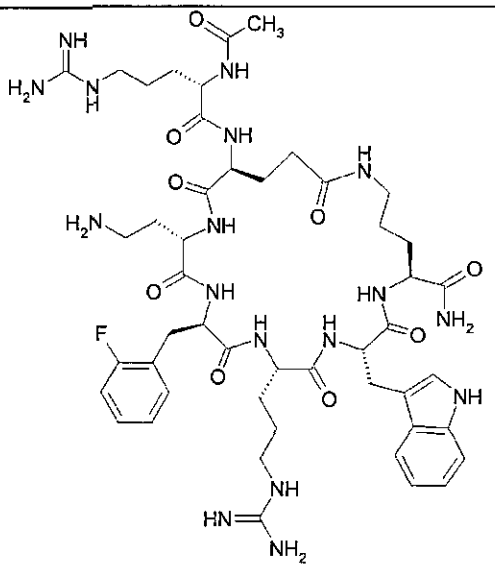
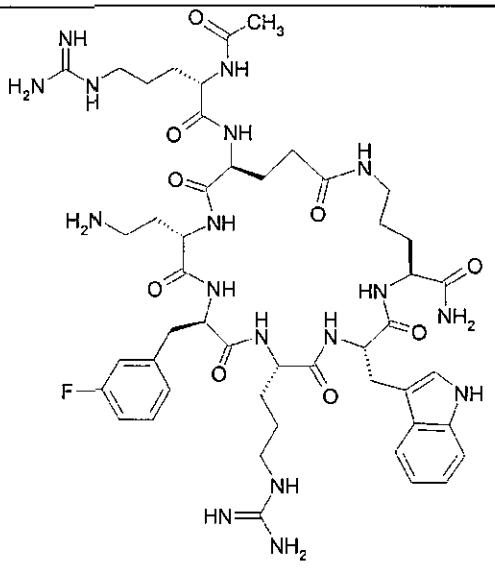
The peptide yield was 16%.

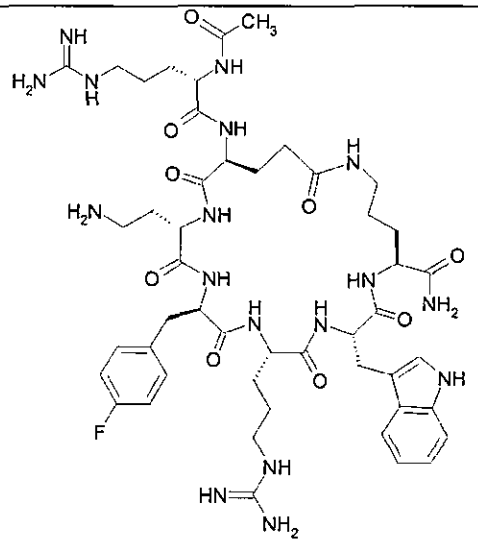
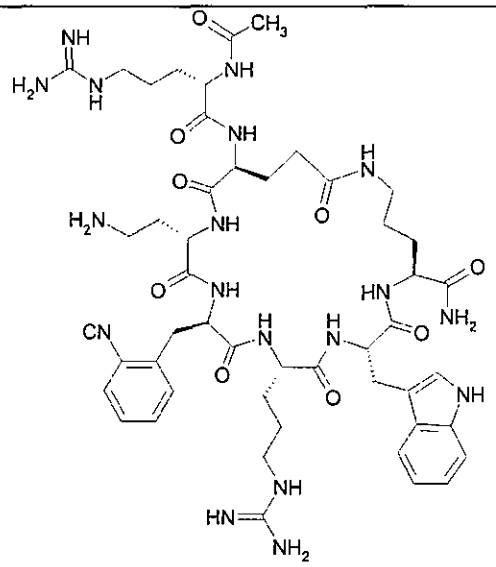
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
1		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:6)	3	1
2		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:7)	0.75	1

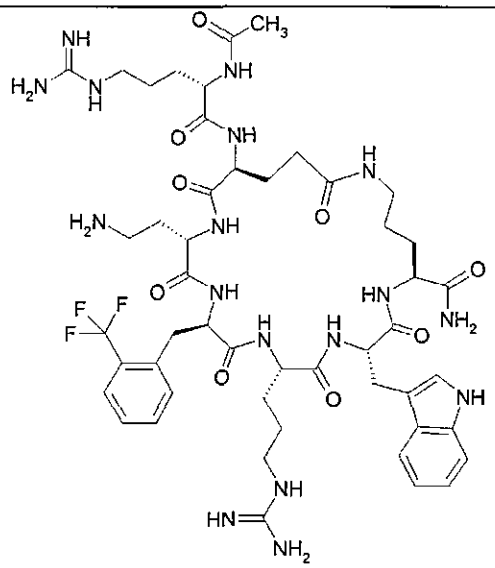
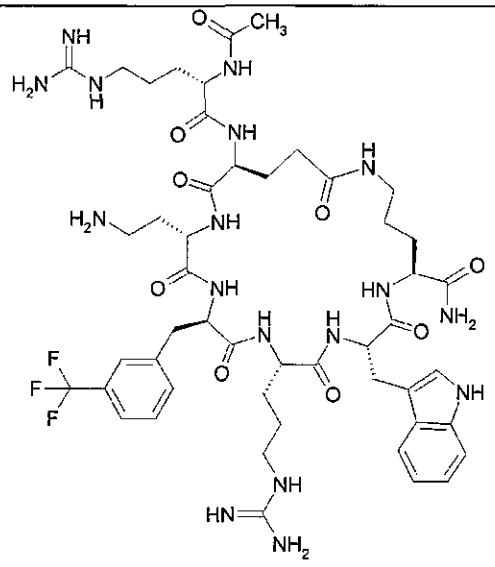
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
3		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:8)	0.08	0.3
4		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2,4-diCl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:9)	0.055	0.5

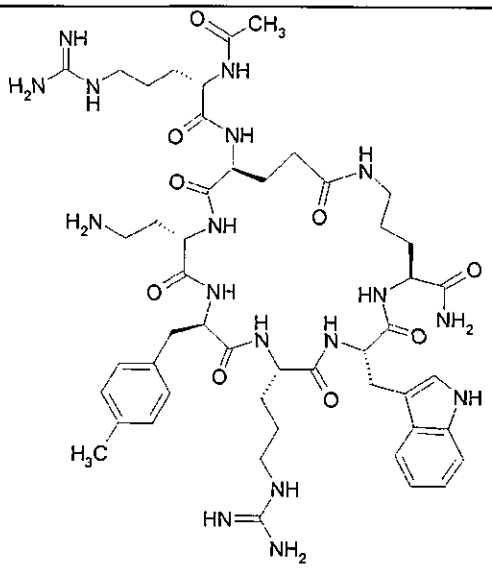
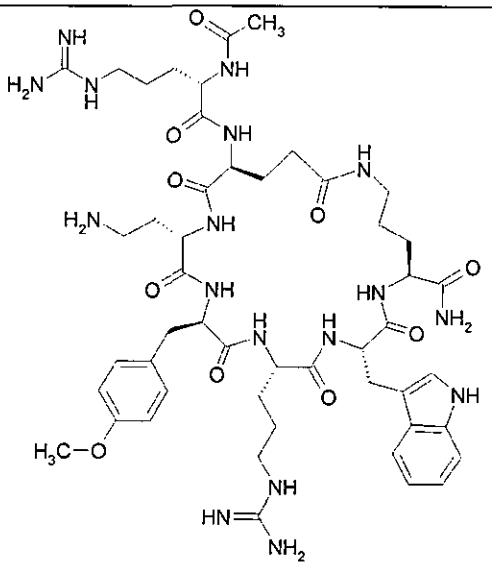
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
5		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diCl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:10)	0.055	0.2
6		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:11)	33	43

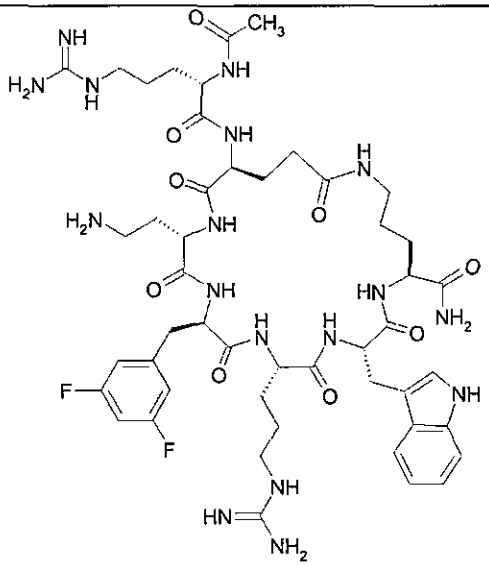
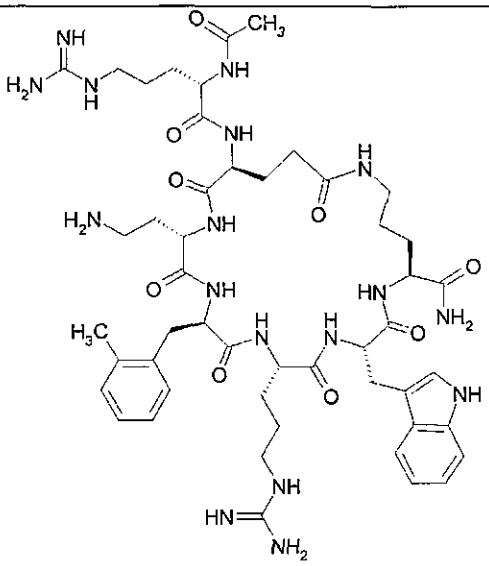
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
7		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:12)	0.833	5
8		Ac-Arg-*cyclo*(Glu-Dab-D-Tyr-Arg-Trp-Orn)-NH_2 (SEQ ID NO:13)	75	15

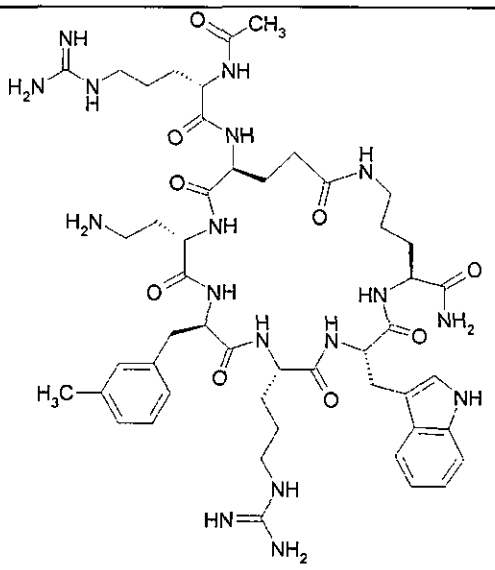
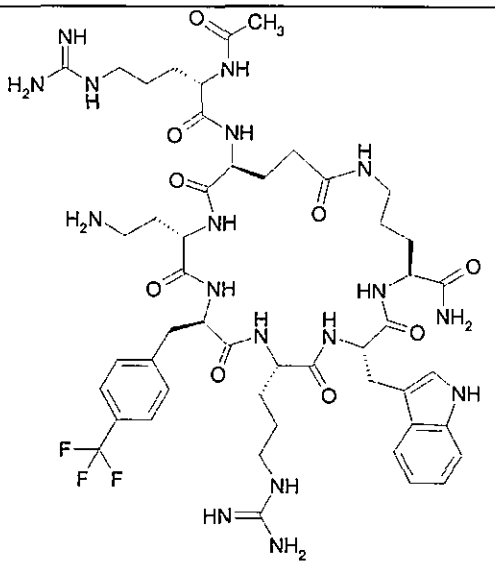
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
9		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:14)	0.7	0.8
10		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:15	2	2

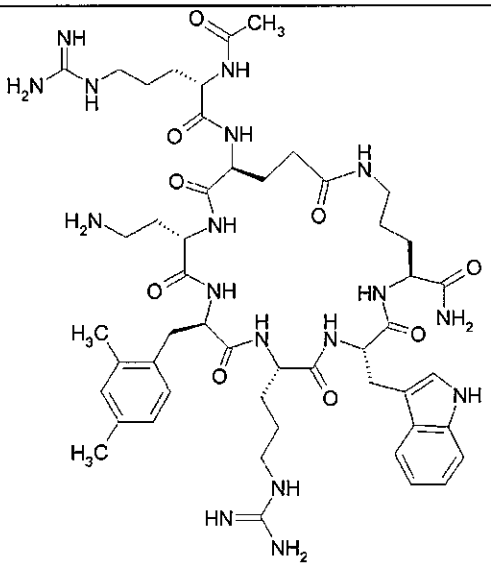
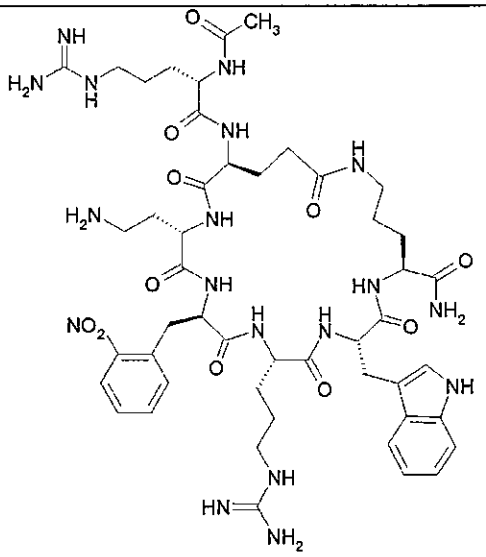
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
11		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:16)	0.25	0.5
12		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:17)	45	35

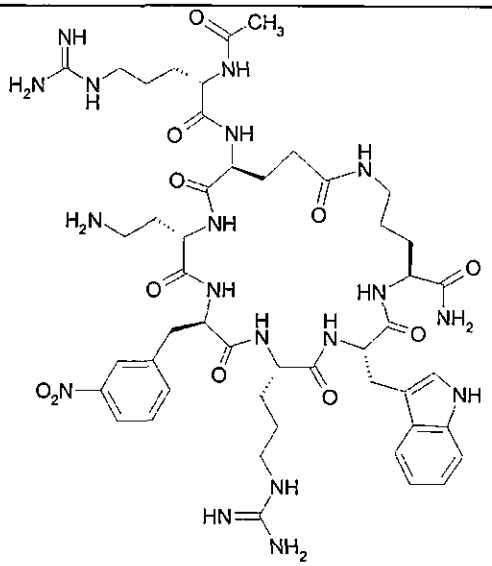
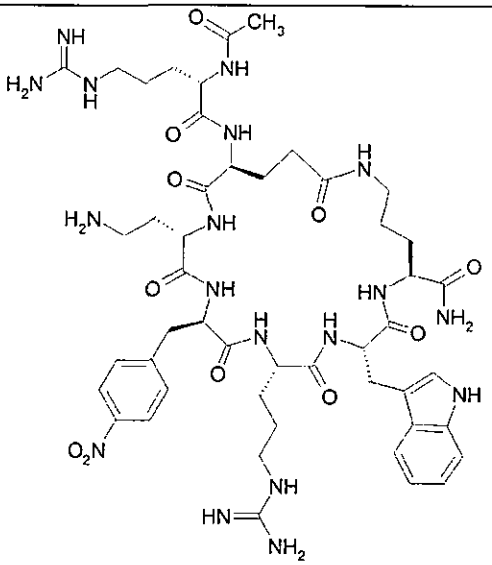
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
13		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:18)	0.6	3
14		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:19)	0.95	9

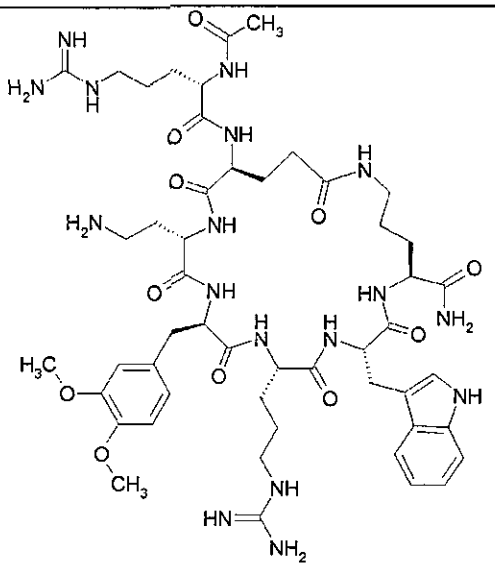
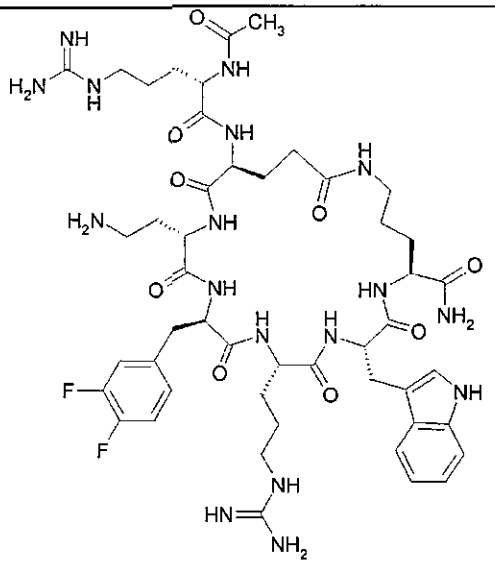
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
15		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:20)	0.145	0.75
16		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-OMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:21)	0.25	0.15

