The present invention relates to novel peptide compounds which are effective in modulating one or more melanocortin receptor types, to the use of the compounds in therapy, to methods of treatment comprising administration of the compounds to patients in need thereof, and to the use of the compounds in the manufacture of medicaments. The compounds of the invention are of particular interest in relation to the treatment of obesity as well as a variety of diseases or conditions associated with obesity.
NOVEL PEPTIDES FOR USE IN THE TREATMENT OF OBESITY

FIELD OF THE INVENTION

The present invention relates to novel peptides which are specific to one or more melanocortin receptors and which exert a prolonged activity, to the use of said peptides in therapy, to methods of treatment comprising administration of said peptides to patients, and to the use of said peptides in the manufacture of medicaments.

BACKGROUND OF THE INVENTION

Obesity is a well known risk factor for the development of many very common diseases such as atherosclerosis, hypertension, type 2 diabetes (non-insulin dependent diabetes mellitus (NIDDM)), dyslipidaemia, coronary heart disease, and osteoarthritis and various malignancies. It also causes considerable problems through reduced motility and decreased quality of life. The incidence of obesity and thereby also these diseases is increasing throughout the entire industrialised world. Only a few pharmacological treatments are available to date, namely Sibutramine (Abbot; acting via serotonergic and noradrenaline mechanisms), Orlistat (Roche Pharm; reducing fat uptake from the gut,) and Acomplia (rimonabant; Sanofi-Aventis; CB1 endocannabinoid receptor antagonist; approved in EU in June 2006). However, due to the important effect of obesity as a risk factor in serious and even fatal and common diseases there is still a need for pharmaceutical compounds useful in the treatment of obesity.

The term obesity implies an excess of adipose tissue. In this context, obesity is best viewed as any degree of excess adiposity that imparts a health risk. The distinction between normal and obese individuals can only be approximated, but the health risk imparted by obesity is probably a continuum with increasing adiposity. However, in the context of the present invention, individuals with a Body Mass Index (BMI = body weight in kilograms divided by the square of the height in meters) above 25 are to be regarded as obese.

Even mild obesity increases the risk for premature death, diabetes, hypertension, atherosclerosis, gallbladder disease and certain types of cancer. In the industrialized western world the prevalence of obesity has increased significantly in the past few decades. Because of the
high prevalence of obesity and its health consequences, its treatment should be a high public health priority.

When energy intake exceeds energy expenditure, the excess calories are stored in adipose tissue, and if this net positive balance is prolonged, obesity results, i.e. there are two components to weight balance, and an abnormality on either side (intake or expenditure) can lead to obesity.

Proopiomelanocortin (POMC) is the precursor for β-endorphin and melanocortin peptides, including melanocyte stimulating hormone (α-MSH) and adrenocorticotropic (ACTH). POMC is expressed in several peripheral and central tissues including melanocytes, the pituitary, and neurons of the hypothalamus. The POMC precursor is processed differently in different tissues, resulting in the expression of different melanocortin peptides depending on the site of expression. In the anterior lobe of the pituitary, mainly ACTH is produced whereas in the intermediate lobe and the hypothalamic neurons the major peptides are α-MSH, β-MSH, des-acetyl-α-MSH and β-endorphin. Several of the melanocortin peptides, including ACTH and α-MSH, have been demonstrated to have appetite-suppressing activity when administered to rats by intracerebroventricular injection [Vergoni et al, European Journal of Pharmacology 179, 347-355 (1990)]. An appetite-suppressing effect is also obtained with the artificial cyclic α-MSH analogue, MT-II.

A family of five melanocortin receptor subtypes has been identified (melanocortin receptor 1-5, also called MC1, MC2, MC3, MC4 and MC5). The MC1, MC2 and MC5 are mainly expressed in peripheral tissues, whereas MC3 and MC4 are mainly centrally expressed; MC3 are, however, also expressed in several peripheral tissues. In addition to being involved in energy homeostasis, MC3 receptors have also been suggested to be involved in several inflammatory diseases. An MC3 agonist could have a positive effect on such diseases, e.g. gouty arthritis. MC5 are mainly peripherally expressed, and have been suggested to be involved in exocrine secretion and in inflammation. MC4 have been shown to be involved in the regulation of body weight and feeding behavior, as MC4 knock-out mice develop obesity [Huzar et al., Cell 88, 131-141 (1997)]. Furthermore, studies of either ectopic central expression of agouti protein (MC1, MC3 and MC4 antagonist) or over-expression of an endogenously occurring MC3 and MC4 antagonist (agouti gene related protein, AGRP) in mouse brain demonstrated that the over-expression of these two antagonists led to the development of obesity [Kleibig et al., PNAS 92, 4728-4732 (1995)]. Moreover, icv injection of a C-terminal
fragment of AGRP increases feeding and antagonizes the inhibitory effect of α-MSH on food intake.

In humans, several cases of families with obesity which is presumably due to frame shift mutations in MC4 have been described [see, e.g., Yeo et al., Nature Genetics 20, 111-112 (1998); Vaisse et al., Nature Genetics 20, 113-114 (1998)]. Mutations in the gene encoding the MC4 receptor appear to be the most abundant monogenic cause of obesity [Farooqi et al., New England Journal of Medicine 384, 1085-1095 (2003)]

In conclusion, a MC4 agonist could serve as an anorectic drug and/or energy expenditure increasing drug and be useful in the treatment of obesity or obesity-related diseases, as well as in the treatment of other diseases, disorders or conditions which may be ameliorated by activation of MC4.

MC4 antagonists may be useful for treatment of cachexia or anorexia, and for treatment of waisting in frail elderly patients. Furthermore, MC4 antagonists may be used for treatment of chronic pain, neuropathy and neurogenic inflammation.

A large number of patent applications disclose various classes of non-peptidic small molecules as melanocortin receptor modulators; examples hereof are WO 03/009850, WO 03/007949 and WO 02/081443.

The use of peptides as melanocortin receptor modulators is disclosed in a number of patent documents, e.g. WO 03/006620, US 5731,408 and WO 98/27113. Hadley [Pigment Cell Res., 4, 180-185, (1991)] reports a prolonged effect of specific melanotropic peptides conjugated to fatty acids, the prolongation effected by a transformation of the modulators from being reversibly acting to being irreversibly acting being caused by the conjugated fatty acids.

SUMMARY OF THE INVENTION

The present inventors have surprisingly found that specific peptide conjugates have a high modulating effect on one or more melanocortin receptors, i.e. the MC1, MC2, MC3, MC4 or MC5. Accordingly, the invention relates, inter alia, to compounds (more particularly compounds acting as melanocortin receptor agonists or antagonists) of formula I:

T-A-L-P [I]
wherein

T represents tetrazol-5-yl;

A represents a straight-chain, branched and/or cyclic C₆₋₂₀alkyl, C₆₋₂₀alkenyl or C₆₋₂₀alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxy and aryl;

L is a bond or a chemical structure covalently linking A and P; and

P represents a peptide structure comprising at least six α-amino acid residues.

Another aspect of the invention relates to compounds having the formula II:

\[
R^1-C(=O)-R^2-C(=O)-R^3-S-Z^1-Z^2-Z^3-Z^4-Z^5-Z^6-c[X^1-X^2-X^3-X^4-X^5]-R^4
\]

wherein

R¹ represents tetrazol-5-yl or carboxy;

R² represents a straight-chain, branched and/or cyclic C₆₋₂₀alkyl, C₆₋₂₀alkenyl or C₆₋₂₀alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxy and aryl;

R³ is absent or represents -NH-S(=O)₂(CH₂)₃-5-C(=O)- or a peptide fragment comprising one or two amino acid residues and containing at least one carboxy group;

S¹ is absent or represents a 4-aminobutyric acid residue, Gly, β-Ala, or a glycolether-based structure according to one of the formulas IIIa-IIIg.

\[
\begin{align*}
-\text{HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_2 & \text{[IIIa]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_3 & \text{[IIib]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_4 & \text{[IIic]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_5 & \text{[IIId]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_6 & \text{[IIle]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_7 & \text{[IIIf]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_8 & \text{[IIig]} \\
-\text{[HN-CH}_2&-\text{CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-C(=O)-]}_9 & \text{[IIih]} \\
\end{align*}
\]

Z¹ is absent or represents Gly, β-Ala, Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Glu, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;

Z² is absent or represents Gly, β-Ala, Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Glu, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;

Z³ represents Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Glu, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;

Z⁴ represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Tyr, Glu, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab or Orn;
Z\(^5\) represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla,
His, homoArg, Arg, Lys, Dab, Dap or Orn;

Z\(^6\) represents Ala, D-Ala, Val, Val, Leu, D-Leu, He, D-Ile, Met, D-Met, Nle or D-Nle;

X\(^1\) represents Glu, Asp, Cys, homoCys, Lys, Orn, Dap or Dap;

X\(^2\) represents His, Cit, Dab, Dap, Cgl, Cha, Val, He, tBuGly, Leu, Tyr, Glu, Ala, Nle, Met,
Met(O), Met(O\(_2\)), Gln, Gln(alkyl), Gln(aryl), Asn, Asn(alkyl), Asn(aryl), Ser, Thr, Cys, Pro,
Hyp, Tic, 2-PyAla, 3-PyAla, 4-PyAla, (2-thienyl)alanine, 3-(thienyl)alanine, (4-thiazolyl)Ala,
(2-furyl)alanine, (3-furyl)alanine or Phe, wherein one or more hydrogens on the phenyl moiety
of the Phe in question may optionally and independently be substituted by a substituent
selected among halogen, hydroxy, alkoxy, nitro, benzoyl, methyl, trifluoromethyl, amino and
cyano;