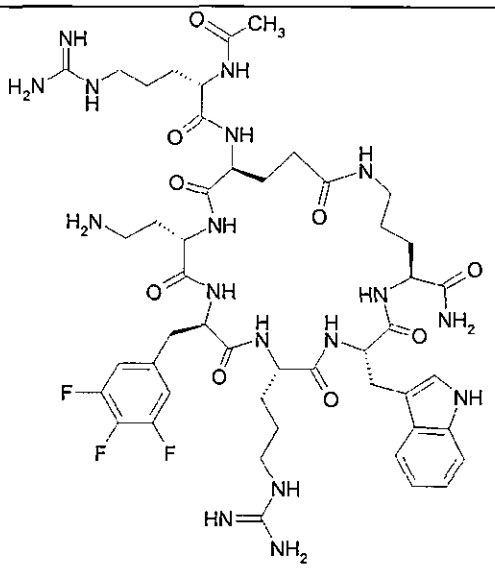
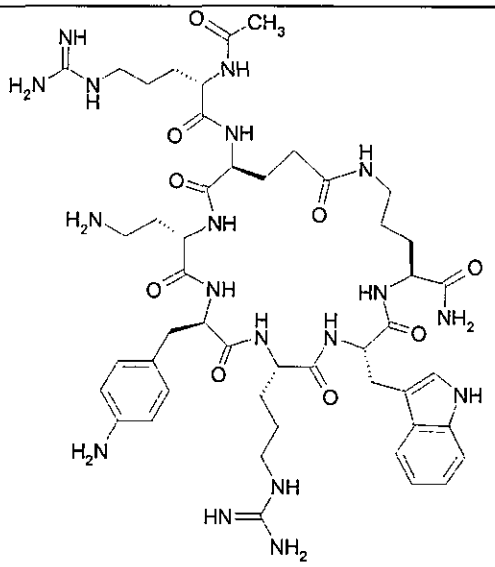
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
17		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,5-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:22)	2	-
18		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:23)	0.5	-

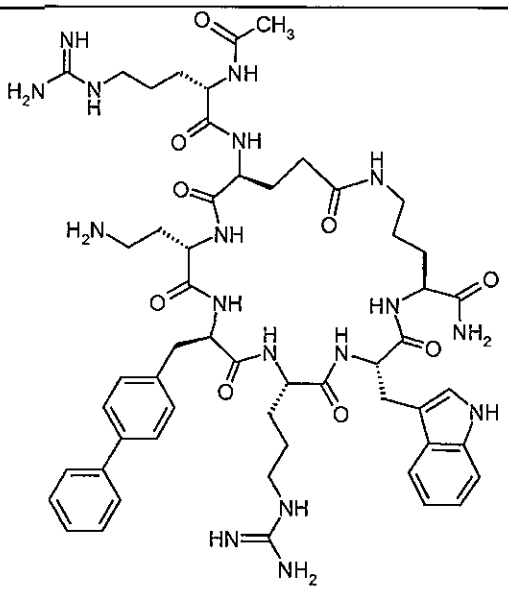
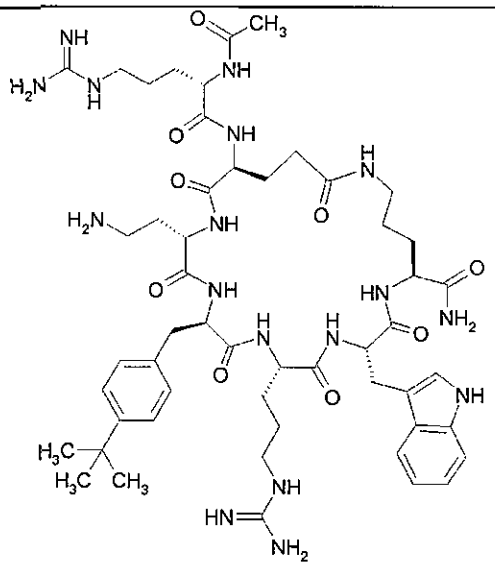
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
19		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:24)	0.4	-
20		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:25)	0.06	-

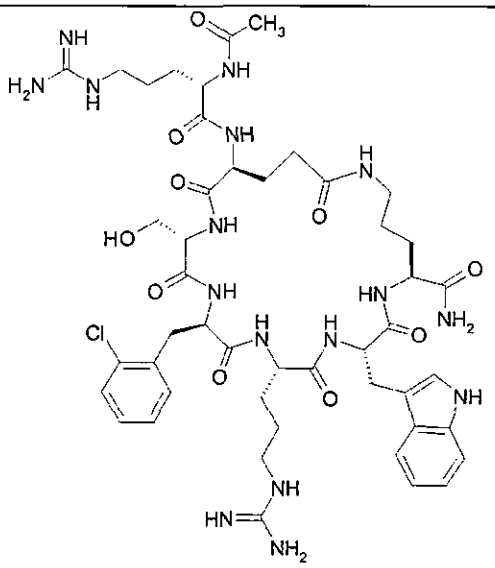
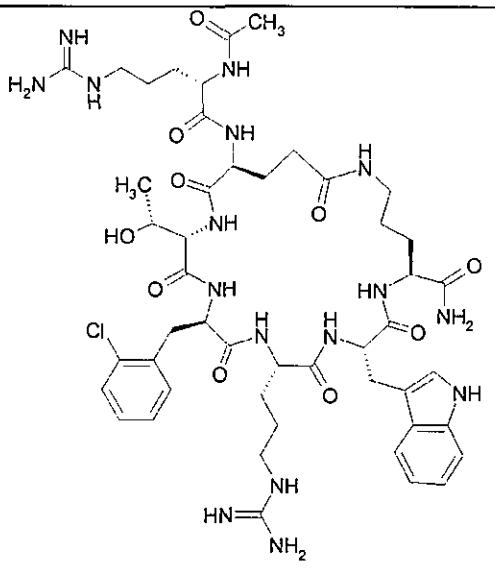
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
21		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2,4-diMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:26)	0.085	-
22		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(2-NO_2)-Arg-Trp-Orn-NH_2 (SEQ ID NO:27)	11	-

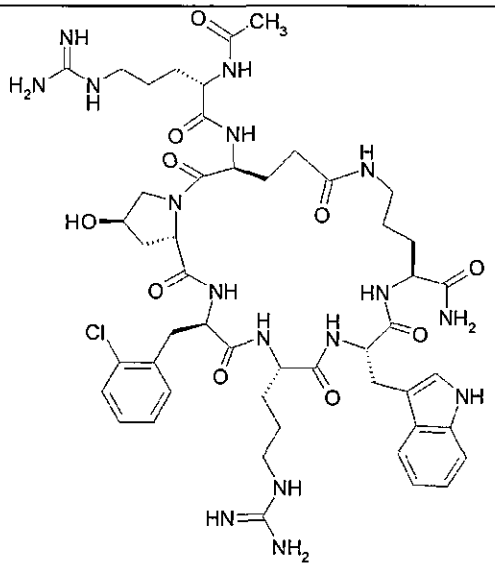
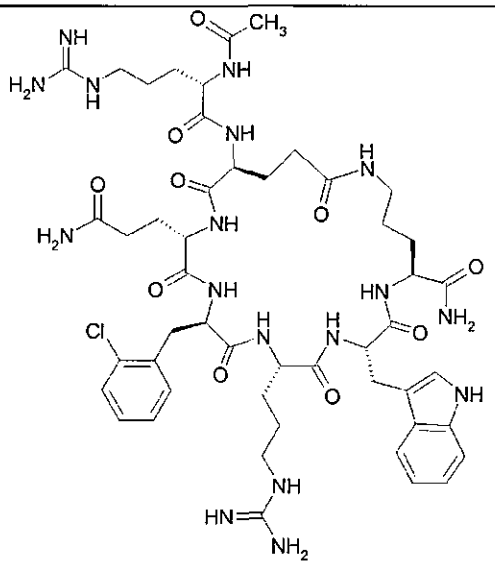
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
23		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-NO_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:28)	0.7	-
24		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-NO_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:29)	0.45	-

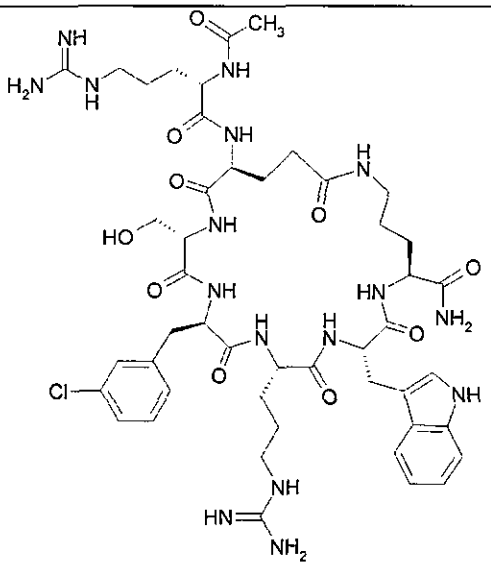
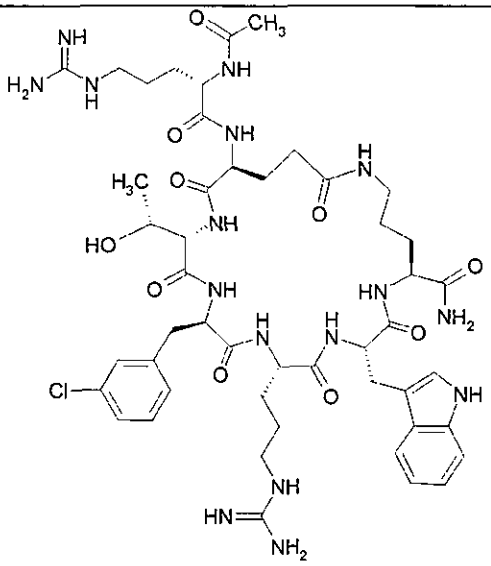
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
25		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diOMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:30)	35	-
26		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:31)	0.3	-

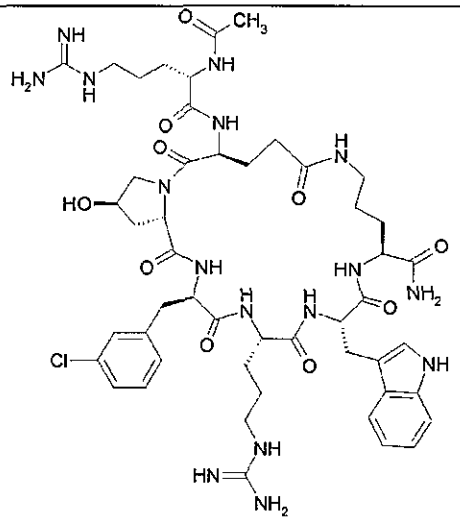
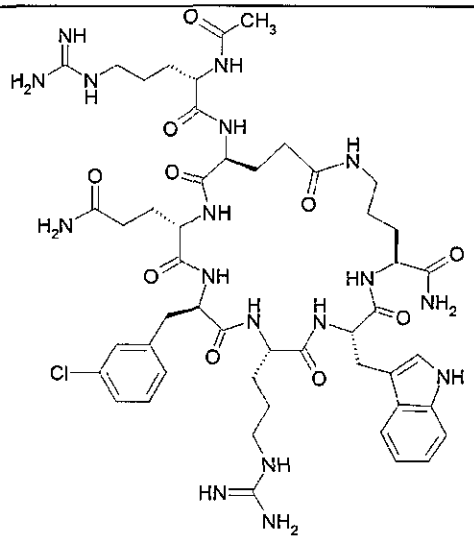
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
27		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:32)	0.667	-
28		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-NH_2)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:33)	48	-

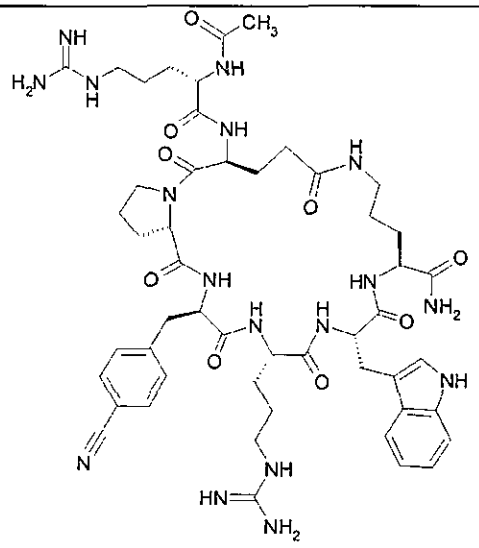
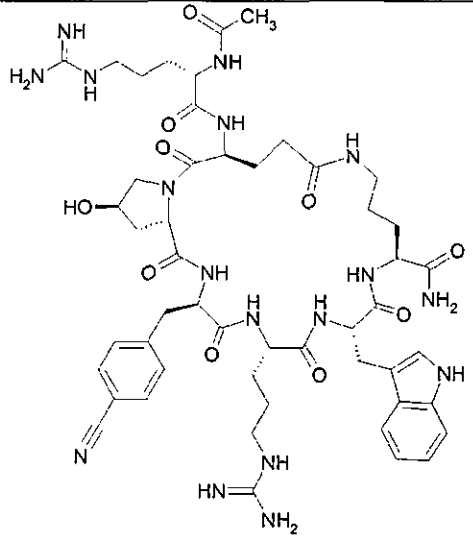
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
29		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-Ph)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:34)	0.5	-
30		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(4-tBu)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:35)	4	-

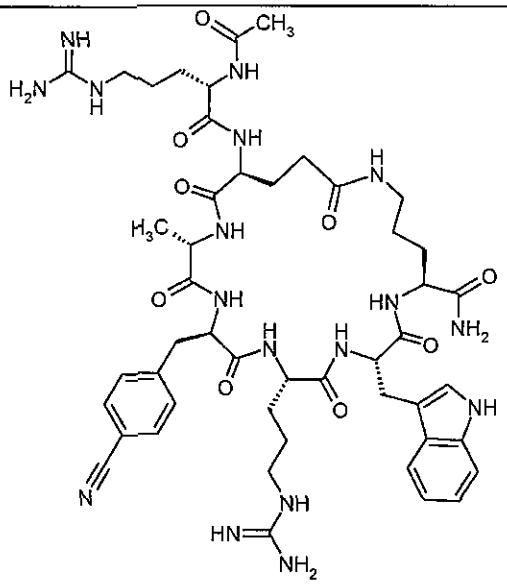
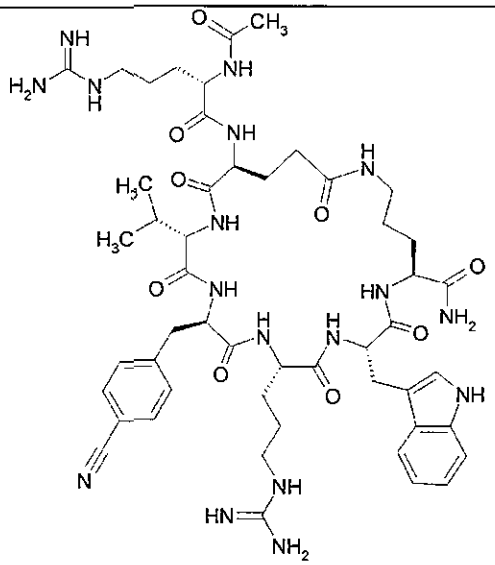
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
31		Ac-Arg-*cyclo*(Glu-Ser-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:36)	8	300
32		Ac-Arg-*cyclo*(Glu-Thr-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:37)	11	540