X\(^3\) represents D-Phe, wherein one or more hydrogens on the phenyl moiety in D-Phe may
optionally and independently be substituted by a substituent selected among halogen, hydroxy,
alkoxy, nitro, methyl, trifluoromethyl and cyano;

X\(^4\) represents Trp, 2-Nal, (3-benzo[b]thienyl)alanine or (S)-2,3,4,9-tetrahydro-1 H-β-carboline
3-carboxylic acid;

X\(^5\) represents Glu, Asp, Cys, homoCys, Lys, Orn, Dap or Dap;

wherein X\(^1\) and X\(^5\) are joined, rendering the compound of formula \(\text{II} \) cyclic, either via a disul-
ride bridge deriving from X\(^1\) and X\(^5\) both independently being Cys or homoCys, or via an am-
ide bond formed between a carboxylic acid in the side-chain of X\(^1\) and an amino group in the
side-chain of X\(^5\), or between a carboxylic acid in the side-chain of X\(^5\) and an amino group in
the side-chain of X\(^1\);

R\(^4\) represents OR\(^-\) or N(R\(^{\prime}\))\(_2\), wherein each R\(^{\prime}\) independently represents hydrogen or rep-
sents Ci\(_6\)-alkyl, C\(_2\)-alkenyl or C\(_2\)-alkynyl which may optionally be substituted with one or
more amino or hydroxy;

with the proviso that the compound of formula \(\text{II} \) is not 15-carboxypentadecanoyl-Gly-Ser-
Gln-His-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH\(_2\) or

2-[2-{15-carboxypentadecanoylaminooxy(ethoxy)ethoxyacetyl-Ser-Gln-Ser-Nle-c[Glu-Hyp-D-
Phe-Arg-Trp-Lys]-NH\(_2\) or

Yet another aspect of the invention relates to compounds having the formula IVa, IVb or IVc:

\[
\begin{align*}
R^1\cdot R^2\cdot C(=O)\cdot R^3\cdot S^2\cdot Z^4\cdot Z^6\cdot c[X^1\cdot X^2\cdot X^3\cdot \text{Arg} \cdot X^4\cdot X^5] R^4 & \quad \text{[IVa]} \\
R^1\cdot R^2\cdot C(=O)\cdot R^3\cdot S^2\cdot Z^4\cdot Z^6\cdot c[X^1\cdot X^2\cdot X^3\cdot \text{Arg} \cdot X^4\cdot X^5] R^4 & \quad \text{[IVb]} \\
R^1\cdot R^2\cdot C(=O)\cdot R^3\cdot S^2\cdot Z^6\cdot c[X^1\cdot X^2\cdot X^3\cdot \text{Arg} \cdot X^4\cdot X^5] R^4 & \quad \text{[IVc]} \\
\end{align*}
\]
wherein

R\(^1\) represents tetrazol-5-yl or carboxy;
R\(^2\) represents a straight-chain, branched and/or cyclic C\(_{6-2}\)alkyl, C\(_{6-2}\)alkenyl or C\(_{6-2}\)alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxyl and aryl;
R\(^3\) is absent or represents -NH-S(=O)\(_2\)CH\(_2\)3-5-C(=O)- or a peptide fragment comprising one or two amino acid residues and containing at least one carboxy group;
S\(^2\) represents a glycolether-based structure according to one of the formulas Illa-Ilg;

\[
\begin{align*}
-HN-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-C(=O)- & \quad [\text{Illa}] \\
-HN-CH_2-CH-O-CH_2-CH-O-C(=O) \quad & \quad [\text{IIb}] \\
-HN-CH_2-CH-O-CH_2-CH-O-C(=O) \quad & \quad [\text{Ilc}] \\
-HN-CH_2-CH-O-CH_2-CH-NH-C(=O)-CH_2-CH-O-C(=O) \quad & \quad [\text{IId}] \\
-HN-CH_2-CH-O-CH_2-CH-O-C(=O) \quad & \quad [\text{IIe}] \\
-HN-CH_2-CH-O-CH_2-CH-O-C(=O) \quad & \quad [\text{IIf}] \\
H-NH-C(=O)-CH_2-O-CH_2-C(=O)- & \quad [\text{Ilg}] \\
H-NH-C(=O)-CH_2-O-CH_2-C(=O)- & \quad [\text{Ilh}]
\end{align*}
\]

Z\(^4\) represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Tyr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab, Dap or Orn;
Z\(^5\) represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab, Dap or Orn;

X\(^1\) represents Glu, Asp, Cys, homoCys, Lys, Orn, Dab or Dap;
X\(^2\) represents His, Cit, Dap, Dab, Cgl, Cha, Val, He, D-Ile, Met, D-Met, Nle or D-Nle;
X\(^3\) represents D-Phe, wherein one or more hydrogens on the phenyl moiety of the Phe in question may optionally and independently be substituted by a substituent selected among halogen, hydroxy, alkoxy, nitro, benzoyl, methyl, trifluoromethyl, amino and cyano;
wherein X^1 and X^5 are joined, rendering the compound of formula IVa, IVb or IVc cyclic, either via a disulfide bridge deriving from X^1 and X^5 both independently being Cys or homoCys, or via an amide bond formed between a carboxylic acid in the side-chain of X^1 and an amino group in the side-chain of X^5, or between a carboxylic acid in the side-chain of X^5 and an amino group in the side-chain of X^1;

R^4 represents OR' or N(R')_2, wherein each R' independently represents hydrogen or represents Ci-alkyl, C_2-alkenyl or C_2-alkynyl which may optionally be substituted with one or more amino or hydroxy;

with the proviso that the compound of formula IVa, IVb or IVc is not 2-[2-(15-carboxypentadecanoylamino)ethoxy]ethoxyacetyl-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2; and pharmaceutically acceptable salts, prodrugs and solvates thereof.

The invention further relates to the use of compounds of the invention in therapy, to pharmaceutical compositions comprising compounds of the invention, and to the use of compounds of the invention in the manufacture of medicaments.

DEFINITIONS

The use of a prefix of the type "C_{xy}" preceding the name of a radical, such as in C_{xy}alkyl (e.g. C_{6-2}alkyl) is intended to indicate a radical of the designated type having from x to y carbon atoms.

The term "alkyl" as used herein refers to a straight-chain, branched and/or cyclic, saturated monovalent hydrocarbon radical.

The term "alkenyl" as used herein refers to a straight-chain, branched and/or cyclic, monovalent hydrocarbon radical comprising at least one carbon-carbon double bond.

The term "alkynyl" as used herein refers to a straight-chain, branched and/or cyclic, monovalent hydrocarbon radical comprising at least one carbon-carbon triple bond, and it may optionally also comprise one or more carbon-carbon double bonds.

The term "alkoxy" as used herein is intended to indicate a radical of the formula -OR', wherein R' is alkyl as indicated above.
In the present context, the term "aryl" is intended to indicate a carbocyclic aromatic ring radical or a fused aromatic ring system radical wherein at least one of the rings is aromatic. Typical aryl groups include phenyl, biphenyl, naphthyl, and the like.

The term "halogen" is intended to indicate members of the 7th main group of the periodic table of the elements, which includes fluorine, chlorine, bromine and iodine (corresponding to fluoro, chloro, bromo and iodo substituents, respectively).

The term "tetrazol-5-yl" is intended to indicate 1H-tetrazol-5-yl or 2H-tetrazol-5-yl.

In the present context, common rules for peptide nomenclature based on the three letter amino acid code apply, unless exceptions are specifically indicated. Briefly, the central portion of the amino acid structure is represented by the three letter code (e.g. Ala, Lys) and L-configuration is assumed, unless D-configuration is specifically indicated by "D-" followed by the three letter code (e.g. D-Ala, D-Lys). A substituent at the amino group replaces one hydrogen atom and its name is placed before the three letter code, whereas a C-terminal substituent replaces the carboxylic hydroxy group and its name appears after the three letter code. For example, "acetyl-Gly-Gly-NH₂" represents CH₃-C(=O)-NH-CH₂-C(=O)-NH-CH₂-C(=O)-NH₂. Unless indicated otherwise, amino acids with additional amino or carboxy groups in the side chains (such as Lys, Orn, Dap, Glu, Asp and others) are connected to their neighboring groups by amide bonds formed at the N-2 (α-nitrogen) atom and the C-1 (C=O) carbon atom.

When two amino acids are said to be bridged, it is intended to indicate that functional groups in the side chains of the two respective amino acids have reacted to form a covalent bond.

In the present context, the term "agonist" is intended to indicate a substance (ligand) that activates the receptor type in question.

In the present context, the term "antagonist" is intended to indicate a substance (ligand) that blocks, neutralizes or counteracts the effect of an agonist.

More specifically, receptor ligands may be classified as follows:
Receptor agonists, which activate the receptor; partial agonists also activate the receptor, but with lower efficacy than full agonists. A partial agonist will behave as a receptor partial antagonist, partially inhibiting the effect of a full agonist.

Receptor neutral antagonists, which block the action of an agonist, but do not affect the receptor-constitutive activity.