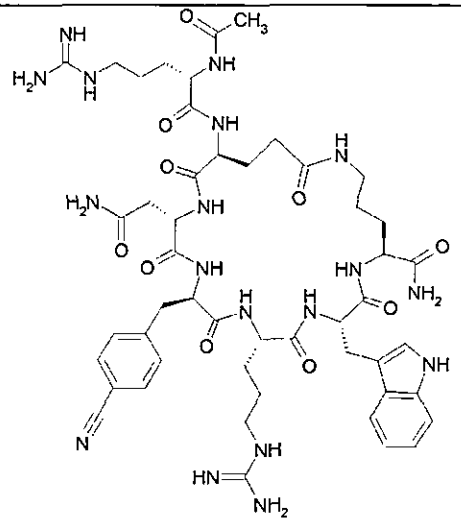
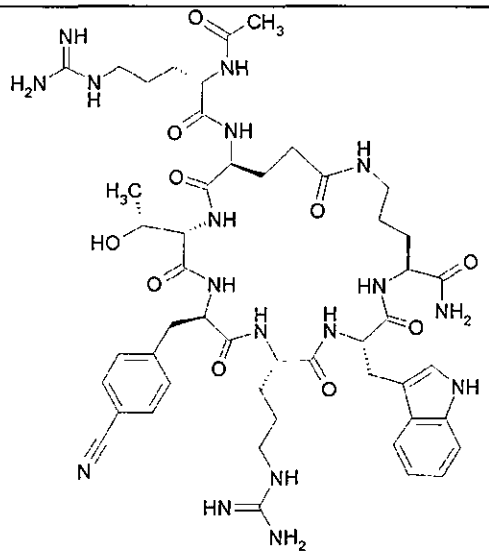
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
33		Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:38)	1	143
34		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:39)	7	128

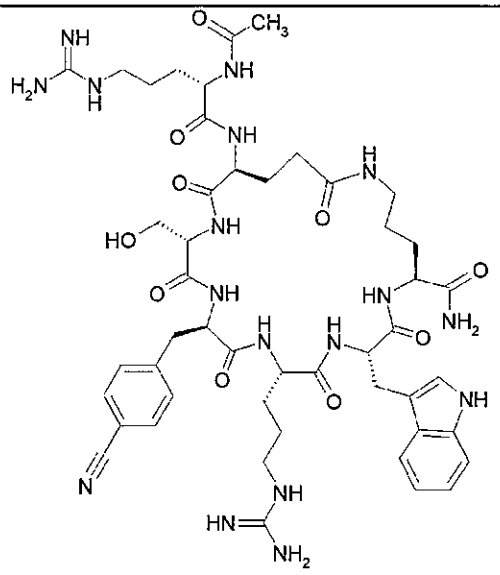
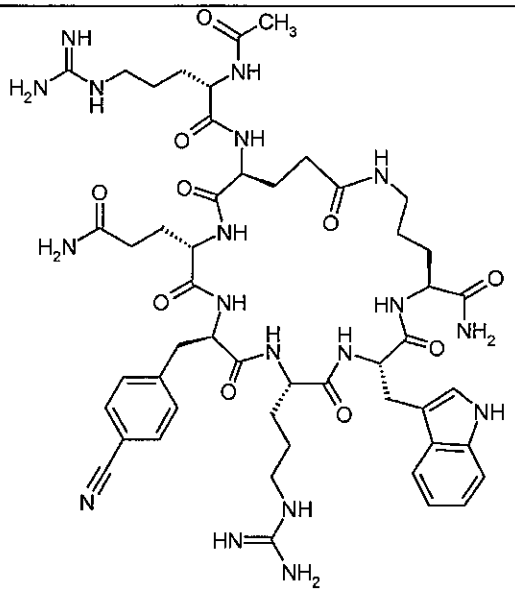
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
35		Ac-Arg-*cyclo*(Glu-Scr-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:40)	10	810
36		Ac-Arg-*cyclo*(Glu-Thr-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:41)	6	380

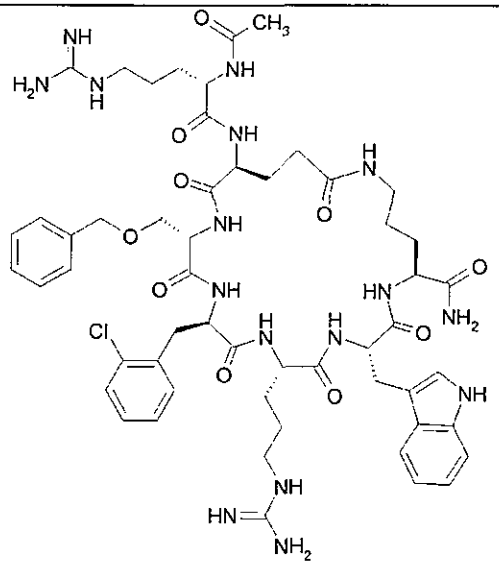
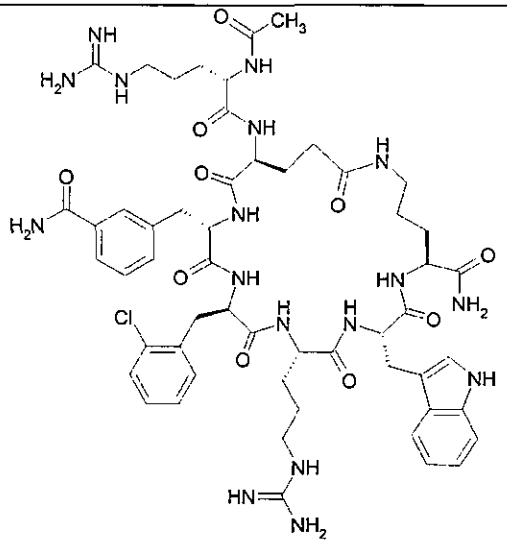
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
37		Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:42)	2	430
38		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:43)	3	290

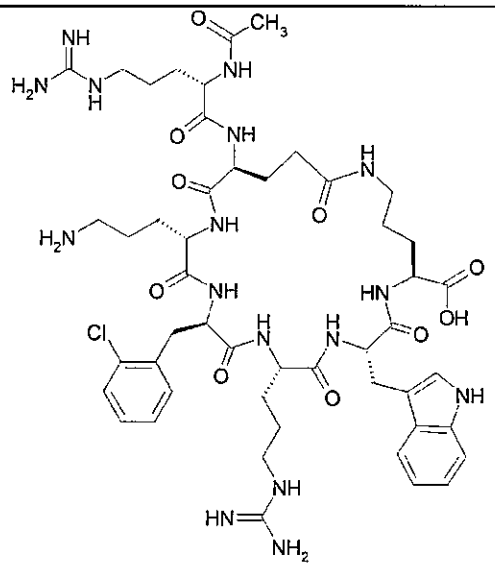
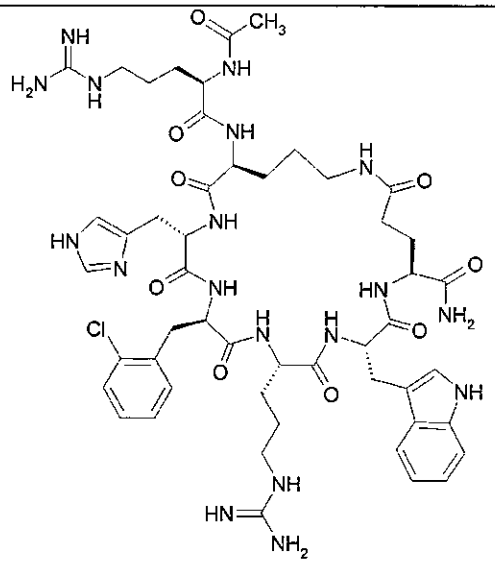
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
39		Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:44)	4	1550
40		Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:45)	2	880

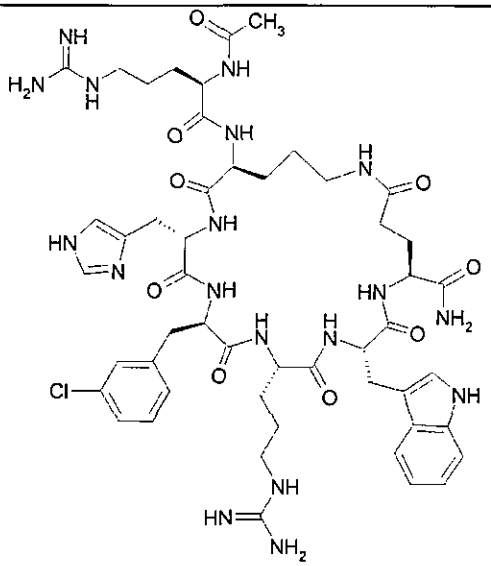
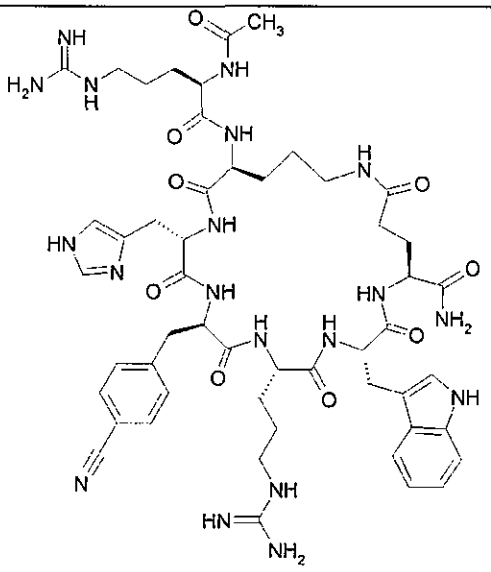
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
41		Ac-Arg-*cyclo*(Glu-Ala-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:46)	3	4300
42		Ac-Arg-*cyclo*(Glu-Val-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:47)	6	420

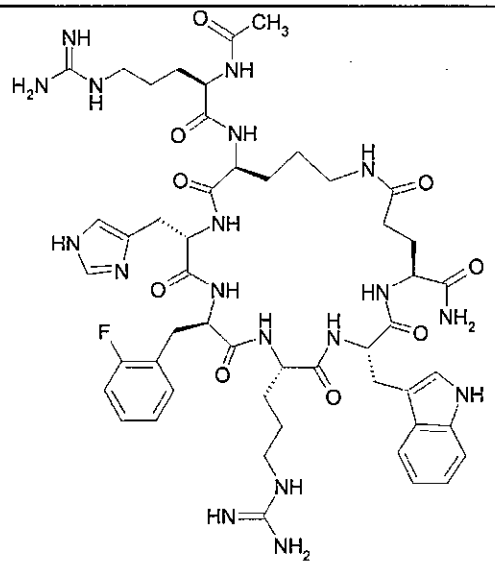
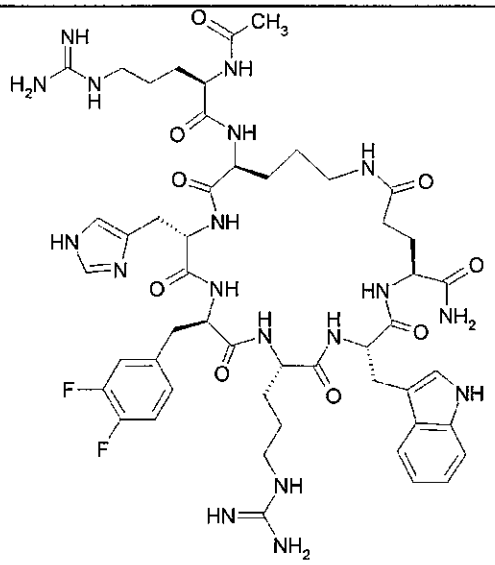
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
43		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:48)	5	870
44		Ac-Arg-*cyclo*(Glu-Thr-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:49)	18	1625

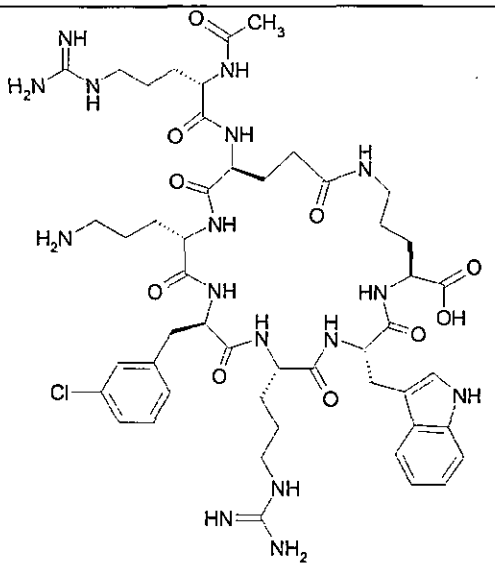
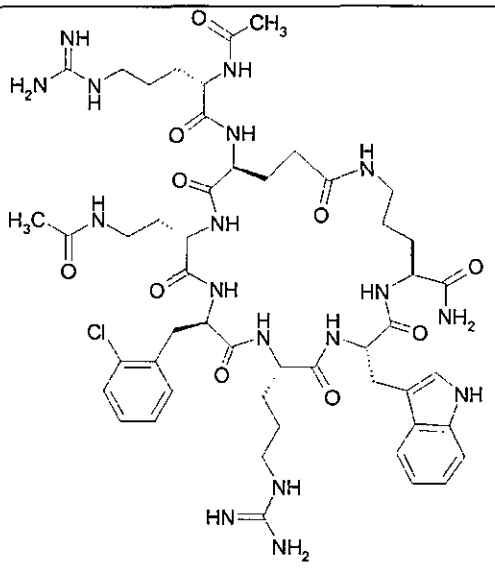
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
45		Ac-Arg-*cyclo*(Glu-Ser-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:50)	10	10000
46		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:51)	4	425

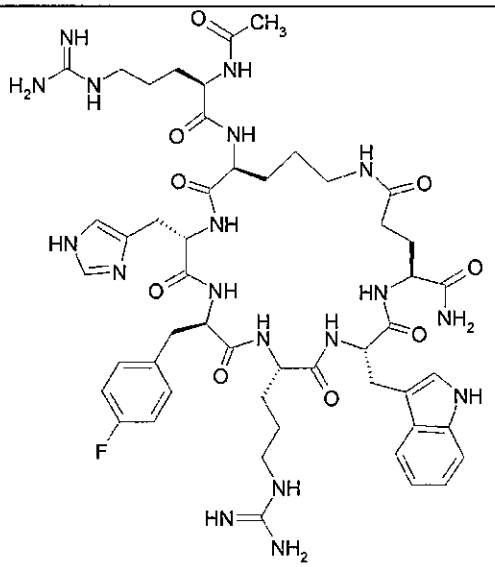
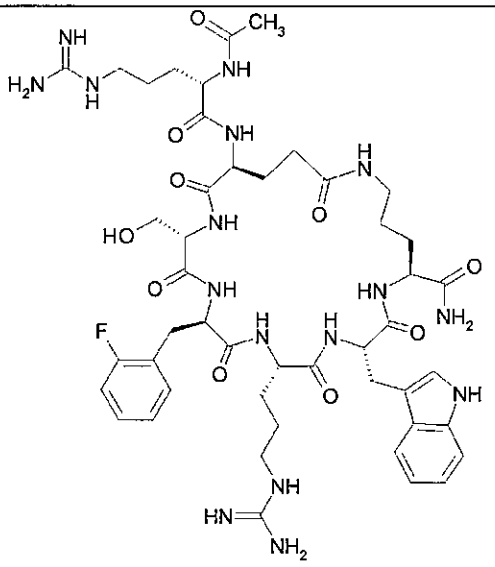
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
47		Ac-Arg-*cyclo*(Glu-Ser(Bzl)-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:52)	0.3	25
48		Ac-Arg-*cyclo*(Glu-Phe(3-C(=O)-NH_2)-D-Phe(2-Cl)-D-Phe-Arg-Trp-Orn)-NH_2 (SEQ ID NO:53)	15	545