Receptor inverse agonists, which block the action of an agonist and at the same time attenuate the receptor-constitutive activity. A full inverse agonist will attenuate the receptor-constitutive activity completely; a partial inverse agonist will attenuate the receptor-constitutive activity to a lesser extent.

As used herein the term "antagonist" includes neutral antagonists and partial antagonists, as well as inverse agonists. The term "agonist" includes full agonists as well as partial agonists.

In the present context, the term "pharmacologically acceptable salt" is intended to indicate a salt which is not harmful to the patient. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric and nitric acids, and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bis(methylene)salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. (1977) 66, 2, which is incorporated herein by reference. Examples of relevant metal salts include lithium, sodium, potassium and magnesium salts, and the like. Examples of alkylated ammonium salts include methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts, and the like.

As used herein, the term "therapeutically effective amount" of a compound refers to an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of a given disease.
and/or its complications. An amount adequate to accomplish this is defined as a "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury, as well as on the weight and general state of the subject. It will be understood that determination of an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix, all of which is within the level of ordinary skill of a trained physician or veterinarian.

The terms "treatment", "treating" and other variants thereof as used herein refer to the management and care of a patient for the purpose of combating a condition, such as a disease or a disorder. The terms are intended to include the full spectrum of treatments for a given condition from which the patient is suffering, such as administration of the active compound(s) in question to alleviate symptoms or complications thereof, to delay the progression of the disease, disorder or condition, to cure or eliminate the disease, disorder or condition, and/or to prevent the condition, in that prevention is to be understood as the management and care of a patient for the purpose of combating the disease, condition, or disorder, and includes the administration of the active compound(s) in question to prevent the onset of symptoms or complications. The patient to be treated is preferably a mammal, in particular a human being, but treatment of other animals, such as dogs, cats, cows, horses, sheep, goats or pigs, is within the scope of the invention.

As used herein, the term "solvate" refers to a complex of defined stoichiometry formed between a solute (in casu, a compound according to the present invention) and a solvent. Solvents may include, by way of example, water, ethanol, or acetic acid.

The amino acid abbreviations used in the present context have the following meanings:
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>β-Ala</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Asn</td>
<td>Asparagines</td>
</tr>
<tr>
<td>Asn(alkyl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Asn(aryl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>β-Asp</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
</tbody>
</table>

*α*-nitrogen and β-carboxy group form the amide bonds to the two neighboring residues
| **Cha** | \[
\begin{align*}
H_2N & \quad \text{COOH} \\
\text{cyclohexylalanine}
\end{align*}
\] |
| **Cgl** | \[
\begin{align*}
H_2N & \quad \text{COOH} \\
\text{cyclohexylglycine}
\end{align*}
\] |
| **Cit** | Citrulline |
| **Cys** | Cysteine |
| **Dab** | (S)-2,4-diaminobutyric acid |
| **Dap** | (S)-2,3-diaminopropionic acid |
| **D-β-Asp** | \[
\begin{align*}
H_2N & \quad \text{COOH} \\
\alpha\text{-nitrogen and } \beta\text{-carboxy group form the amide bonds to the two neighboring residues}
\end{align*}
\] |
| **D-γ-Glu** | \[
\begin{align*}
H_2N & \quad \text{COOH} \\
\alpha\text{-nitrogen and } \gamma\text{-carboxy group form the amide bonds to the two neighboring residues}
\end{align*}
\] |
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Phe</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ![D-Phe](image)
<p>|
| Gln | Glutamine |
| Gln(alkyl) |
| <img src="image" alt="Gln(alkyl)" /> |
| Gln(aryl) |
| <img src="image" alt="Gln(aryl)" /> |
| Glu | glutamic acid |
| γ-Glu |
| <img src="image" alt="γ-Glu" /> |
| Gly | Glycine |
| His | Histidine |</p>
<table>
<thead>
<tr>
<th>Protein</th>
<th>Chemical Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>homoArg</td>
<td><img src="image" alt="homoArg structure" /></td>
<td>homo-arginine</td>
</tr>
<tr>
<td>homoCys</td>
<td><img src="image" alt="homoCys structure" /></td>
<td>homo-cysteine</td>
</tr>
<tr>
<td>homoSer</td>
<td><img src="image" alt="homoSer structure" /></td>
<td>homo-serine</td>
</tr>
<tr>
<td>Hyp</td>
<td>4-hydroxyproline</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
<td></td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Structure</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Met(O)</td>
<td><img src="image1" alt="Met(O)" /></td>
<td></td>
</tr>
<tr>
<td>Met(O₂)</td>
<td><img src="image2" alt="Met(O₂)" /></td>
<td></td>
</tr>
<tr>
<td>2-Nal</td>
<td><img src="image3" alt="2-Nal" /></td>
<td></td>
</tr>
<tr>
<td>Nle</td>
<td><img src="image4" alt="Nle" /></td>
<td></td>
</tr>
<tr>
<td>Orn</td>
<td>Ornithine</td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Praline</td>
<td></td>
</tr>
<tr>
<td>2-PyAla</td>
<td><img src="image5" alt="2-PyAla" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Structure</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>3-PyAla</td>
<td><img src="image1.png" alt="Structure" /></td>
<td></td>
</tr>
<tr>
<td>4-PyAla</td>
<td><img src="image2.png" alt="Structure" /></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td><img src="image3.png" alt="Structure" /> Serine</td>
<td></td>
</tr>
<tr>
<td>tBuGly</td>
<td><img src="image4.png" alt="Structure" /> <em>tert</em>-butylglycine</td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td><img src="image5.png" alt="Structure" /> Threonine</td>
<td></td>
</tr>
<tr>
<td>(4-thiazolyl)Ala</td>
<td><img src="image6.png" alt="Structure" /></td>
<td></td>
</tr>
<tr>
<td>Tic</td>
<td><img src="image7.png" alt="Structure" /></td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td><img src="image8.png" alt="Structure" /> Tyrosine</td>
<td></td>
</tr>
</tbody>
</table>
Amino acid abbreviations beginning with D-followed by a three letter code, such as D-Ser, D-His and so on, refer to the D-enantiomer of the corresponding amino acid, for example D-serine, D-histidine and so on.

## DESCRIPTION OF THE INVENTION

In certain embodiments of compounds of the present invention, the moiety T-A in formula I represents 10-(tetrazol-5-yl)decyl, 11-(tetrazol-5-yl)undecyl, 12-(tetrazol-5-yl)dodecyl, 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecey, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl, 17-(tetrazol-5-yl)heptadecyl; 18-(tetrazol-5-yl)octadecyl or 19-(tetrazol-5-yl)nonadecyl.

In certain embodiments of compounds of the present invention, S₁ in formula II is absent.

In further embodiments of compounds of the invention, S₁ in formula II represents a structure according to formula IIa.

In additional embodiments of compounds of the invention, S₁ in formula II represents a structure according to formula IIb.

In still further embodiments of compounds of the invention, S₁ in formula II represents a structure according to formula IIc.

In certain other embodiments of compounds of the invention, Z₁ in formula II is absent, or Z₁ in formula II represents Gly.

In further embodiments of compounds of the invention, Z₂ in formula II represents Ser, Thr, Gln, Gly or His, such as Ser or Thr.

In additional embodiments of compounds of the invention, Z₃ in formula II represents Gln, D-Gln, Asn, D-Asn, Ser or D-Ser.
In further embodiments of compounds of the invention, S² in formula IVa, IVb or IVc represents a structure according to formula IIIa or formula IIIb.

In some embodiments of compounds of the invention, the moiety R¹-R² (i.e. R¹ and R² taken together) in formula II or in formula IVa, IVb or IVc represents 10-(tetrazol-5-yl)decyl, 11-(tetrazol-5-yl)undecyl, 12-(tetrazol-5-yl)dodecyl, 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecyl, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl, 17-(tetrazol-5-yl)heptadecyl, 18-(tetrazol-5-yl)octadecyl or 19-(tetrazol-5-yl)nonadecyl, such as 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecyl, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl or 17-(tetrazol-5-yl)heptadecyl, e.g. 15-(tetrazol-5-yl)pentadecyl.

In other embodiments, the moiety R¹-R² (i.e. R¹ and R² taken together) in formula II or in formula IVa, IVb or IVc represents 12-carboxydodecyl, 13-carboxytridecyl, 14-carboxytetradecyl, 15-carboxypentadecyl, 16-carboxyhexadecyl, 17-carboxyheptadecyl, 18-carboxyoctadecyl or 19-carboxynonadecyl, such as 14-carboxytetradecyl or 16-carboxytetradecyl.

In certain embodiments of compounds of the invention, R³ in formula II or in formula IVa, IVb or IVc is absent. In other embodiments, R³ in formula II or in formula IVa, IVb or IVc represents -NH-S(=O)₂-(CH₂)₃-C(=O)- or Glu, D-Glu, γ-Glu, D-γ-Glu, Asp, D-Asp, β-Asp, D-β-Asp or Gly-γ-Glu. In some embodiments, R³ in formula II or in formula IVa, IVb or IVc represents -NH-S(=O)₂-(CH₂)₃-C(=O)-. In other embodiments, R³ represents D-Glu, γ-Glu, β-Asp or Gly-γ-Glu.

In additional embodiments of compounds of the invention, Z⁴ in formula II or in formula IVa represents Ser, homoSer, Gln, Asn, Tyr, His, Arg, homoArg, Lys, Orn, Dab or Dap, such as Ser, His, Arg or Dap.

In further embodiments of compounds of the invention, Z⁵ in formula II or in formula IVa or IVb represents Ser, homoSer, Thr, Pro, His, Hyp, Lys, Orn, Dab or Dap, such as Ser, His or Dap.

In certain embodiments of compounds of the invention, Z⁶ in formula II or in formula IVa, IVb or IVc represents Ala, Val, Leu, His, Met or Nle, such as Nle.
In additional embodiments of compounds of the invention, X² in formula II or in formula IVa, IVb or IVc represents Ser, Hyp, Cit, Dap, Asn, Gln or (4-thiazolyl)Ala, such as Hyp, Dap, Cit or Gln, e.g. Hyp.

In a group of embodiments of compounds of the invention, X¹ is Glu, X³ is D-Phe, X⁴ is Trp and X⁵ is Lys. In another group of embodiments, X¹ is Asp, X³ is D-Phe, X⁴ is Trp and X⁵ is Lys.

In a particular group of embodiments of compounds of the invention, R⁴ in formula II or in formula IVa, IVb or IVc is NH₂. In another group of embodiments, R⁴ is OH.

Specific examples of compounds according to the present invention are the following, each of which individually constitutes an embodiment of a compound of the invention:

16-(Tgtrazol-5-yl)h⁶xad⁶canoyl-Gly-Thr-Gln-S⁶r-Nlg⁶-c[Glu-Hyp-D-Ph⁶-Arg-Tg⁶- Lys]-NH₂

16-(Tgtrazol-5-yl)h⁶xad⁶canoyl-Gly-Thr-Gln-Dap-S⁶r-Nlg⁶-c[Glu-Hyp-D-Ph⁶-Arg-Tg⁶-Lys]-NH₂

;
{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Thr-Gln-Dap-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Ser-Nle-c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH$_2$

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$
4-(16-(T<sup>θ</sup>tetrazol-5-yl)hexadecanoylsulfamoyl)butanoyl-Gly-Ser-D-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH<sub>2</sub>


10 (2-[2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy]ethoxy)acetyl-His-Nle-c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH<sub>2</sub>
\[
\text{the compound:}
\]

\[
\text{the compound:}
\]
the compound:

the compound:

the compound:

the compound:
(2-{2-{2-{2-{[(R)-4-Carboxy-2-(1H-tetrazol-5-yl)hexadecanoylamino]butanoylamino}-ethoxy}ethoxy}acetylamino}ethoxy)ethoxy)acetyl-Ser-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

the compound:

the compound:

5

the compound:

the compound:

10

the compound:
i 5-Carboxypentaclecanoyl-Gly-Ser-Ser-Tyr-Thr-Nle-ctGlu-Hyp-D-Phe-Arg-Trp-Lysl-NHg

5 \{2-[2-(15-Carboxypentadecanoylamino)ethoxy]ethoxy}acetyl-Ser-Tyr-Hyp-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2

10 \{2-[2-(15-Carboxypentadecanoylamino)ethoxy]ethoxy}acetyl-Asn-Asn-Pro-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2

(2-{2-[(R)-4-Carboxy-2-(16-(tetrazol-5-yl)hexadecanoylamino)butanoylamino]ethoxy}θthoxy)acθtyl-Gly-Sθr-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2

15

(2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]acetyl-Gly-Ser-Gln-Dap-Sor-NIlc[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

{2-[2-[2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy]acetylamino}ethoxy)acetyl-Ser-Gln-His-Dap-Nle-ctGlu-Hyp-D-Ph β-Arg-Trp-Lysl-NHg
(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

5  

10  
(2-{4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂
4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoyl-Gly-S^θr-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH\textsubscript{2}

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]


\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]


\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH} & \quad \text{NH}_2 & \quad \text{O} & \quad \text{H} \\
\text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{S} & \quad \text{O} & \quad \text{N} & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

{2-[2-[16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]acetyl-Gly-Ser-Gln-His-His-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂}

{2-[2-[16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]acetyl-Gly-Ser-Gln-His-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂}
\[2-\{2-(16-(\text{trazol}-5-\text{yl})\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\text{acetyl-Gly-Ser-Gln-His-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH}_2\]

\[4-(15-\text{Carboxypentadecanoylsulfamoyl})\text{butanoyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Ph\theta-Arg-Trp-Lys]-NH}_2\]

\[(2-\{2-[(S)-4-\text{Carboxy-4-(17-carboxyheptadecanoylamino)butanoylamino}]\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Ph\theta-Arg-Trp-Lys]-NH}_2\]

(2-[(S)-3-Carboxy-3-(1 7-carboxyheptadecanoylamino)propanoylamino]ethoxy)ethoxy)-acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

{2-[2-(1 6-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Thr-Nle-c[Glu-Hyp-D-Ph θ -Arg-Trp-Lys]-NH$_2$
\{2-\{2-(16-(T^\theta\text{trazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Dab-Nl}\theta-\text{c[Glu-Hyp-D-Phe-Arg-Trp-Lys]}-\text{NH}_2

\{2-\{2-(16-(T^\theta\text{trazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-homoS\theta-r-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]}-\text{NH}_2

\{2-\{2-(16-(T^\theta\text{trazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Orn-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]}-\text{NH}_2

; and
{2-[2-(1 6-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-4-PyAla-Nle-c{Glu-Hyp-D-Phe-Arg-Trp-Lys}-NH$_2$

The present invention also encompasses combinations of two or more embodiments of compounds of the invention as outlined above.

In one aspect of the present invention, the compound of the invention is an agonist of a melanocortin receptor, notably an agonist of MC4. In another aspect of the invention, the compound is a selective agonist of MC4. In this context, selectivity is to be understood in relation to the activity of the compound with respect to MC1, MC3 and/or MC5. If a compound is a significantly more potent as a MC4 agonist than as a MC1, MC3 and/or MC5 agonist, it is deemed to be a selective MC4 agonist. The binding affinity of a compound with respect to MC1 and MC4 may be determined by comparing the IC50 from an MC1 binding assay as described below under "Assay IV" (MC1) with IC50 from an MC4 binding assay as described below under "Assay V" (MC4). If a compound is more than 10 times, such as more than 50 times, e.g. more than 100 times more potent with respect to MC4 than with respect to MC1, it is deemed to be a selective MC4 agonist with respect to MC1. The agonistic potency of a compound with respect to MC3, MC4 and MC5 may be determined in functional assays as described in "Assay II" (MC 3 and MC5) and "Assay III" (MC4). If a compound is more than 10 times, such as more than 50 times, e.g. more than 100 times more potent with respect to MC4 than with respect to MC3, it is deemed to be a selective MC4 agonist with respect to MC3. If a compound is more than 10 times, such as more than 50 times, e.g. more than 100 times more potent with respect to MC4 than with respect to MC5, it is deemed to be a selective MC4 agonist with respect to MC5. In a particular aspect, the compound of the present invention is a selective MC4 agonist with respect to MC1, with respect to MC3, with respect to MC5, with respect to MC1 and MC3, with respect to MC1 and MC5, with respect to MC3 and MC5 or with respect to MC1, MC3 and MC5.
In another aspect of the present invention, the compound of the invention is a selective MC4 agonist and a MC3 antagonist. In this context, a compound is deemed to be a selective MC4 agonist and a MC3 antagonist if it is a selective MC4 agonist with respect to MC1 and MC5 as discussed above, and it antagonizes MC3 as determined as described in "Assay II". In the latter assay, a compound exhibiting an IC_{50} value of less than 100 nM, such as less than 10 nM, e.g. less than 5 nM, such as less than 1 nM, is deemed to be a MC3 antagonist.

In a further aspect of the present invention, the compound of the present invention is both a selective MC3 agonist and a selective MC4 agonist. In this context, a compound is deemed to be a selective MC3 and MC4 agonist if it is significantly more potent as an agonist towards MC3 and MC4 than as an agonist toward MC1 and MC5. The selectivity of a compound with respect to MC1 and MC3 may be determined by comparing the potency determined for MC1 as described in "Assay IV" with the potency for MC3 determined as described in "Assay II". If a compound is more than 10 times, such as more than 50 times, e.g. more than 100 times more potent with respect to MC3 than with respect to MC1, it is deemed to be a selective MC3 agonist with respect to MC1. The selectivity of a compound with respect to MC3 and MC5 may be determined by comparing the potency determined as described in "Assay II". If a compound is more than 10 times, such as more the 50 times, e.g. more than 100 times more potent with respect to MC3 than with respect to MC5, it is deemed to be a selective MC3 agonist with respect to MC5. The MC4 selectivity of a compound with respect to MC3 and MC5 is determined as discussed above.

Compounds of the present invention may exert a protracted effect, i.e. the period of time in which they exert a biological activity is prolonged. A protracting effect may be evaluated in a slightly modified "Assay I" in a comparison between a compound of the present invention and the corresponding compound wherein R^1 is hydrogen. The experiment is allowed to continue for a period of time, T, until the rats have eaten as much as they did prior to the experiment. T values for compounds of the present invention and the corresponding compounds wherein R^1 is hydrogen are measured, and the difference ΔT is calculated. Compounds of the present invention giving rise to ΔT above 3 hours, such as above 7 hours, such as above 12 hours, such as above 12 hours, such as above 24 hours, such as above 48 hours, such as above 72 hours, are deemed to exert a protracted effect. Alternatively, a protracting effect may be evaluated in an indirect albumin-binding assay, in which K_i determined for binding in the presence of ovalbumin is compared with the the EC_{50} value determined in the presence of
HSA [see Assay VII in the PHARMACOLOGICAL METHODS section (vide infra) for a description of a suitable assay procedure].