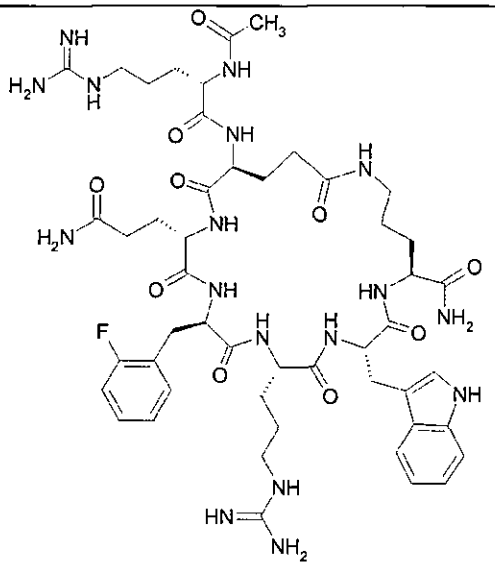
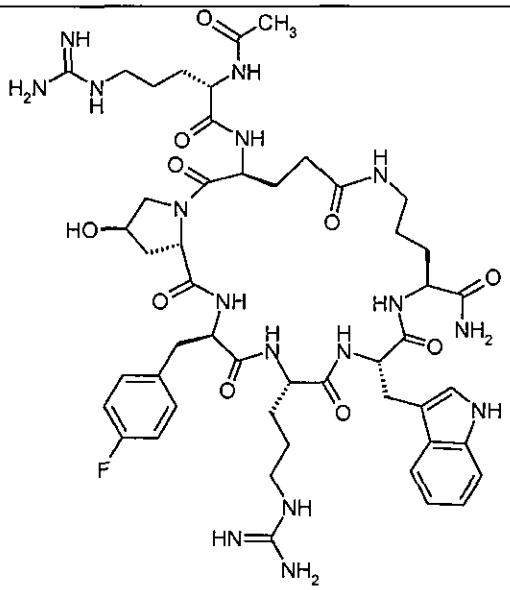
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
49		Ac-Arg-*cyclo*(Glu-Orn-D-Phe(2-Cl)-Arg-Trp-Orn)-OH (SEQ ID NO:54)	8	8
50		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(2-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:55)	1	2

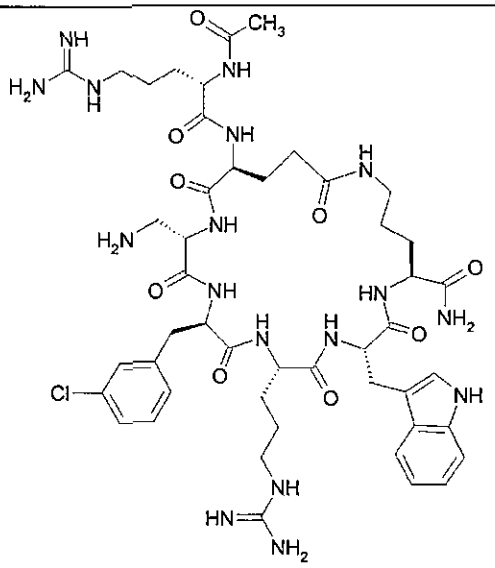
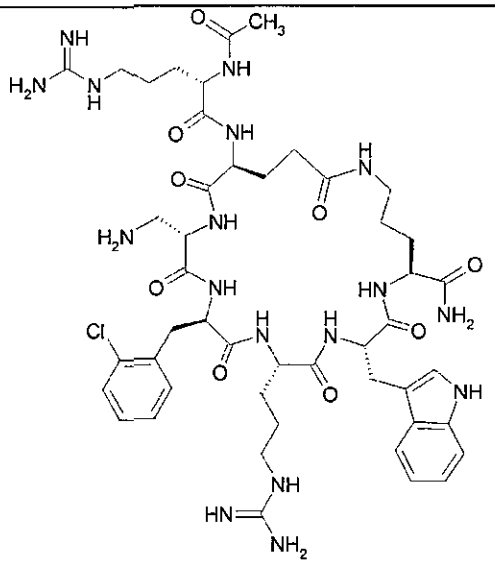
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
51		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(3-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:56)	0.6	3
52		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(4-CN)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:57)	0.6	6

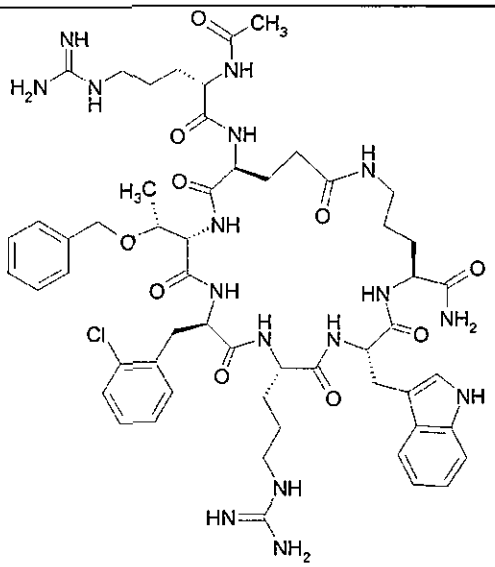
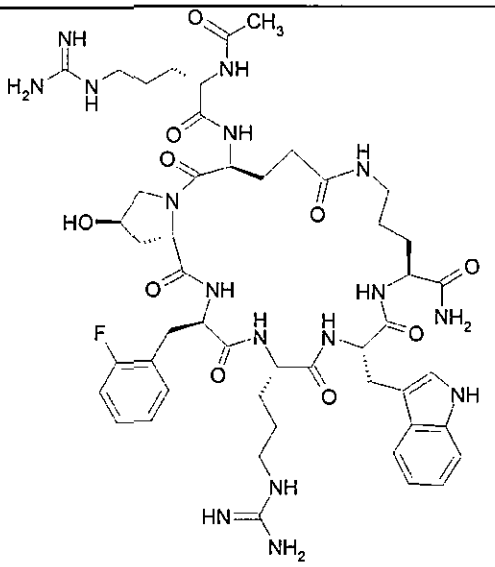
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
53		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(2-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:58)	0.95	0.75
54		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(3,4-diF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:59)	0.25	0.7

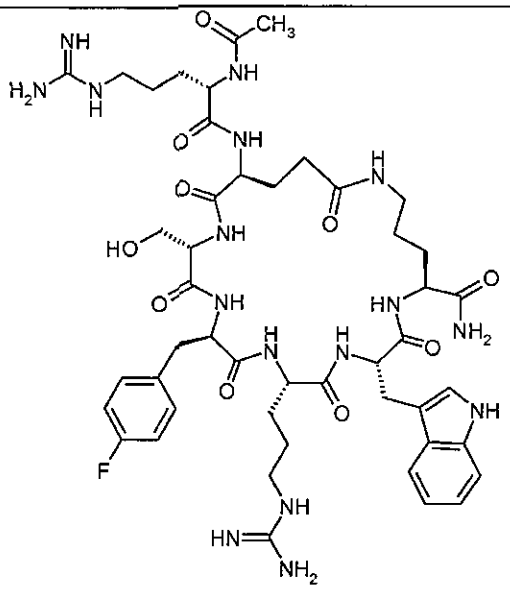
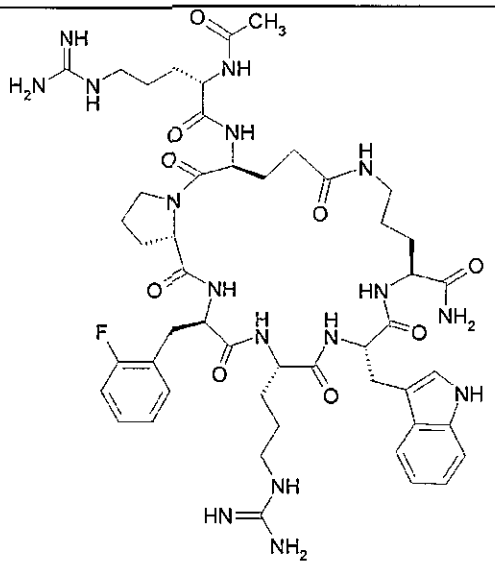
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
55		Ac-Arg-*cyclo*(Glu-Orn-D-Phe(3-Cl)-Arg-Trp-Orn)-OH (SEQ ID NO:60)	20	23
56		Ac-Arg-*cyclo*(Glu-Dab(Acetyl)-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:61)	11	30

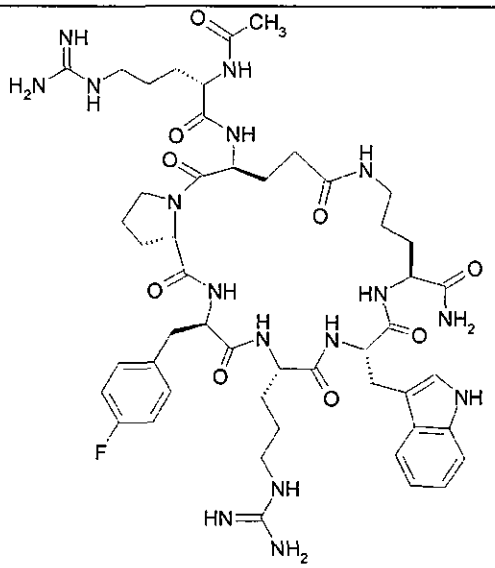
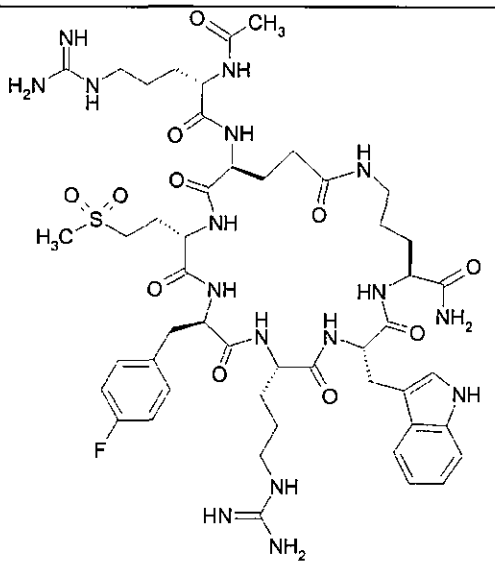
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
57		Ac-D-Arg-*cyclo*(Orn-His-D-Phe(4-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:62)	0.65	1
58		Ac-Arg-*cyclo*(Glu-Ser-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:63)	15	138

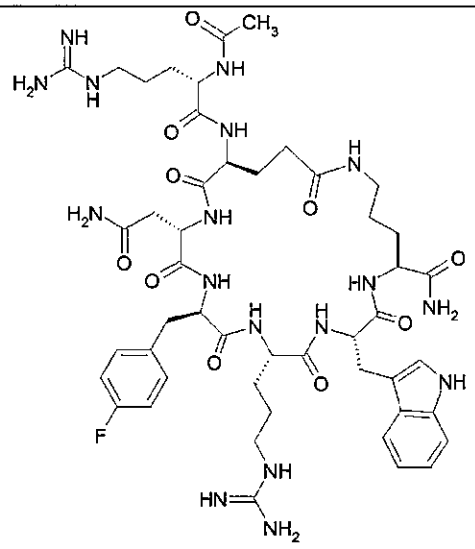
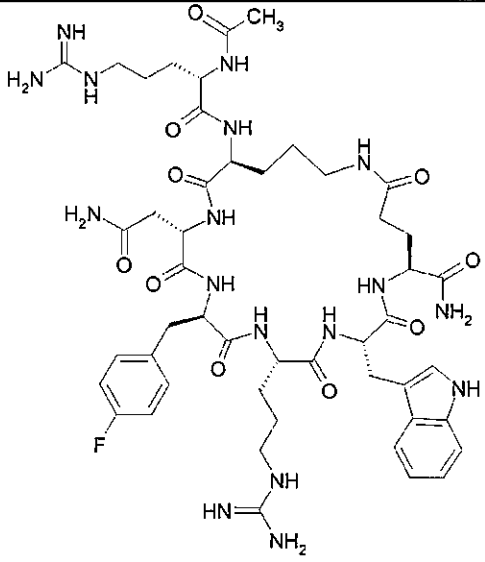
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
59		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:64)	7	58
60		Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:65)	1	89

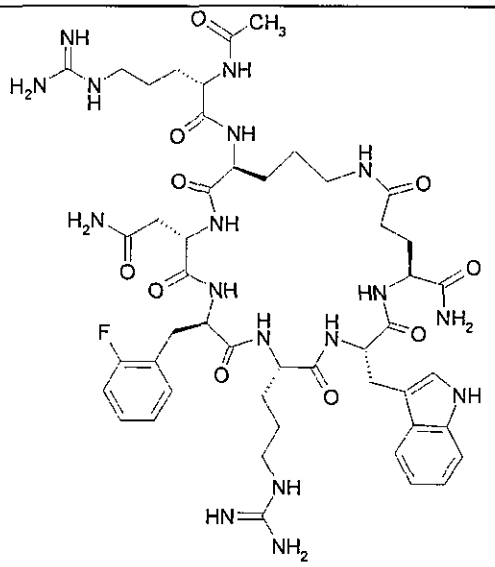
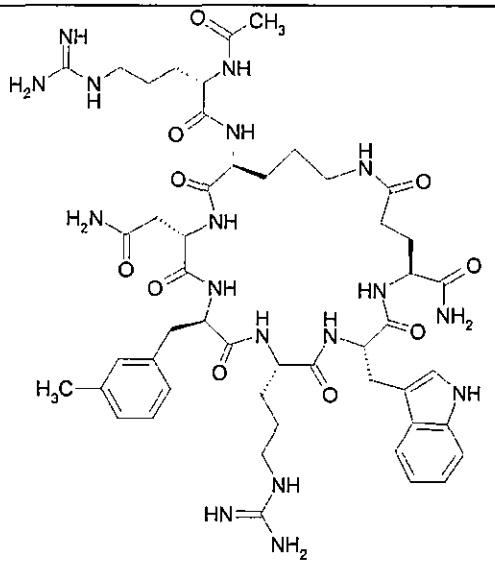
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
61		Ac-Arg-*cyclo*(Glu-Dap-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:66)	4	25
62		Ac-Arg-*cyclo*(Glu-Dap-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:67)	9	28

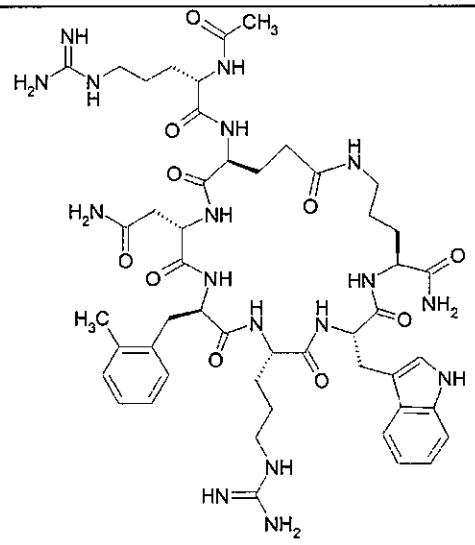
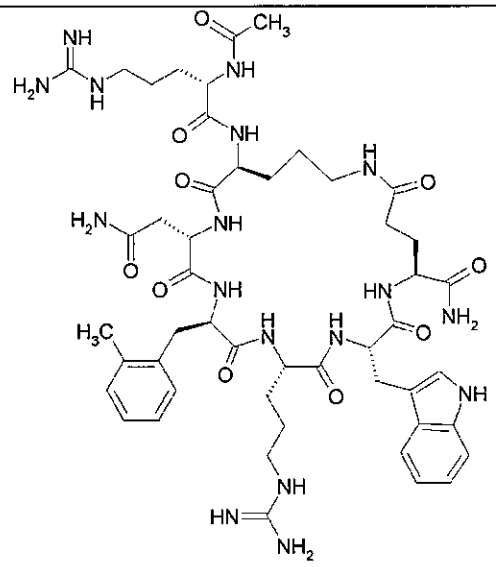
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
63		Ac-Arg-*cyclo*(Glu-Thr(Bzl)-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:68)	0.35	12
64		Ac-Arg-*cyclo*(Glu-Hyp-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:69)	3	53

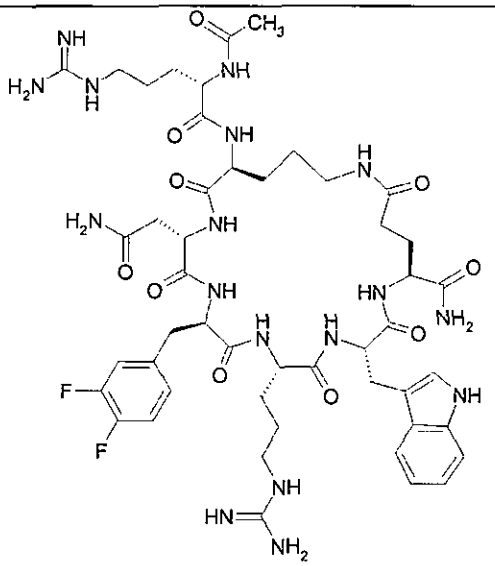
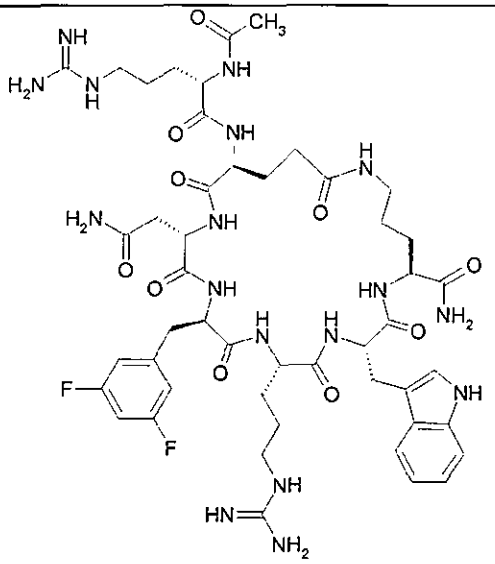
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
65		Ac-Arg-*cyclo*(Glu-Ser-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:70)	5	43
66		Ac-Arg-*cyclo*(Glu-Pro-D-Phe(2-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:71)	6	95