Compounds of the present invention modulate melanocortin receptors, and they are therefore believed to be particularly suited for the treatment of diseases or states which can be treated by a modulation of melanocortin receptor activity. In particular, compounds of the present invention are believed to be suited for the treatment of diseases or states via activation of MC4.

In one aspect, the present invention relates to a method of agonizing or activating MC4 in a subject, the method comprising administering to the subject an effective amount of a compound of the present invention (i.e. a compound of formula I, II, IVa, IVb or IVc).

In another aspect, the invention provides a method of delaying the progression from impaired glucose tolerance (IGT) to type 2 diabetes, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

In a further aspect, the invention provides a method of delaying the progression from type 2 diabetes to insulin-requiring diabetes, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

In an additional aspect, the invention relates to a method of treating obesity or preventing overweight, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

In a still further aspect, the present invention provides a method of regulating appetite, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

Another aspect of the invention relates to a method of inducing satiety, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.
A further aspect of the invention relates to a method of preventing weight regain after successfully having lost weight, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

Yet another aspect of the invention relates to a method of increasing energy expenditure, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

Still further aspects of the invention include the following:

a method of treating a disease or state related to overweight or obesity, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention;

a method of treating bulimia, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention;

a method of treating a disease or state selected from atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction and risk of premature death, the method comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

In particular, compounds of the present invention may be suited for the treatment of diseases in obese or overweight patients. Accordingly, the present invention also provides a method of treating, in an obese patient, a disease or state selected from type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction and risk of premature death in obese patients, the method comprising administering to an obese patient in need thereof an effective amount of a compound of the present invention.

In addition, MC4 agonists could have a positive effect on insulin sensitivity, on drug abuse by modulating the reward system and on hemorrhagic shock. Furthermore, MC3 and MC4 agonists have antipyretic effects, and both have been suggested to be involved in peripheral nerve regeneration. MC4 agonists are also known to reduce stress response. In addition to
treatment drug abuse, treating or preventing hemorrhagic shock, and reducing stress response, compounds of the invention may also be of value in treating alcohol abuse, treating stroke, treating ischemia and protecting against neuronal damage.

In all of the therapeutic methods or indications disclosed above, the compound of the present invention may be administered alone. However, it may also be administered in combination with one or more additional therapeutically active agents, substances or compounds, either sequentially or concomitantly.

A typical dosage of a compound of the invention when employed in a method according to the present invention is in the range of from about 0.001 to about 100 mg/kg body weight per day, preferably from about 0.01 to about 50 mg/kg body weight per day, such as from about 0.05 to about 10 mg/kg body weight per day, administered in one or more doses, such as from 1 to 3 doses. The exact dosage will depend upon the frequency and mode of administration, the sex, age, weight and general condition of the subject treated, the nature and severity of the condition treated, any concomitant diseases to be treated and other factors evident to those skilled in the art.

Compounds of the invention may conveniently be formulated in unit dosage form using techniques well known to those skilled in the art. A typical unit dosage form intended for oral administration one or more times per day, such as from one to three times per day, may suitably contain from 0.05 to about 1000 mg, preferably from about 0.1 to about 500 mg, such as from about 0.5 mg to about 200 mg of a compound of the invention.

Compounds of the invention comprise compounds that are believed to be well-suited to administration with longer intervals than, for example, once daily. Thus, appropriately formulated compounds of the invention may be suitable for, e.g., twice-weekly or once-weekly administration by a suitable route of administration, such as one of the routes disclosed herein.

In a further aspect, the invention relates to a pharmaceutical composition comprising a compound of the present invention, optionally in combination with one or more additional therapeutically active compounds or substances and/or together with one or more pharmaceutically acceptable carriers or excipients. The composition may suitably be in unit dosage form comprising from about 0.05 mg to about 1000 mg, such as from about 0.1 mg to about 500 mg, e.g. from about 0.5 mg to about 200 mg, of a compound of the present invention.
The present invention also relates to the use of a compound of the present invention, optionally in combination with one or more additional therapeutically active compounds or substances, in the manufacture of a medicament for the treatment of a disease or condition selected from overweight or obesity, bulimia, atherosclerosis, hypertension, type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction and risk of premature death.

The invention also relates to the use of a compound of the present invention, optionally in combination with one or more additional therapeutically active compounds or substances, in the manufacture of a medicament effective in: delaying the progression from IGT to type 2 diabetes; delaying the progression from type 2 diabetes to insulin-requiring diabetes; regulating appetite; inducing satiety; preventing weight regain after successfully having lost weight; or increasing energy expenditure.

As described above, compounds of the present invention may be administered or applied in combination with one or more additional therapeutically active compounds or substances. Suitable additional compounds or substances may be selected, for example, from antidiabetic agents, antihyperlipidemic agents, antiobesity agents, antihypertensive agents and agents for the treatment of complications resulting from, or associated with, diabetes.

Suitable antidiabetic agents include insulin, insulin derivatives or analogues, GLP-1 (glucagon like peptide-1) derivatives or analogues [such as those disclosed in WO 98/08871 (Novo Nordisk A/S), which is incorporated herein by reference, or other GLP-1 analogues such as Byetta (exenatide; Eli Lilly/Amylin)] as well as orally active hypoglycemic agents.

Suitable orally active hypoglycemic agents include: imidazolines; sulfonylureas; biguanides; meglitinides; oxadiazolidinediones; thiazolidinediones; insulin sensitizers; α-glucosidase inhibitors; agents acting on the ATP-dependent potassium channel of the pancreatic β-cells, e.g. potassium channel openers such as those disclosed in WO 97/26265, WO 99/03861 and WO 00/37474 (Novo Nordisk A/S) which are incorporated herein by reference; potassium channel openers such as ormitiglinide; potassium channel blockers such as nateglinide or BTS-67582; glucagon antagonists such as those disclosed in WO 99/01423 and WO 00/39088 (Novo Nordisk A/S and Agouron Pharmaceuticals, Inc.), all of which are incorporated herein by reference; GLP-1 agonists such as those disclosed in WO 00/42026 (Novo
Nordisk A/S and Agouron Pharmaceuticals, Inc.), which are incorporated herein by reference; DPP-IV (dipeptidyl peptidase-IV) inhibitors; PTPase (protein tyrosine phosphatase) inhibitors; glucokinase activators, such as those described in WO 02/08209 to Hoffmann La Roche; inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis; glucose uptake modulators; GSK-3 (glycogen synthase kinase-3) inhibitors; compounds modifying lipid metabolism, such as antihyperlipidemic agents and antilipidemic agents; compounds lowering food intake; as well as PPAR (peroxisome proliferator-activated receptor) agonists and RXR (retinoid X receptor) agonists such as ALRT-268, LG-1268 or LG-1069.

Other examples of suitable additional therapeutically active substances include insulin or insulin analogues; sulfonylureas, e.g. tolbutamide, chlorpropamide, tolazamide, glibenclamide, glipizide, glimepiride, glicazide or glyburide; biguanides, e.g. metformin; and meglitinides, e.g. repaglinide or senaglinide/nateglinide.

Further examples of suitable additional therapeutically active substances include thiazolidinedione insulin sensitizers, e.g. troglitazone, ciglitazone, pioglitazone, rosiglitazone, isaglitazone, darglitazone, englitazone, CS-01 1/CI-1 037 or T 174, or the compounds disclosed in WO 97/41 097 (DRF-2344), WO 97/41 119, WO 97/41 120, WO 00/41 121 and WO 98/45292 (Dr. Reddy’s Research Foundation), the contents of all of which are incorporated herein by reference.

Additional examples of suitable additional therapeutically active substances include insulin sensitizers, e.g. GI 262570, YM-440, MCC-555, JTT-501, AR-H039242, KRP-297, GW-409544, CRE-1 6336, AR-H049020, LY510929, MBX-1 02, CLX-0940, GW-501 516 and the compounds disclosed in WO 99/1 931 3 (NN622/DRF-2725), WO 00/5041 4, WO 00/631 91, WO 00/63192 and WO 00/631 93 (Dr. Reddy’s Research Foundation), and in WO 00/23425, WO 00/2341 5, WO 00/23451, WO 00/23445, WO 00/2341 7, WO 00/2341 6, WO 00/631 53, WO 00/631 96, WO 00/63209, WO 00/631 90 and WO 00/631 89 (Novo Nordisk A/S), the contents of all of which are incorporated herein by reference.

Still further examples of suitable additional therapeutically active substances include:

α-glucosidase inhibitors, e.g. voglibose, emiglitate, miglitol or acarbose;

α-glucosidase inhibitors, e.g. voglibose, emiglitate, miglitol or acarbose;
glycogen phosphorylase inhibitors, e.g. the compounds described in WO 97/09040 (Novo Nordisk A/S);
glucokinase activators;
agents acting on the ATP-dependent potassium channel of the pancreatic β-cells, e.g. tolbutamide, glibenclamide, glipizide, glicazide, BTS-67582 or repaglinide;

Other suitable additional therapeutically active substances include antihyperlipidemic agents and antilipidemic agents, e.g. cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol or dextrothyroxine.

Further agents which are suitable as additional therapeutically active substances include antiobesity agents and appetite-regulating agents. Such substances may be selected from the group consisting of CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, MC3 (melanocortin receptor 3) agonists, MC3 antagonists, MC4 (melanocortin receptor 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, β3 adrenergic agonists such as CL-31 6243, AJ-9677, GW-0604, LY362884, LY377267 or AZ-40140, MC1 (melanocortin receptor 1) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin reuptake inhibitors (e.g. fluoxetine, seroxat or citalopram), serotonin and norepinephrine reuptake inhibitors, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth factors such as prolactin or placental lactogen, growth hormone releasing compounds, TRH (thyrotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, chemical uncouplers, leptin agonists, DA (dopamine) agonists (bromocriptin, doprexin), lipase/amylase inhibitors, PPAR modulators, RXR modulators, TR β agonists, adrenergic CNS stimulating agents, AGRP (agouti-related protein) inhibitors, histamine H3 receptor antagonists such as those disclosed in WO 00/42023, WO 00/63208 and WO 00/64884, the contents of all of which are incorporated herein by reference, exendin-4, GLP-1 agonists and ciliary neurotrophic factor.

Further suitable antiobesity agents are bupropion (antidepressant), topiramate (anticonvulsant), ecopipam (dopamine D1/D5 antagonist), naltrexone (opioid antagonist), and peptide YY3-36 (Batterham et al, Nature 418, 650-654 (2002)).
Among embodiments of suitable antiobesity agents for use in a method of the invention as additional therapeutically active substances in combination with a compound of the invention are leptin and analogues or derivatives of leptin.

A further embodiment of a suitable antiobesity agent is peptide YY$_{36}$.

Additional embodiments of suitable antiobesity agents are serotonin and norepinephrine re-uptake inhibitors, e.g. sibutramine.

Other embodiments of suitable antiobesity agents are lipase inhibitors, e.g. orlistat.

Still further embodiments of suitable antiobesity agents are adrenergic CNS stimulating agents, e.g. dexamphetamine, amphetamine, phentermine, mazindol, phendimetrazine, diethylpropion, fenfluramine or dexfenfluramine.

Other examples of suitable additional therapeutically active compounds include antihypertensive agents. Examples of antihypertensive agents are β-blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α-blockers such as doxazosin, urapidil, prazosin and terazosin.

In certain embodiments of the uses and methods of the present invention, the compound of the present invention may be administered or applied in combination with more than one of the above-mentioned, suitable additional therapeutically active compounds or substances, e.g. in combination with: metformin and a sulfonyleurea such as glyburide; a sulfonyleurea and acarbose; nateglinide and metformin; acarbose and metformin; a sulfonyleurea, metformin and troglitazone; insulin and a sulfonyleurea; insulin and metformin; insulin, metformin and a sulfonyleurea; insulin and troglitazone; insulin and lovastatin; etc.

**PHARMACEUTICAL COMPOSITIONS**

As already mentioned, one aspect of the present invention provides a pharmaceutical composition (formulation) comprising a compound of the present invention. Appropriate embodi-
merits of such formulations will often contain a compound of the invention in a concentration of from $10^{-3}$ mg/ml to 200 mg/ml, such as, e.g., from $10^{-1}$ mg/ml to 100 mg/ml. The pH in such a formulation of the invention will typically be in the range of 2.0 to 10.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizer(s) and/or surfactant(s). In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation, i.e. formulation comprising water, and the term "aqueous formulation" in the present context may normally be taken to indicate a formulation comprising at least 50% by weight (w/w) of water. Such a formulation is typically a solution or a suspension. An aqueous formulation of the invention in the form of an aqueous solution will normally comprise at least 50% (w/w) of water. Likewise, an aqueous formulation of the invention in the form of an aqueous suspension will normally comprise at least 50% (w/w) of water.

In another embodiment, a pharmaceutical composition (formulation) of the invention may be a freeze-dried (i.e. lyophilized) formulation intended for reconstitution by the physician or the patient via addition of solvents and/or diluents prior to use.

In a further embodiment, a pharmaceutical composition (formulation) of the invention may be a dried formulation (e.g. freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect, the invention relates to a pharmaceutical composition (formulation) comprising an aqueous solution of a compound of the present invention, and a buffer, wherein the compound of the invention is present in a concentration of 0.1 - 100 mg/ml or above, and wherein the formulation has a pH from about 2.0 to about 10.0.

In another embodiment of the invention, the pH of the formulation has a value selected from the list consisting of 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9 and 10.0.

In a further embodiment, the buffer in a buffered pharmaceutical composition of the invention may comprise one or more buffer substances selected from the group consisting of sodium acetate, sodium carbonate, citrates, glycylglycine, histidine, glycine, lysine, arginine, sodium
dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, tris(hydroxymethyl)aminomethane (TRIS), bicine, tricine, maleic acid, succinates, maleic acid, fumaric acid, tartaric acid and aspartic acid. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In another embodiment, a pharmaceutical composition of the invention may comprise a pharmaceutically acceptable preservative, e.g. one or more preservatives selected from the group consisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, thiomersal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride and chlorphenesine (3p-chlorphenoxypypropane-1,2-diol). Each one of these specific preservatives constitutes an alternative embodiment of the invention. In a further embodiment of the invention the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In still further embodiments of such a pharmaceutical composition of the invention, the preservative is present in a concentration in the range of 0.1 mg/ml to 5 mg/ml, a concentration in the range of 5 mg/ml to 10 mg/ml, or a concentration in the range of 10 mg/ml to 20 mg/ml. The use of a preservative in pharmaceutical compositions is well known to the skilled person. For convenience, reference is made in this respect to Remington: The Science and Practice of Pharmacy, 20th edition, 2000.

In a further embodiment of the invention the formulation further comprises a tonicity-adjusting agent, i.e. a substance added for the purpose of adjusting the tonicity (osmotic pressure) of a liquid formulation (notably an aqueous formulation) or a reconstituted freeze-dried formulation of the invention to a desired level, normally such that the resulting, final liquid formulation is isotonic or substantially isotonic. Suitable tonicity-adjusting agents may be selected from the group consisting of salts (e.g. sodium chloride), sugars and sugar alcohols (e.g. mannitol), amino acids (e.g. glycine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan or threonine), alditols [e.g. glycerol (glycerine), 1,2-propanediol (propylene glycol), 1,3-propanediol or 1,3-butanediol], polyethylene glycols (e.g. PEG 400) and mixtures thereof.

Any sugar, such as a mono-, di- or polysaccharide, or a water-soluble glucan, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch or carboxymethylcellulose-sodium, may be used; in one embodiment, sucrose may be
employed. Sugar alcohols (polyols derived from mono-, di-, oligo- or polysaccharides) include, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabinol. In one embodiment, the sugar alcohol employed is mannitol. Sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid composition (formulation) and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the concentration of sugar or sugar alcohol is between about 1 mg/ml and about 150 mg/ml.

In further embodiments, the tonicity-adjusting agent is present in a concentration of from 1 mg/ml to 50 mg/ml, such as from 1 mg/ml to 7 mg/ml, from 8 mg/ml to 24 mg/ml, or from 25 mg/ml to 50 mg/ml. A pharmaceutical composition of the invention containing any of the tonicity-adjusting agents specifically mentioned above constitutes an embodiment of the invention. The use of a tonicity-adjusting agent in pharmaceutical compositions is well known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy, 20th edition, 2000.*

In a still further embodiment of a pharmaceutical composition (formulation) of the invention, the formulation further comprises a chelating agent. Suitable chelating agents may be selected, for example, from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. The concentration of chelating agent will suitably be in the range from 0.1 mg/ml to 5 mg/ml, such as from 0.1 mg/ml to 2 mg/ml or from 2 mg/ml to 5 mg/ml. A pharmaceutical composition of the invention containing any of the chelating agents specifically mentioned above constitutes an embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy, 20th edition, 2000.*

In another embodiment of a pharmaceutical composition (formulation) of the invention, the formulation further comprises a stabilizer. The use of a stabilizer in pharmaceutical compositions is well known to the skilled person. For convenience, reference is made to Remington: *The Science and Practice of Pharmacy, 20th edition, 2000.*

More particularly, particularly useful compositions of the invention include stabilized liquid pharmaceutical compositions whose therapeutically active components include an oligo- or
polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical formulations. By "aggregate formation" is meant the formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution, as the result of a physical interaction between the oligo- or polypeptide molecules. The term "during storage" refers to the fact that a liquid pharmaceutical composition or formulation, once prepared, is not normally administered to a subject immediately. Rather, following preparation, it is packaged for storage, whether in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is meant the product obtained when a liquid pharmaceutical composition or formulation is dried by freeze-drying (i.e., lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38: 48-59), by spray-drying [see, e.g., Masters (1991) in Spray-Drying Handbook (5th edn.; Longman Scientific and Technical, Essex, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18: 1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11: 12-20], or by air-drying [see, e.g., Carpenter and Crowe (1988) Cryobiology 25: 459-470; and Roser (1991) Biopharm. 4: 47-53]. Aggregate formation by an oligo- or polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that peptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate formation may cause other problems, such as blockage of tubing, membranes or pumps when the oligo- or polypeptide-containing pharmaceutical composition is administered using an infusion system.