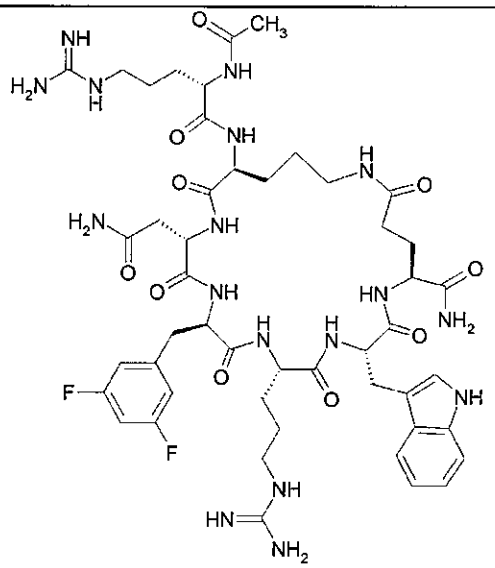
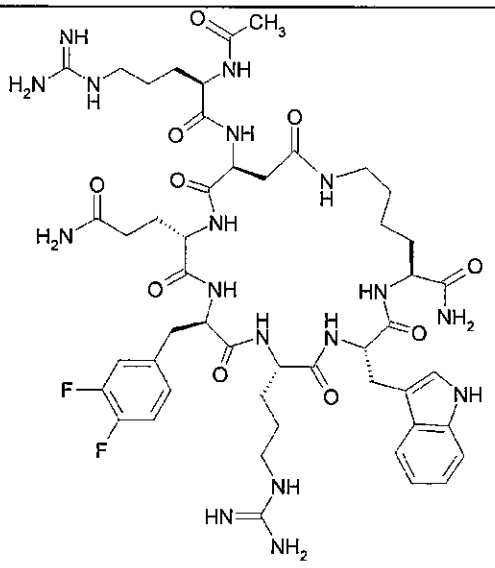
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
67		Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:72)	5	130
68		Ac-Arg-*cyclo*(Glu-Met(O_2)-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:73)	2	33

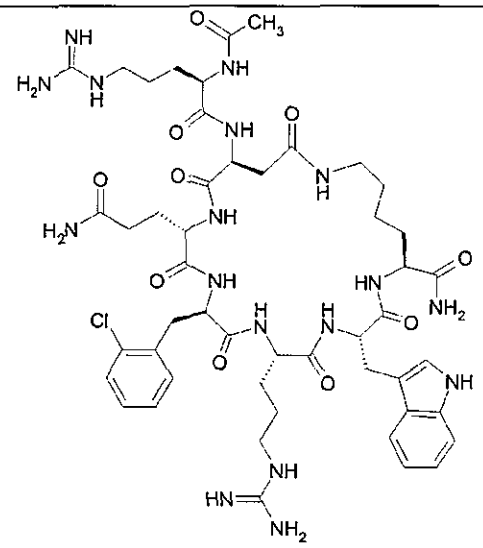
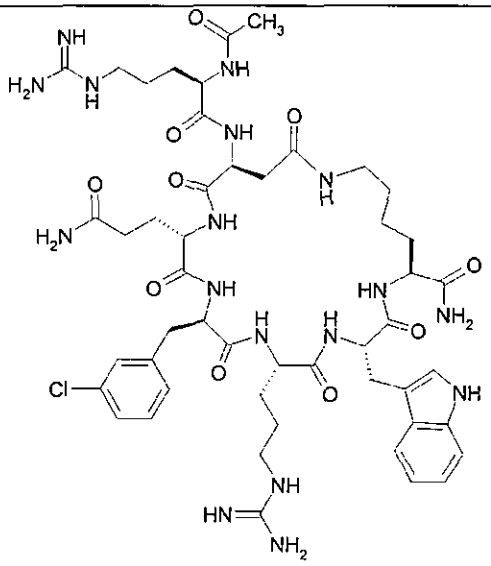
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
69		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:74)	3	150
70		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(4-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:75)	3	170

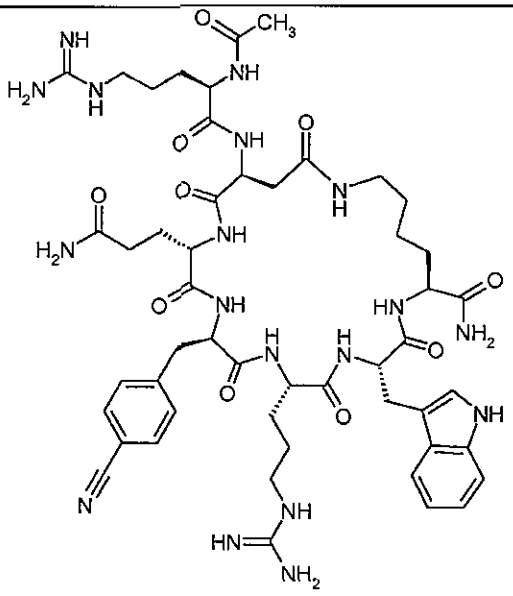
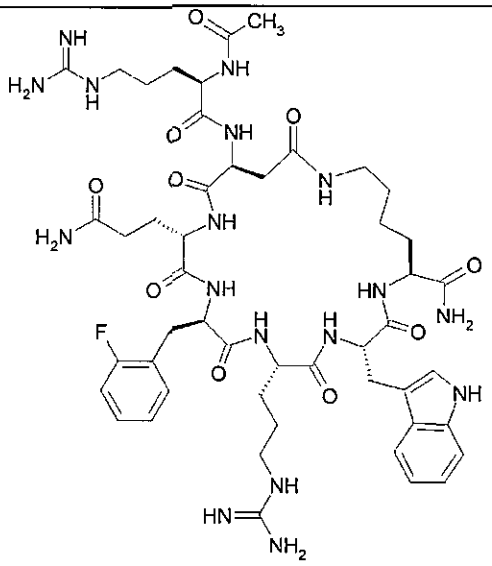
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
71		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:76)	9	335
72		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:77)	4	2300

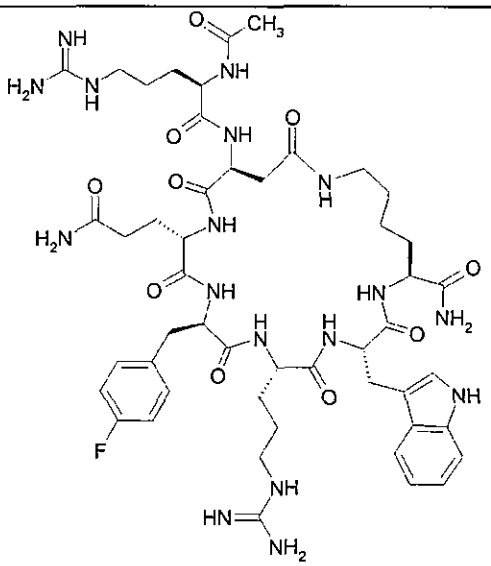
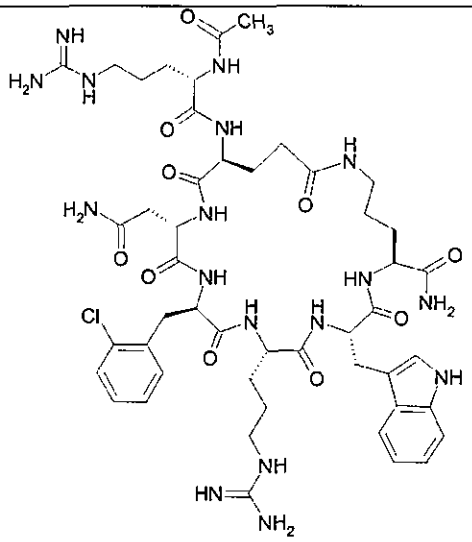
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
73		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:78)	4	160
74		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:79)	5	405

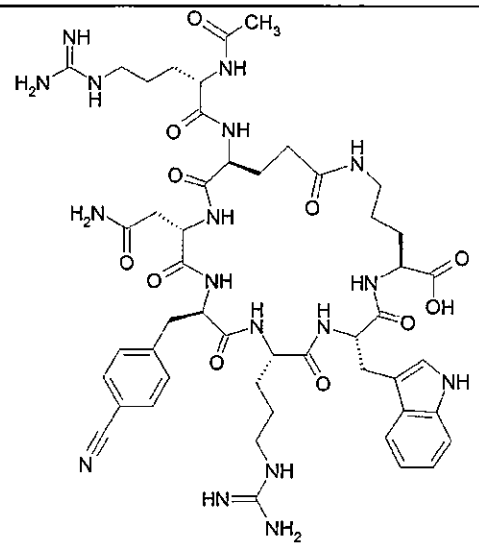
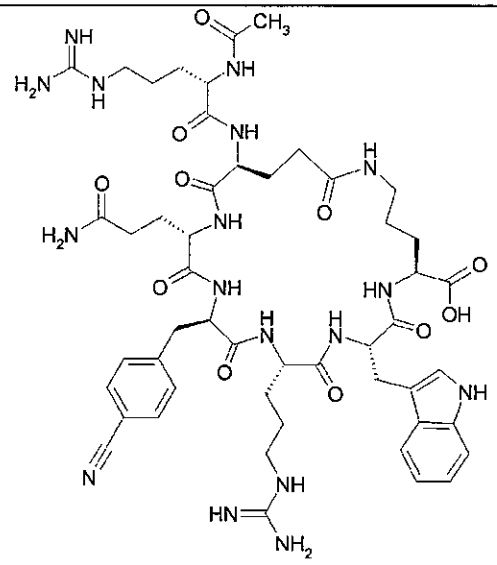
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
75		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,4-diF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:80)	3	190
76		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(3,5-diF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:81)	20	260

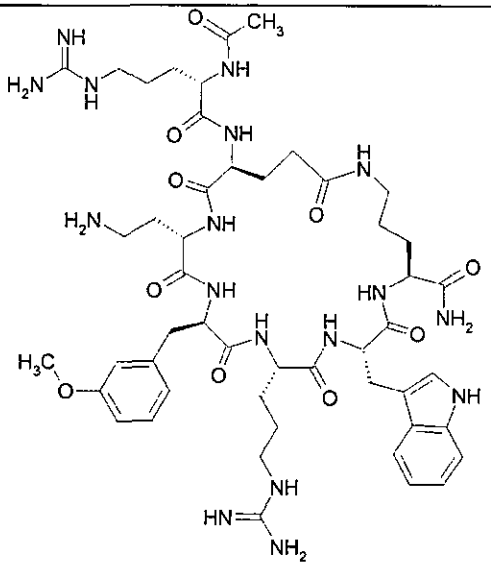
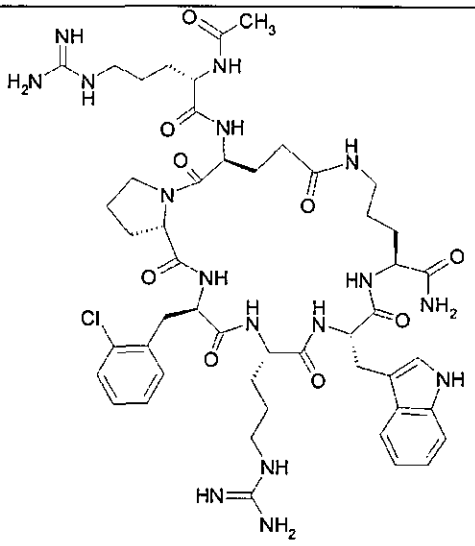
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
77		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,5-diF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:82)	8	190
78		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(3,4-diF)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:83)	8	515

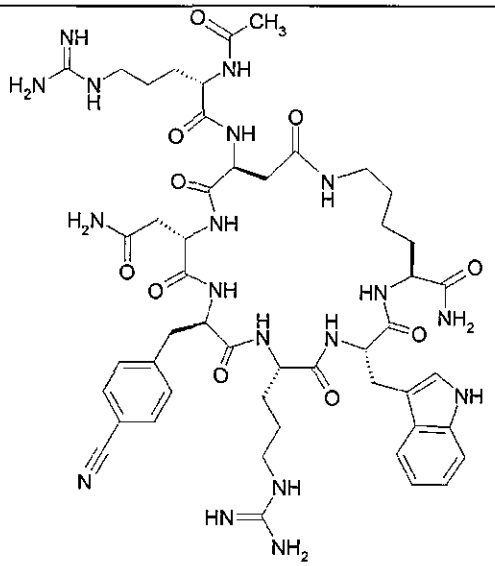
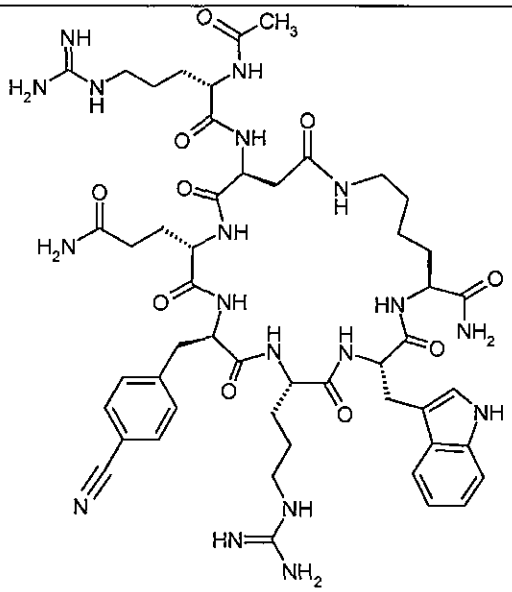
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
79		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(2-Cl)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:84)	10	395
80		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(3-Cl)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:85)	7	1750

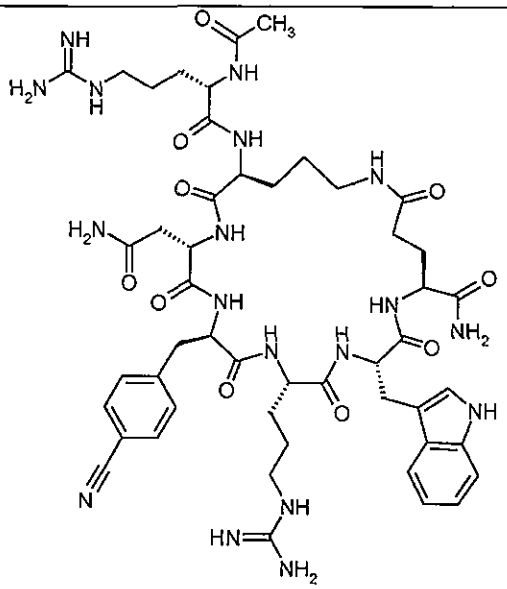
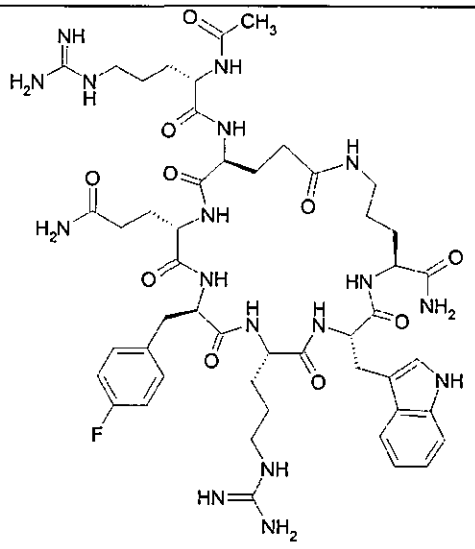
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
81		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(4-CN)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:86)	13	10000
82		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(2-F)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:87)	10	220