A pharmaceutical composition of the invention may further comprise an amount of an amino acid base sufficient to decrease aggregate formation by the oligo- or polypeptide during storage of the composition. By "amino acid base" is meant an amino acid, or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids for use in preparing a composition of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid and glutamic acid. Any stereoisomer (i.e., L, D, or mixtures thereof) of a particular amino acid (e.g. methionine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan or threonine, and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment, the L-stereoisomer of an amino acid is used. Compositions of the invention may
also be formulated with analogues of these amino acids. By "amino acid analogue" is meant a derivative of a naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the oligo- or polypeptide during storage of liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl-L-arginine. Suitable methionine analogues include ethionine and buthionine, and suitable cysteine analogues include S-methyl-L-cysteine. As with the amino acids *perse*, amino acid analogues are incorporated into compositions of the invention in either their free base form or their salt form. In a further embodiment of the invention, the amino acids or amino acid analogues are incorporated in a concentration which is sufficient to prevent or delay aggregation of the oligo-or polypeptide.

In a particular embodiment of the invention, methionine (or another sulfur-containing amino acid or amino acid analogue) may be incorporated in a composition of the invention to inhibit oxidation of methionine residues to methionine sulfoxide when the oligo- or polypeptide acting as the therapeutic agent is a peptide comprising at least one methionine residue susceptible to such oxidation. The term "inhibit" in this context refers to minimization of accumulation of methionine-oxidized species over time. Inhibition of methionine oxidation results in increased retention of the oligo- or polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of methionine residues such that the amount of methionine sulfoxide is acceptable to regulatory agencies. Typically, this means that no more than from about 10% to about 30% of forms of the oligo- or polypeptide wherein methionine is sulfoxidated are present. In general, this can be achieved by incorporating methionine in the composition such that the ratio of added methionine to methionine residues ranges from about 1:1 to about 1000:1, such as from about 10:1 to about 100:1.

In a further embodiment of the invention the formulation further comprises a stabilizer selected from high-molecular-weight polymers and low-molecular-weight compounds. Thus, for example, the stabilizer may be selected from substances such as polyethylene glycol (e.g. PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose and derivatives thereof (e.g. HPC, HPC-SL, HPC-L or HPMC), cyclodextrins, sulfur-containing substances such as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and various salts (e.g. sodium chloride). A pharmaceutical composition of the invention containing any of the stabilizers specifically mentioned above constitutes an embodiment of the invention.
Pharmaceutical compositions of the present invention may also comprise additional stabilizing agents which further enhance stability of a therapeutically active oligo- or polypeptide therein. Stabilizing agents of particular interest in the context of the present invention include, but are not limited to: methionine and EDTA, which protect the peptide against methionine oxidation; and surfactants, notably nonionic surfactants which protect the polypeptide against aggregation or degradation associated with freeze-thawing or mechanical shearing.

Thus, in a further embodiment of the invention, the pharmaceutical formulation comprises a surfactant, particularly a nonionic surfactant. Examples thereof include ethoxylated castor oil, polyglycolyzed glycerides, acetylated monoglycerides, sorbitan fatty acid esters, polyoxypropylene-polyoxyethylene block polymers (e.g. poloxamers such as Pluronic® F68, poloxamer 188 and 407, Triton X-100), polyoxyethylene sorbitan fatty acid esters, polyoxyethylene and polyethylene derivatives such as alkylated and alkoxylated derivatives (Tweens, e.g. Tween-20, Tween-40, Tween-80 and Brij-35), monoglycerides or ethoxylated derivatives thereof, diglycerides or polyoxyethylene derivatives thereof, alcohols, glycerol, lectins and phospholipids (e.g. phosphatidyl-serine, phosphatidyl-choline, phosphatidyl-ethanolamine, phosphatidyl-inositol, diphaspatidyl-glycerol and sphingomyelin), derivatives of phospholipids (e.g. dipalmitoyl phosphatidic acid) and lysophospholipids (e.g. palmitoyl lysophosphatidyl-L-serine and 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine) and alkyl, alkyl ester and alkyl ether derivatives of lysophosphatidyl and phosphatidylcholines, e.g. lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, i.e. cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, and the positively charged DODAC, DOTMA, DCP, BISHOP, lysophosphatidylserine and lysophosphatidylthreonine, and glycerophospholipids (e.g. cephalins), glycerolglycolipids (e.g. galactopyranoside), sphingolipids (e.g. ceramides, gangliosides), dodecylphosphocholine, hen egg lysolecithin, fusidic acid derivatives (e.g. sodium tauro-dihydrofusidate, etc.), long-chain fatty acids (e.g. oleic acid or caprylic acid) and salts thereof, acylcarnitines and derivatives, Nα-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, Nα-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, Nα-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, DSS (docusate sodium, CAS registry no. [577-1 1-7]), docusate

...
calcium, CAS registry no. [128-49-4]), docusate potassium, CAS registry no. [7491-09-0]), SDS (sodium dodecyl sulfate or sodium lauryl sulfate), sodium caprylate, cholic acid or derivatives thereof, bile acids and salts thereof and glycine or taurine conjugates, ursodeoxycholic acid, sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulfonates) monovalent surfactants, zwitterionic surfactants (e.g. N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propylldimethylammonio-1-propanesulfonate, cationic surfactants (quaternary ammonium bases) (e.g. cetyltrimethylammonium bromide, cetylpyridinium chloride), non-ionic surfactants (e.g. Dodecyl β-D-glucopyranoside), poloxamines (e.g. Tetronic's), which are tetrafunctional block copolymers derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine. The surfactant may also be selected from imidazoline derivatives and mixtures thereof. A pharmaceutical composition of the invention containing any of the surfactants specifically mentioned above constitutes an embodiment of the invention.


Additional ingredients may also be present in a pharmaceutical composition (formulation) of the present invention. Such additional ingredients may include, for example, wetting agents, emulsifiers, antioxidants, bulking agents, metal ions, oleaginous vehicles, proteins (e.g. human serum albumin, gelatine or other proteins) and a zwitterionic species (e.g. an amino acid such as betaine, taurine, arginine, glycine, lysine or histidine). Such additional ingredients should, of course, not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Pharmaceutical compositions containing a compound according to the present invention may be administered to a patient in need of such treatment at several sites, for example at topical sites (e.g. skin and mucosal sites), at sites which bypass absorption (e.g. via administration in an artery, in a vein or in the heart), and at sites which involve absorption (e.g. in the skin, under the skin, in a muscle or in the abdomen).

Administration of pharmaceutical compositions according to the invention to patients in need thereof may be via several routes of administration. These include, for example, lingual, sub-
lingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary (for example through the bronchioles and alveoli or a combination thereof), epidermal, dermal, transdermal, vaginal, rectal, ocular (for example through the conjunctiva), uretal and parenteral.

Compositions of the present invention may be administered in various dosage forms, for example in the form of solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules (e.g. hard gelatine capsules or soft gelatine capsules), suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, opthalmic ointments, opthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solutions, in situ transforming solutions (for example in situ gelling, in situ setting, in situ precipitating or in situ crystallizing), infusion solutions or implants.

Compositions of the invention may further be compounded in, or bound to, e.g. via covalent, hydrophobic or electrostatic interactions, a drug carrier, drug delivery system or advanced drug delivery system in order to further enhance the stability of the compound of the present invention, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance, or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to: polymers, for example cellulose and derivatives; polysaccharides, for example dextran and derivatives, starch and derivatives; polyvinyl alcohol; acrylate and methacrylate polymers; polyactic and polyglycolic acid and block copolymers thereof; polyethylene glycols; carrier proteins, for example albumin; gels, for example thermogelling systems, such as block co-polymeric systems well known to those skilled in the art; micelles; liposomes; microspheres; nanoparticulates; liquid crystals and dispersions thereof; L2 phase and dispersions thereof well known to those skilled in the art of phase behavior in lipid-water systems; polymeric micelles; multiple emulsions (self-emulsifying, self-microemulsifying); cyclodextrins and derivatives thereof; and dendrimers.

Compositions of the present invention are useful in the formulation of solids, semisolids, powders and solutions for pulmonary administration of a compound of the present invention, using, for example, a metered dose inhaler, dry powder inhaler or a nebulizer, all of which are devices well known to those skilled in the art.
Compositions of the present invention are useful in the formulation of controlled-release, sustained-release, protracted, retarded or slow-release drug delivery systems. Compositions of the invention are thus of value in the formulation of parenteral controlled-release and sustained-release systems well known to those skilled in the art (both types of systems leading to a many-fold reduction in the number of administrations required).

Of particular value are controlled-release and sustained-release systems for subcutaneous administration. Without limiting the scope of the invention, examples of useful controlled release systems and compositions are those containing hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles.

Methods for producing controlled-release systems useful for compositions of the present invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high-pressure homogenisation, encapsulation, spray-drying, microencapsulation, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made in this context to *Handbook of Pharmaceutical Controlled Release* (Wise, D.L., ed. Marcel Dekker, New York, 2000), and *Drugs and the Pharmaceutical Sciences*, vol. 99: *Protein Formulation and Delivery* (MacNally, E.J., ed. Marcel Dekker, New York, 2000).