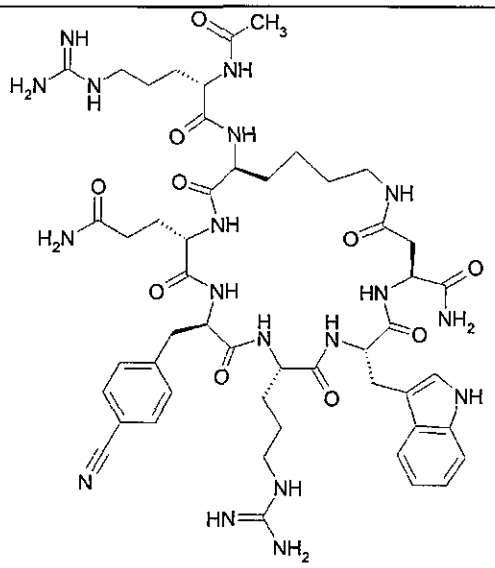
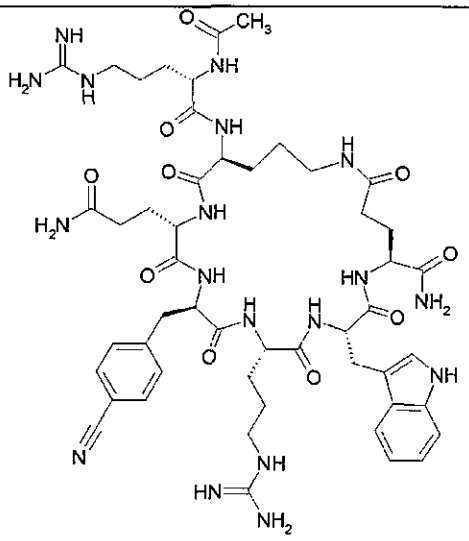
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
83		Ac-D-Arg-*cyclo*(Asp-Gln-D-Phe(4-F)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:88)	6	260
84		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:89)	7	245

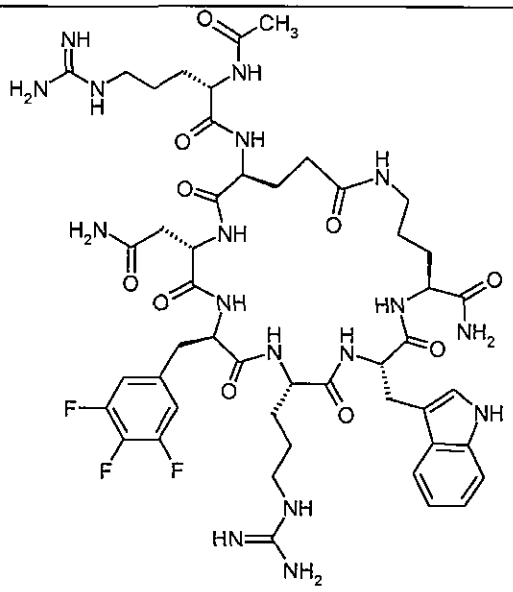
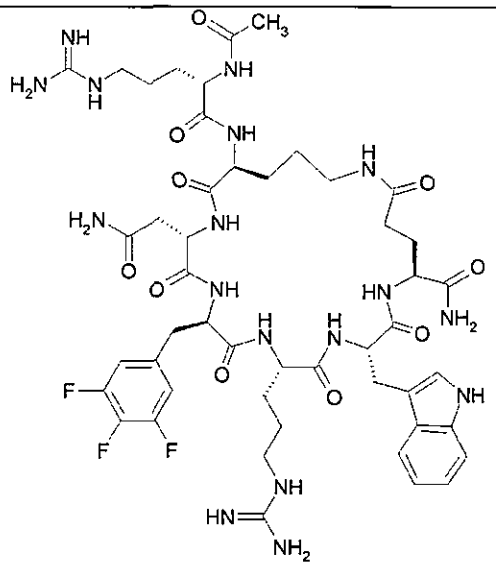
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
85		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:90)	64	10000
86		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:91)	40	3968

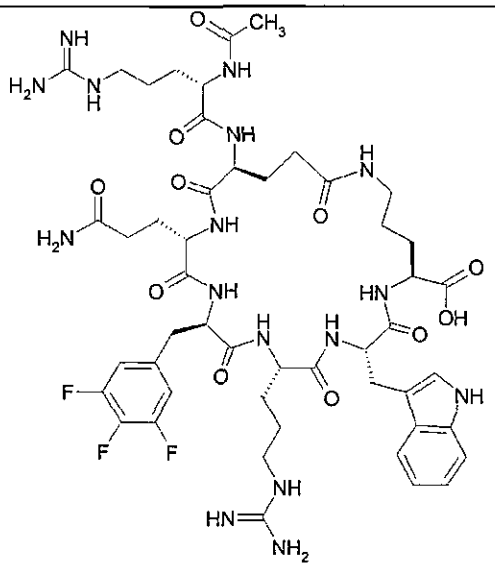
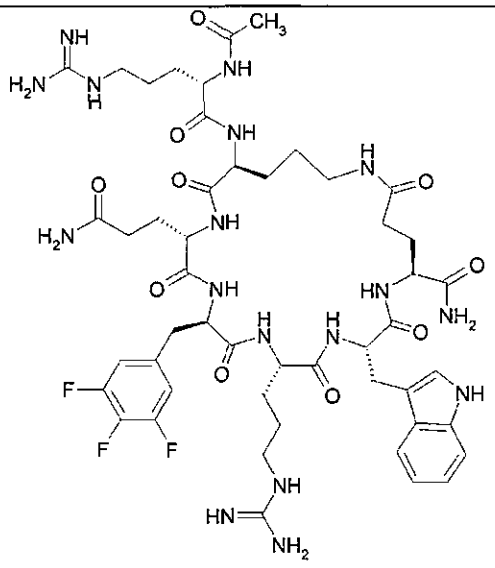
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
87		Ac-Arg-*cyclo*(Glu-Dab-D-Phe(3-OMe)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:92)	3	-
88		Ac-Arg-*cyclo*(Glu-Pro-D-Phe(2-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:93)	5	-

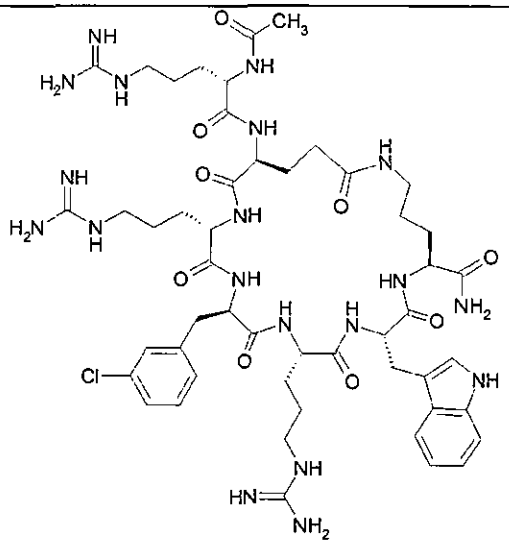
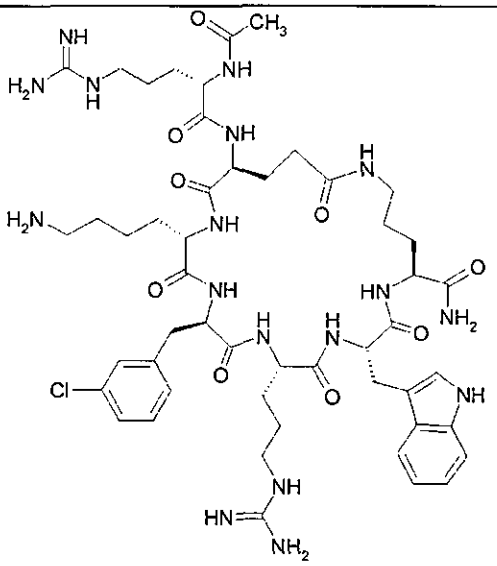
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
89		Ac-Arg-*cyclo*(Asp-Asn-D-Phe(4-CN)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:94)	48	-
90		Ac-Arg-*cyclo*(Asp-Gln-D-Phe(4-CN)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:95)	5	-

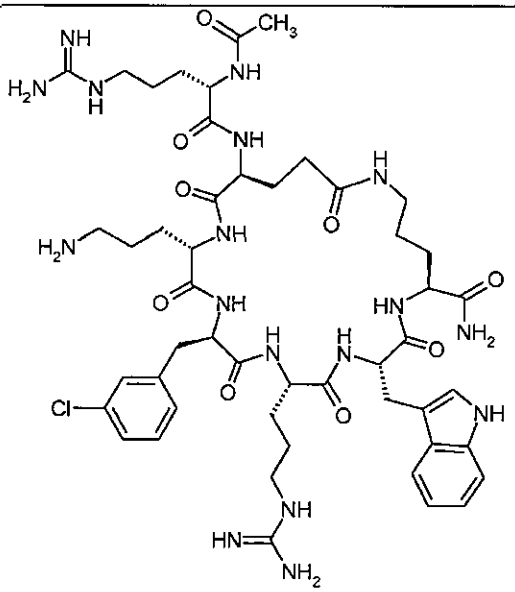
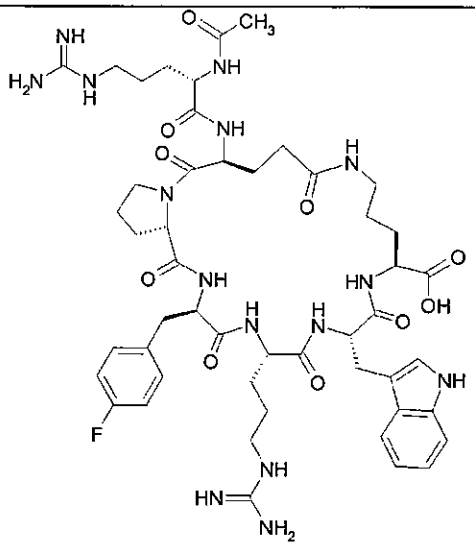
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
91		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(4-CN)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:96)	4	-
92		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:97)	2	-

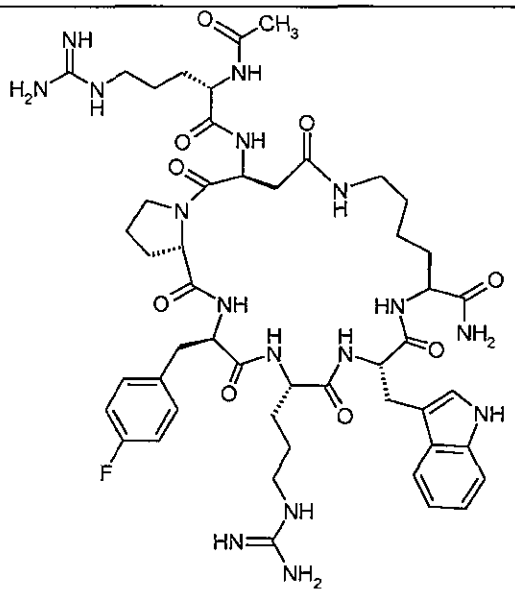
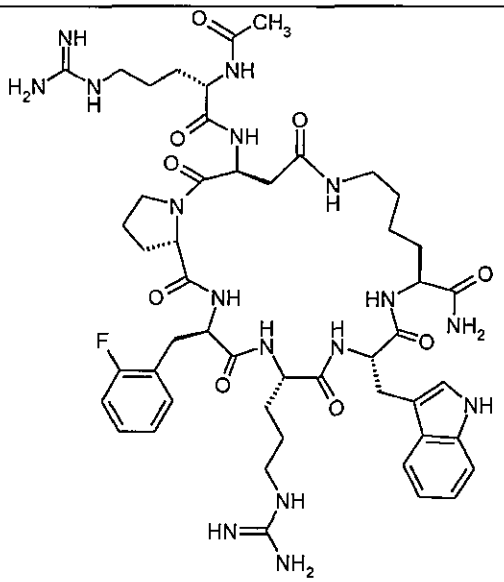
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
93		Ac-Arg-*cyclo*(Lys-Gln-D-Phe(4-CN)-Arg-Trp-Asp)-NH_2 (SEQ ID NO:98)	1	-
94		Ac-Arg-*cyclo*(Orn-Gln-D-Phe(4-CN)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:99)	0.9	-

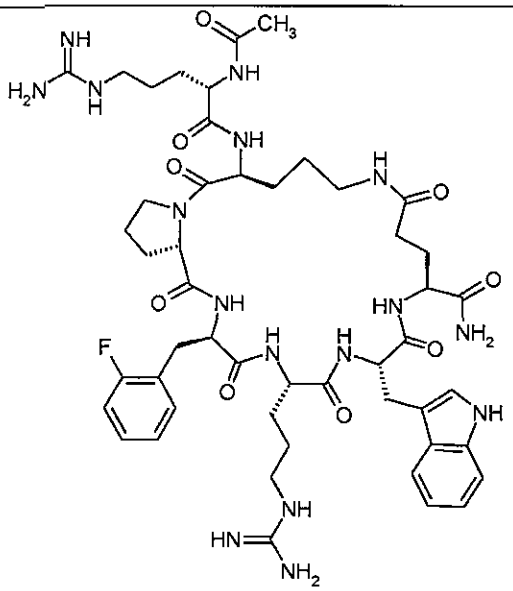
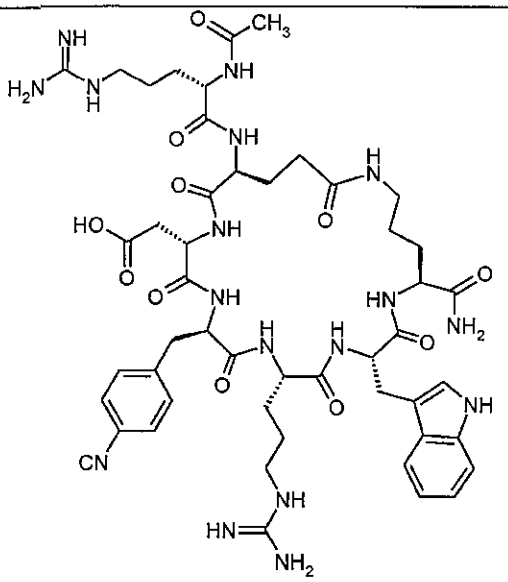
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
95		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:100)	3	-
96		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(3,4,5-triF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:101)	2	-

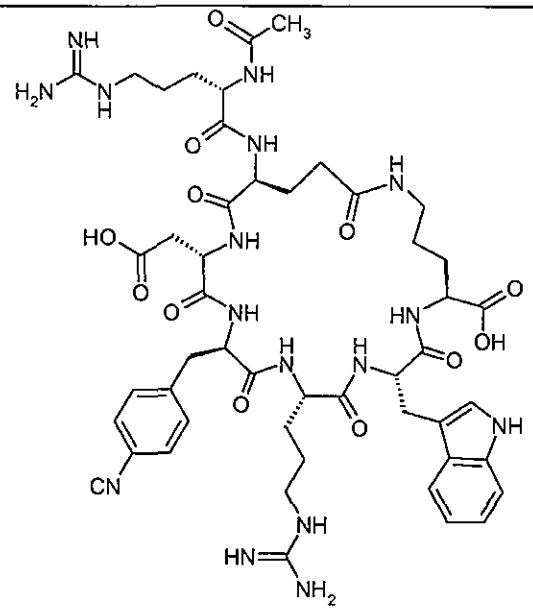
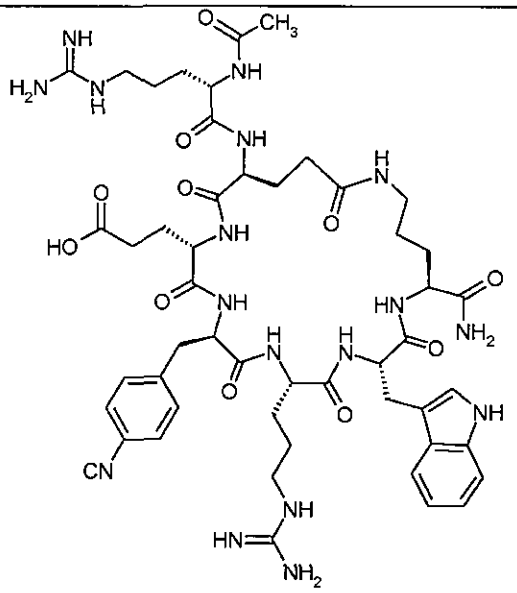
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
97		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(3,4,5-triF)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:102)	0.75	-
98		Ac-Arg-*cyclo*(Orn-Gln-D-Phe(3,4,5-triF)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:103)	0.75	-

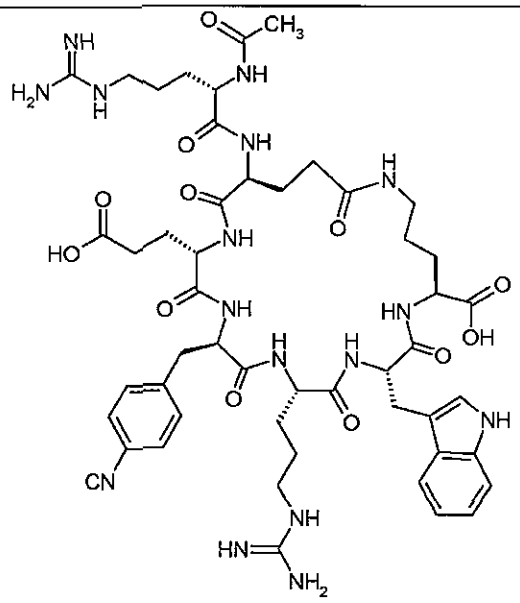
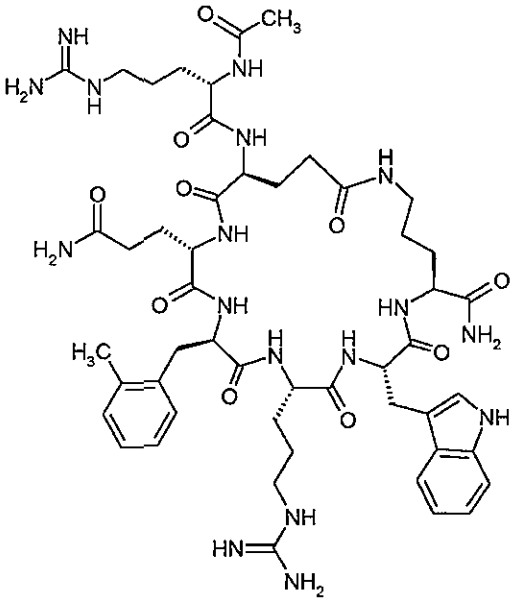
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
99		Ac-Arg-*cyclo*(Glu-Arg-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:104)	0.5	-
100		Ac-Arg-*cyclo*(Glu-Lys-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:105)	4	-

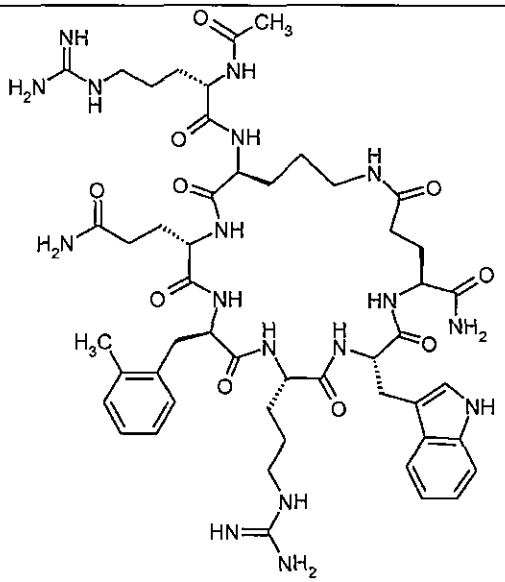
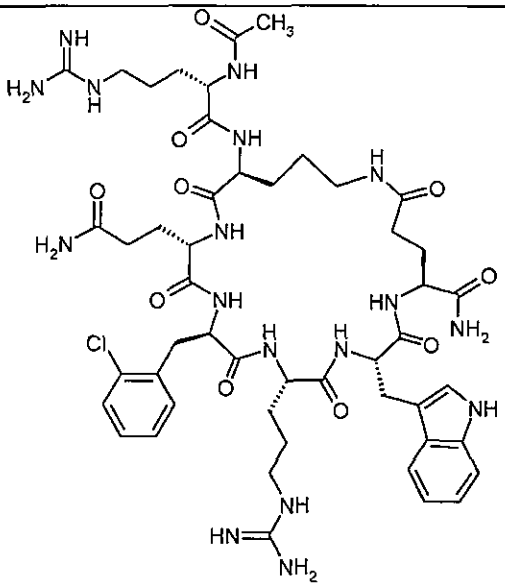
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
101		Ac-Arg-*cyclo*(Glu-Orn-D-Phe(3-Cl)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:106)	1	-
102		Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-OH (SEQ ID NO:107)	30	730

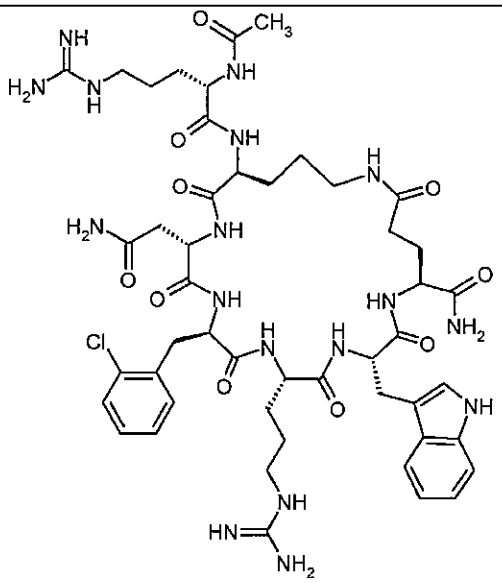
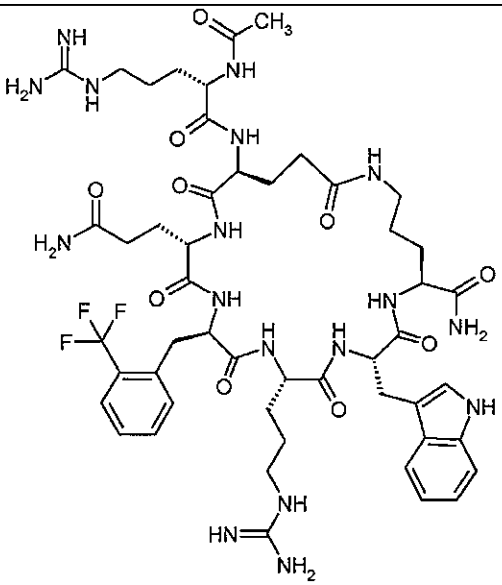
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
103		Ac-Arg-*cyclo*(Asp-Pro-D-Phe(4-F)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:108)	4	-
104		Ac-Arg-*cyclo*(Asp-Pro-D-Phe(2-F)-Arg-Trp-Lys)-NH_2 (SEQ ID NO:109)	78	-

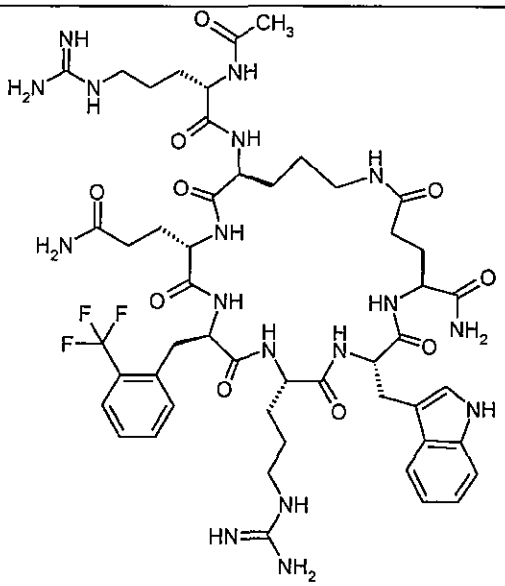
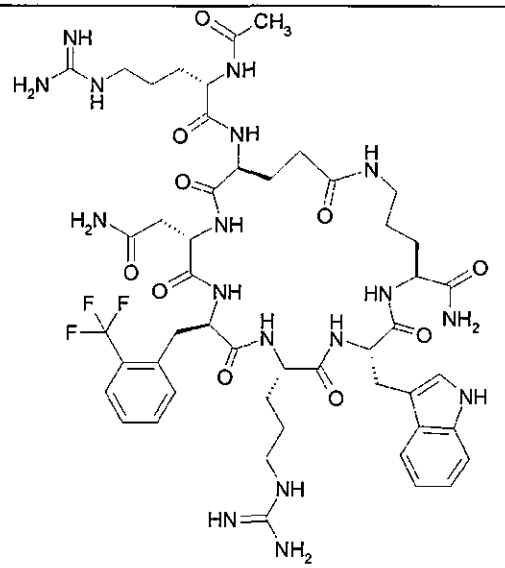
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
105		Ac-Arg-*cyclo*(Orn-Pro-D-Phe(2-F)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:110)	7	-
106		Ac-Arg-*cyclo*(Glu-Asp-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:111)	647	10000

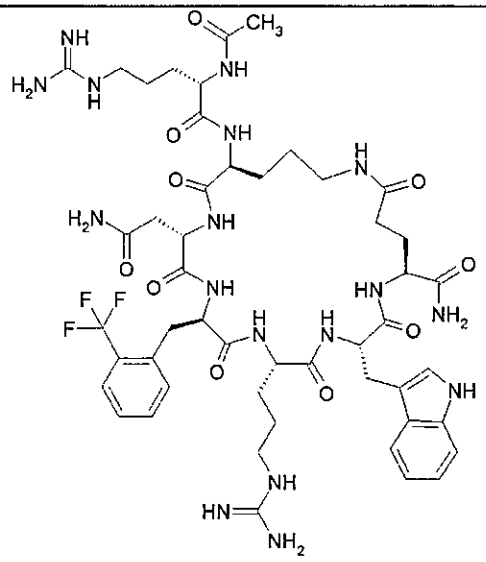
No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
107		Ac-Arg-*cyclo*(Glu-Asp-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:112)	6463	10000
108		Ac-Arg-*cyclo*(Glu-Glu-D-Phe(4-CN)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:113)	128	10000

No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
109		Ac-Arg-*cyclo*(Glu-Glu-D-Phe(4-CN)-Arg-Trp-Orn)-OH (SEQ ID NO:114)	1090	10000
110		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-Me)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:115)	5	105

No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
111		Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-Me)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:116)	6	145
112		Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:117)	4	83

No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
113		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-Cl)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:118)	7	255
114		Ac-Arg-*cyclo*(Glu-Gln-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:119)	8	93

No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
115		Ac-Arg-*cyclo*(Orn-Gln-D-Phe(2-CF_3)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:120)	4	235
116		Ac-Arg-*cyclo*(Glu-Asn-D-Phe(2-CF_3)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:121)	7	380

No.	Structure	Amino Acid Sequence	MC4-R Ki (nM)	MC1-R Ki (nM)
117		Ac-Arg-*cyclo*(Orn-Asn-D-Phe(2-CF_3)-Arg-Trp-Glu)-NH_2 (SEQ ID NO:122)	5	540

7.0 Tests and Assays Employed in Evaluation of the Peptides of the Present Invention.

The melanocortin receptor-specific peptides of this invention may be tested by a variety of assay systems and animal models to determine binding, functional status and efficacy.

7.1 Competitive Inhibition Assay using [I^{125}]-NDP-α-MSH.

A competitive inhibition binding assay was performed for exemplified peptides according to the invention using membrane homogenates prepared from HEK-293 cells that express recombinant hMC4-R, hMC3-R, or hMC5-R, and from B-16 mouse melanoma cells (containing endogenous MC1-R). In some instances, HEK-293 cells that express recombinant hMC1-R were employed. Assays were performed in 96 well GF/B Millipore multiscreen filtration plates (MAFB NOB10) pre-coated with 0.5% bovine serum albumin (Fraction V). Membrane homogenates were incubated with 0.2nM (for hMC4-R) 0.4 nM (for MC3-R and MC5-R) or 0.1 nM (for mouse B16 MC1-R or hMC1-R) [I^{125}]-NDP-α-MSH (Perkin Elmer) and increasing concentrations of test peptides of the present invention in buffer containing 25 mM HEPES buffer (pH 7.5) with 100 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.3 mM 1,10-phenanthroline, and 0.2% bovine serum albumin. After incubation for 60 minutes at 37° C, the assay mixture was filtered and the

membranes washed three times with ice-cold buffer. Filters were dried and counted in a gamma counter for bound radioactivity. Non-specific binding was measured by inhibition of binding of [I^{125}]-NDP-α-MSH in the presence of 1 μM NDP-α-MSH. Maximal specific binding (100%) was defined as the difference in radioactivity (cpm) bound to cell membranes in the absence and presence of 1 μM NDP-α-MSH. Radioactivity (cpm) obtained in the presence of test peptides was normalized with respect to 100% specific binding to determine the percent inhibition of [I^{125}]-NDP-α-MSH binding. Each assay was conducted in triplicate and the actual mean values are described, with results less than 0% reported as 0%. Ki values for test peptides of the present invention were determined using Graph-Pad Prism® curve-fitting software. Results from this assay are presented herein (see 6.1). For some peptides, a mean value of more than two values is presented.

7.2 Assay for Agonist Activity.

Accumulation of intracellular cAMP was examined as a measure of the ability of peptides of the present invention to elicit a functional response in HEK-293 cells that express MC4-R. Confluent HEK-293 cells that express recombinant hMC4-R were detached from culture plates by incubation in enzyme-free cell dissociation buffer. Dispersed cells were suspended in Earle's Balanced Salt Solution containing 10 mM HEPES (pH 7.5), 1 mM $MgCl_2$, 1mM glutamine, 0.5% albumin and 0.3 mM 3-isobutyl-1-methyl-xanthine (IBMX), a phosphodiesterase inhibitor. The cells were plated in 96-well plates at a density of 0.5 x 10^5 cells per well and pre-incubated for 10 minutes. Cells were exposed for 15 minutes at 37° C to peptides of the present invention dissolved in DMSO (final DMSO concentration of 1%) at a concentration range of 0.05 - 5000 nM in a total assay volume of 200 μL. NDP-α-MSH was used as the reference agonist. cAMP levels were determined by an HTRF® cAMP cell-based assay system from Cisbio Bioassays utilizing cryptate-labeled anti-cAMP and d2-labeled cAMP, with plates read on a Perkin-Elmer Victor plate reader at 665 and 620nM. Data analysis was performed by nonlinear regression analysis with Graph-Pad Prism® software. The maximum efficacies of test peptides of the present invention were compared to that achieved by the reference melanocortin agonist NDP-α-MSH, used as full agonist benchmark in this context.

7.3 High and Low Density hMC4-R Functional Assay.

A HEK293 cell line transfected with human MC4-R (Palatin Technologies, US, with license from the University of Michigan) was used. The human MC4-R was

introduced to HEK293 by using the T-REx™ System, Invitrogen. The T-REx™ System employs a tetracycline-regulated mammalian expression system that uses regulatory elements from the *E. coli* Tn*10*-encoded tetracycline (Tet) resistance operon. By use of the T-REx™ System, expression of the gene of interest, the human MC4-R gene, is repressed in the absence of tetracycline or doxycycline and induced in the presence of tetracycline or doxycycline (see T-REx™ System Manual, published by Invitrogen).

HEK293-T-REx-MC4-R cells were cultured in DMEM (Gibco 11965), supplemented with L-Glutamine (Gibco 25030), 10% fetal bovine serum (FBS), 200 µg/mL Zeocin (Invitrogen 46-0072) and 6 µg/mL Blasticidin (Invitrogen 46-1120) in 5% CO_2 and 95% humidity at 37°C. T-150 flasks of cells at 75% confluence were incubated with two concentrations of doxycycline (0.1 ng/mL to provide a low density hMC4-R system and 10 ng/mL to provide a high density hMC4-R system) in 5% CO_2 at 37°C for 16-18 hours to induce MC4-R expression. On the day of the assay, the cells were washed with PBS (Gibco 14190) and harvested using cell dissociation buffer (Gibco 13150-016), then centrifuged and resuspended in Hanks' Balanced Salt Solution (+Ca, +Mg) (Gibco 14025), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4) (Sigma H0887), 1mM L-Glutamine (Gibco 25030), 1 mg/mL bovine serum albumin (BSA) (Sigma A3311) and 0.3 mM 3-isobutyl-1-methyl-xanthine (IBMX). Cells were then counted and volume was adjusted to 2.5 x 10^5 cells per ml.

The cells were then dispensed into 96-well plates (BD 353916) in 198 µL (about 5 x 10^4) cells/well and incubated for 10 minutes at 37°C. The compound to be tested was diluted with DMSO to a final concentration of 1 mM. Serial dilution was prepared in polypropylene removable 12-well library tube strips (VWR cat# 83009-682). 120 µL of the 1 mM compound stock was pipetted in the second column on the plate. Using the Janus liquid handler the compound was serially diluted 1:10 (25 µL compound + 225 µL DMSO) to a total of 9 concentrations (representing final assay concentrations ranging from 10^{-5} to 10^{-13} M).

2 µL of the standard, [Nle^4, D-Phe^7]-alpha-Melanocyte Stimulating Hormone (NDP-α-MSH), or compound was added to the 96-well plate using the Janus robotic system. All assay samples were run in duplicate (i.e. each sample was in two low dox and two high dox plates, respectively). The plates were gently shaken and incubated for 15 minutes at

37° C. The reaction was stopped by adding 15 μL of lysis buffer per well and the plates were shakened for 30 minutes at room temperature.

Agonist stimulation of the MC4-R activates adenylate cyclase, which is an enzyme that catalyses the formation 3′,5′-cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Thus, agonist stimulation of the MC4-R increases the levels of cAMP. cAMP-levels were measured with the cAMP dynamic 2 HTRF kit (CisBio cat# 62AM4PEC; see manual published by CisBio). cAMP levels were normalised against plate controls (1% DMSO for 0%, 400 nM NDP-α-MSH for 100%) and a calibration curve ranging from 712 nM to 0.04 nM cAMP (essentially as described in the CisBio HTRF kit). The plates were incubated on a shaker at room temperature for 1 hour and read on the Perkin-Elmer Victor plate reader at 665 and 620 nm. Fluoresence ratios were then calculated as described in the CisBio HTRF kit, with GraphPad Prism software used to plot the change in fluorescence percent values versus cAMP concentration using the variable slope dose response curve and, based on calculated cAMP concentrations, to determine EC_{50} values and percent activation. Results from this assay are presented herein (see 8.0).

7.4 Food Intake and Body Weight Change.

Change in food intake and body weight was evaluated for selected peptides administered by subcutaneous injection route. Male Sprague-Dawley rats were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). Animals were individually housed in conventional polystyrene hanging cages and maintained on a controlled 12 hour on/off light cycle. Water and pelleted food (ProLab RMH 2500, W.F. Fisher & Son Inc.) was provided *ad libitum*. The rats were dosed subcutaneously with vehicle (3.2% mannitol/50mM Tris buffer) or selected peptides (1.0 mg/kg). The changes in food intake for the 4 and 24 hour periods after dosing and the changes in body weight for the 24 hour period after dosing were determined. The changes in body weight and food intake for the 48 hour and 72 hour periods after dosing was also measured to determine reversal of changes in body weight and food intake effects back to baseline levels (not shown).

Food intake (FI, 0-4 h and 0-24 h) and body weight (BW, 0-24 h) results from studies with 1.0 mg/kg subcutaneously of Examples 43, 46 and 67 and corresponding vehicle controls run in parallel are shown in Table 2 below, expressed as means ± SEM (n=8-10).

Table 2

Experiment	Treatment	Dose (mg/kg, s.c.)	n	FI 0-4h (g)	FI 0-24h (g)	BW change 0-24h (g)
1	Vehicle		10	10.53 ± 0.78	27.75 ± 1.00	1.49 ± 0.18
1	Ref Ex 2	1.0	10	4.41 ± 0.48	20.84 ± 1.01	-0.38 ± 0.46
1	Ex 43	1.0	10	6.85 ± 0.55	24.44 ± 0.85	0.45 ± 0.35
2	Vehicle		8	7.09 ± 0.39	27.46 ± 1.16	1.96 ± 0.55
2	Ref Ex 2	1.0	8	2.80 ± 0.27	21.81 ± 1.04	0.85 ± 0.45
2	Ex 46	1.0	8	3.71 ± 0.68	20.56 ± 1.14	-0.29 ± 0.55
3	Vehicle		8	7.76 ± 0.53	25.71 ± 0.25	2.25 ± 0.50
3	Ref Ex 2	1.0	8	3.17 ± 0.42	19.98 ± 0.60	-0.27 ± 0.48
3	Ex 67	1.0	8	4.20 ± 0.66	21.51 ± 1.27	-0.05 ± 0.39

7.5 Induction of Penile Erection.

The ability of peptides of the present invention to induce penile erection (PE) in male rats was evaluated with selected peptides. Male Sprague-Dawley rats weighing 250-300 g were kept on a 12 hour on/off light cycle with food and water *ad libitum*. All behavioral studies were performed between 9 a.m. and 4 p.m. Groups of 6-8 rats were administered peptides at a variety of doses via the subcutaneous injection route. Immediately after treatment, rats were placed into individual polystyrene cages (27 cm long, 16 cm wide, and 25 cm high) for behavioral observation, typically by remote video monitoring (High-speed Digital Video Recording System EVS-DSX-16000DVD-H with CCD cameras, Epic Systems Inc., St. Louis, MO, USA) followed by blinded off-line scoring of video recordings. Rats were observed for one hour, and the number of yawns, grooming bouts and PEs were recorded in 10-minute bins.

PE results from studies with 1.0-3.0 mg/kg subcutaneously of Examples 43, 46 and 67, along with Ref Ex 2, are shown in Table 3 below, expressed as total number of PE/rat, mean ± SEM (n=7-11) and number of rats/group showing at least one erection over the study period (% responders). Corresponding vehicle controls (3.2% mannitol/50mM Tris buffer) were run in parallel.

Table 3

Treatment	Dose (mg/kg, s.c.)	n	Total number of PE/rat 0-1h	% Responders
Vehicle		11	1.0 ± 0.27	64
Ref Ex 2	3.0	7	6.0 ± 0.44	100
Ex 43	1.0	7	1.7 ± 0.36	29
	3.0	7	6.0 ± 0.76	100
Ex 46	1.0	7	4.9 ± 1.1	100
	3.0	7	4.4 ± 1.1	86
Ex 67	1.0	7	2.3 ± 0.42	86
	3.0	7	3.3 ± 1.0	100

8.0 High and Low Density hMC4-R Functional Assay Results

Exemplified peptides were tested using the high and low density hMC4-R functional assay as described in Section 7.3 above, with the results as shown in Table 4 below. Table 4 includes both single point values and mean values.

Table 4

Compound	0.1 ng/mL Doxycycline		10 ng/mL Doxycycline	
	EC_{50} (nM)	Intrinsic Activity (%)	EC_{50} (nM)	Intrinsic Activity (%)
Example 1	0.34	91	0.045	96
Example 2	0.56	36	0.095	99
Example 3	0.07	28	0.16	100
Example 4	-	11	0.245	100
Example 5	-	5	0.38	92
Example 6	37	29	2	100
Example 7	0.3	48	0.135	100
Example 8	48	71	0.55	87
Example 9	0.09	91	0.035	89
Example 10	0.45	77	0.17	94
Example 11	0.065	83	0.06	90
Example 12	34	64	0.5	97
Example 13	0.125	85	0.06	96

Compound	0.1 ng/mL Doxycycline		10 ng/mL Doxycycline	
	EC_{50} (nM)	Intrinsic Activity (%)	EC_{50} (nM)	Intrinsic Activity (%)
Example 14	8	16	0.4	93
Example 15	0.165	16	0.2	90
Example 16	0.15	28	0.16	97
Example 17	0.95	64	0.12	95
Example 18	0.14	83	0.085	97
Example 19	0.42	35	0.22	97
Example 20	-	0.5	0.75	53
Example 21	-	8	0.5	97
Example 22	3	78	0.2	93
Example 23	2	17	0.55	95
Example 24	0.7	15	0.45	99
Example 25	75	23	2	94
Example 26	0.125	74	0.12	90
Example 27	0.7	37	0.33	97
Example 28	29	35	0.6	94
Example 29	3	14	0.8	100
Example 30	-	2	25	9
Example 31	7	33	0.313	98
Example 32	18	34	0.543	96
Example 33	1	41	0.153	98
Example 34	1	79	0.137	97
Example 35	-	6	1	84
Example 36	-	8	1	89
Example 37	-	6	0.55	90
Example 38	2	31	0.258	97
Example 39	-	4	1	79
Example 40	-	8	0.5	93
Example 41	-	5	0.8	84
Example 42	-	9	0.5	84

Compound	0.1 ng/mL Doxycycline		10 ng/mL Doxycycline	
	EC_{50} (nM)	Intrinsic Activity (%)	EC_{50} (nM)	Intrinsic Activity (%)
Example 43	1	43	0.177	100
Example 44	11	22	0.617	99
Example 45	-	12	0.45	87
Example 46	0.666	40	0.173	97
Example 47	0.31	36	0.373	100
Example 48	-	6	4	93
Example 49	2	74	0.235	93
Example 50	0.21	94	0.085	94
Example 51	0.64	22	0.4	95
Example 52	0.325	31	0.2	99
Example 53	0.13	86	0.13	90
Example 54	0.075	56	0.075	92
Example 55	10	28	0.8	98
Example 56	5	41	0.3	98
Example 57	0.25	89	0.095	100
Example 58	5	67	0.25	100
Example 59	0.6	90	0.1	98
Example 60	0.45	62	0.14	95
Example 61	-	6	0.95	89
Example 62	16	23	0.47	84
Example 63	0.37	30	0.157	93
Example 64	1	57	0.08	90
Example 65	1	58	0.085	93
Example 66	5	32	0.265	88
Example 67	1	38	0.14	99
Example 68	0.85	55	0.075	91
Example 69	0.25	76	0.095	92
Example 70	0.4	64	0.1	91
Example 71	1	86	0.085	91

Compound	0.1 ng/mL Doxycycline		10 ng/mL Doxycycline	
	EC_{50} (nM)	Intrinsic Activity (%)	EC_{50} (nM)	Intrinsic Activity (%)
Example 72	3	11	0.35	84
Example 73	0.587	86	0.068	102
Example 74	0.943	68	0.11	100
Example 75	0.65	43	0.125	87
Example 76	3	53	0.135	85
Example 77	3	34	0.155	80
Example 78	2	48	0.2	89
Example 79	4	48	0.2	85
Example 80	6	13	0.65	86
Example 81	4	23	0.45	85
Example 82	2	79	0.095	95
Example 83	0.75	78	0.075	94
Example 84	0.633	78	0.09	97
Example 85	15	36	0.707	94
Example 86	9	36	0.483	96
Example 87	1	64	0.065	100
Example 88	3	15	0.35	89
Example 89		6	17	87
Example 90	4	31	0.283	96
Example 91	1	18	0.265	96
Example 92	0.1	81	0.04	89
Example 93	0.3	44	0.17	97
Example 94	0.24	32	0.09	92
Example 95	1	42	0.125	100
Example 96	2	24	0.235	100
Example 97	0.8	49	0.1	100
Example 98	0.45	47	0.14	100
Example 99	0.7	32	0.14	94
Example 100	6	27	0.27	93

Compound	0.1 ng/mL Doxycycline		10 ng/mL Doxycycline	
	EC_{50} (nM)	Intrinsic Activity (%)	EC_{50} (nM)	Intrinsic Activity (%)
Example 101	0.95	36	0.14	93
Example 102	18	30	0.725	97
Example 103	1	32	0.13	100
Example 104	191	16	10	100
Example 105	3	35	0.15	94
Example 106		3	113	77
Example 107		4		64
Example 108		3	9	59
Example 109		2	269	69
Example 110	0.6	84	0.06	105
Example 111	0.575	81	0.053	104
Example 112	0.39	83	0.07	101
Example 113	0.72	80	0.065	100
Example 114	1	73	0.185	117
Example 115	0.8	54	0.17	108
Example 116	0.68	77	0.095	104
Example 117	1	50	0.18	112

It is believed that peptides of the invention exhibiting an intrinsic activity of equal to or greater than about 0.1 (10%), such as greater than 0.2 (20%) or greater than about 0.3 (30%) or greater than about 0.4 (40%) or greater than about 0.5 (50%) or greater than about 0.6 (60%) %) or greater than about 0.7 (70%), or greater than about 0.8 (80%), or greater than about 0.9 (90%), or equal to or greater than about 1.0 (100%), in the above described high density hMC4-R system (i.e. HEK293TRexMC4R cells treated with 10 ng/ml doxycycline), based on maximal stimulation of adenylyl cyclase achievable by the compound in the same high density hMC4-R system where the maximal stimulation achieved by α-MSH or NDP-α-MSH is designated as an intrinsic activity of 1.0 (100%), provide agonist (full or partial) activity on the MC4 receptor.

A majority of the peptides of the Examples which were tested in the above described high density hMC4-R system (i.e. HEK293TRexMC4R cells treated with 10 ng/ml doxycycline) gave in said assay EC_{50} values for cAMP production of less than 0.05 μM, such as less than 0.01 μM, and in particular less than 0.001 μM .

As seen from Table 4, peptides of the invention may lack intrinsic activity in the low density hMC4-R system and be full agonists in the high density hMC4-R system.

As further seen from Table 4, peptides of the invention may be partial agonists in the above described low density hMC4-R system and full agonists in the above described high density hMC4-R system.

As further seen from Table 4, peptides of the invention may be full agonists in both the low density hMC4-R system and in the high density hMC4-R system.

Without being bound by any theory, it is believed that peptides that either lack intrinsic activity or are partial agonists in the above described low density hMC4-R system (i.e. peptides having intrinsic activity of from 0 to 70% in the low density hMC4-R system) and are partial or full agonists in the above described high density hMC4-R system (i.e. peptides having intrinsic activity of equal to or above 10%, or above 70%, in the high density hMC4-R system) will provide no or a reduced level of sexual effects resulting from MC4 receptor activation, e.g. penile erection, compared to peptides being full agonists in both the low and the high density hMC4-R systems. As explained above, these sexual effects are considered unwanted side-effects upon treatment of energy homeostasis and metabolism related, food intake related and/or energy balance and body weight related diseases, disorders and/or conditions.

It is thus believed that peptides according to the invention possess promising MC4 receptor affinity, efficacy and potency to be useful for treatment of diseases, disorders and/or conditions responsive to activation of the MC4 receptor, in particular energy homeostasis and metabolism related (e.g. diabetes), food intake related and/or energy balance and body weight related diseases, disorders and/or conditions, including obesity, overweight and diseases, disorders and/or conditions associated with obesity and/or overweight, such as type 2 diabetes and metabolic syndrome.

Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations

and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover all such modifications and equivalents.

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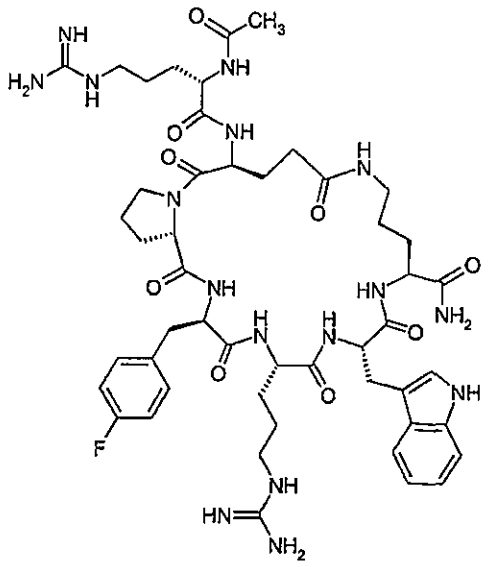
CLAIMS

1. A cyclic peptide which is
Ac-Arg-*cyclo*(Glu-Pro-D-Phe(4-F)-Arg-Trp-Orn)-NH_2 (SEQ ID NO:72);
or a pharmaceutically acceptable salt thereof.

2. A cyclic peptide according to claim 1, which is an acetate salt.

3. A cyclic peptide according to claim 1, which is a trifluoroacetic acid salt.

4. A cyclic peptide having the structure



or a pharmaceutically acceptable salt thereof.

5. A cyclic peptide according to claim 4, which is an acetate salt.

6. A cyclic peptide according to claim 4, which is a trifluoroacetic acid salt.

7. A pharmaceutical composition comprising the cyclic peptide according to either claim 1 or claim 4, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

8. The cyclic peptide according to either claim 1 or claim 4, or a pharmaceutically acceptable salt thereof, for use in the treatment of obesity.

9. Use of the cyclic peptide according to either claim 1 or claim 4, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of obesity.

10. The cyclic peptide according to either claim 1 or claim 4, or a pharmaceutically acceptable salt thereof, for use in reducing food intake, body weight and/or body weight gain.

11. Use of the cyclic peptide according to either claim 1 or claim 4, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for reducing food intake, body weight and/or body weight gain.