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, for example a syringe in the form of a pen device. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is administration of a composition of the invention which is a liquid (typically aqueous) solution or suspension in the form of a nasal or pulmonary spray. As a still further option, a pharmaceutical composition of the invention can be adapted to transdermal administration (e.g. by needle-free injection or via a patch, such as an iontophoretic patch) or transmucosal (e.g. buccal) administration.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. The term "physical stability" in the context of a formulation containing an oligo- or polypeptide refers to the tendency of the peptide to form biologically inactive and/or insoluble aggregates as a result of exposure to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of aqueous
protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation, filled in suitable containers (e.g. cartridges or vials), to mechanical/physical stress (e.g. agitation) at different temperatures for various time periods. Visual inspection of formulations is performed in a sharp focused light with a dark background. The turbidity of a formulation is characterized by a visual score ranking the degree of turbidity, for instance on a scale from 0 to 3 (in that a formulation showing no turbidity corresponds to a visual score 0, whilst a formulation showing visual turbidity in daylight corresponds to visual score 3). A formulation is normally classified physically unstable with respect to aggregation when it shows visual turbidity in daylight. Alternatively, the turbidity of a formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of aqueous oligo- or polypeptide formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the peptide. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the oligo- or polypeptide. One example of a small-molecular spectroscopic probe of this type is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and possibly also other configurations, Thioflavin T gives rise to a new excitation maximum at about 450 nm, and enhanced emission at about 482 nm when bound to a fibril form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths in question.

Other small molecules can be used as probes of the changes in peptide structure from native to non-native states. Examples are the "hydrophobic patch" probes that bind preferentially to exposed hydrophobic patches of a polypeptide. The hydrophobic patches are generally buried within the tertiary structure of a polypeptide in its native state, but become exposed as it begins to unfold or denature. Examples of such small-molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as anthracene, acridine, phenanthroline and the like. Other spectroscopic probes are metal complexes of amino acids, such as cobalt complexes of hydrophobic amino acids, e.g. phenylalanine, leucine, isoleucine, methionine, valine, or the like.

The term "chemical stability" of a pharmaceutical formulation as used herein refers to chemical covalent changes in oligo- or polypeptide structure leading to formation of chemical degradation products with potentially lower biological potency and/or potentially increased immunogenicity compared to the original molecule. Various chemical degradation products can be formed depending on the type and nature of the starting molecule and the environment to which it is exposed. Elimination of chemical degradation can most probably not be com-
pletely avoided and gradually increasing amounts of chemical degradation products may of-

ten be seen during storage and use of oligo- or polypeptide formulations, as is well known to
the person skilled in the art. A commonly encountered degradation process is deamidation, a
process in which the side-chain amide group in glutaminyl or asparaginyl residues is hydro-

lysed to form a free carboxylic acid. Other degradation pathways involve formation of higher
molecular weight transformation products wherein two or more molecules of the starting sub-
stance are covalently bound to each other through transamidation and/or disulfide interac-
tions, leading to formation of covalently bound dimer, oligomer or polymer degradation prod-
ucts (see, e.g., Stability of Protein Pharmaceuticals, Ahern. T.J. & Manning M.C., Plenum
Press, New York 1992). Oxidation (of for instance methionine residues) may be mentioned
as another variant of chemical degradation. The chemical stability of a formulation may be
evaluated by measuring the amounts of chemical degradation products at various time-points
after exposure to different environmental conditions (in that the formation of degradation
products can often be accelerated by, e.g., increasing temperature). The amount of each in-
dividual degradation product is often determined by separation of the degradation products
depending on molecule size and/or charge using various chromatographic techniques (e.g.
SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased
physical stability, increased chemical stability, or increased physical and chemical stability. In
general, a pharmaceutical composition (formulation) must be stable during use and storage
(in compliance with recommended use and storage conditions) until the expiry date is
reached.

A pharmaceutical composition (formulation) of the invention should preferably be stable for
more than 2 weeks of usage and for more than two years of storage, more preferably for
more than 4 weeks of usage and for more than two years of storage, desirably for more than
4 weeks of usage and for more than 3 years of storage, and most preferably for more than 6
weeks of usage and for more than 3 years of storage.

All references, including publications, patent applications and patents, cited herein are
hereby incorporated by reference in their entirety and to the same extent as if each reference
were individually and specifically indicated to be incorporated by reference and were set forth
in its entirety herein (to the maximum extent permitted by law).
Headings and sub-headings are used herein for convenience only, and should not be construed as limiting the invention in any way.

The use of any and all examples, or exemplary language (including "for instance", "for example", "e.g." and "such as") in the present specification is intended merely to better illuminate the invention, and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any non-claimed element as being essential to the practice of the invention.

The citation and incorporation of patent documents herein is done for convenience only, and does not reflect any view of the validity, patentability and/or enforceability of such patent documents.

The present invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto, as permitted by applicable law.

**EXAMPLES**

List of abbreviations employed

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene (1,5-5)</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DIC</td>
<td>diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DIPEA</td>
<td>ethyldiisopropylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-N,N-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EGTA</td>
<td>1,2-di(2-aminoethoxy)ethane-N,N,N',N'-tetraacetic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>fetal calf serum</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenlymethoxy carbonyl</td>
</tr>
<tr>
<td>HEPES</td>
<td>2-[4-(2-hydroxyethyl)-piperazin-1-yl]-ethanesulfonic acid</td>
</tr>
</tbody>
</table>
All compounds of the present invention can be synthesized by those skilled in the art using standard coupling and deprotection steps. A description of all necessary tools and synthetic methods including standard abbreviations for peptide synthesis can be found in "The Fine Art Of Solid Phase Synthesis", 2002/3 Catalogue, Novabiochem. Non-standard procedures and syntheses of special building blocks are described below.

In the examples listed below, Rt values are retention times and the mass values are those detected by the mass spectroscopy (MS) detector and obtained using one of the following HPLC-MS devices (LCMS).
LCMS (system 1)
Agilent 1100 Series, electrospray; column: Waters XTerra® C₁₈ 5 µm 3.0x50mm; wa-
ter/acetonitrile containing 0.05 % TFA; gradient: 5 % → 100 % acetonitrile from 0 to 6.75 min, elution until t = 9.0 min; flow 1.5 ml/min.

LCMS (system 2)
Sciex API-1 50 Ex Quadrupole MS, electrospray, m/z = 200 to m/z = 1500; column: Waters XTerra® MS C₁₈ 5µm 3.0x50mm; elution with a mixture of solution A (water containing 0.1 % TFA) and solution B (acetonitrile containing 0.08 % TFA); gradient: 5 % → 20 % solution B from 1.0 to 3.0 min, 20 % → 50 % solution B from 3.0 to 16.0 min, 50 % → 90 % solution B from 16.0 to 18.0 min, elution until t = 18.0 min; flow 1.5 ml/min.

LCMS (system 3)
Sciex API-1 00 Quadrupole MS, electrospray, m/z = 300 to m/z = 2000; column: Waters XTerra® MS C₁₈ 5µm 3.0x50mm; water/acetonitrile containing 0.05 % TFA; gradient: 5 % → 90 % acetonitrile from 0 to 7.5 min; flow 1.5 ml/min.

MALDI-MS
Molecular weights of the peptides were determined using matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-MS), recorded on a Voyager-DE (Perceptive Biosystems). A matrix of sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid) was used.

A typical example of a synthesis procedure which includes a cyclization step is as follows:

Example 1
^-(Tetrazolylhexadecanoyl-Gly-Thr-Gln-His-Ser-Nle-DLctGlu-Hyp-D-Phe-Arg-Trp-Lysl-NHg
Step A for example 1: protected peptide resin Fmoc-c[Glu-Hyp(tBu)-D-Phe-Arg(Pbf)-Trp-Lys]-NH-Rink linker-polystyrene

Fmoc-Rink resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy-polystyrene resin, Bachem D-2080, Lot 514460; 0.47 mmol/g) was filled into two 60 ml Teflon reactors with frit (per reactor: 4.256 g, 2.0 mmol). The resin in each reactor was washed with 35 ml DCM.

Removal of Fmoc: The resin was shaken with a solution of 20 % piperidine in NMP (30 ml) for 20 min and then washed with NMP/DCM 1:1 (5x 30 ml).

Acylation with Fmoc-Lvs(Mtt)-OH: In a separate glass vial, the Fmoc-amino acid (12.0 mmol) was mixed with NMP (15 ml), DCM (27 ml) and a 1M solution (12.0 ml, 12.0 mmol) of 1-hydroxybenzotriazol (HOBt) in NMP. To the resulting clear solution, DIC (1.872 ml, 12.0 mmol) was quickly added and the solution was shaken immediately thereafter. The solution was left to stand in a closed vial for 30 min. 30 ml (6.0 mmol HOBt ester) of this solution was added to each reactor and the resin was shaken for 2½ hours. Ethyldiisopropylamine (DIPEA) (per reactor 0.514 ml, 2.0 mmol) was added and the mixture was shaken for 18 h. The resin was washed with NMP/DCM 1:1 (4x 30 ml).

Removal of Fmoc: As described above.

Acylation with Fmoc-Trp(Boc)-OH: In a separate glass vial, the Fmoc-amino acid (12.0 mmol) was mixed with NMP (15 ml), DCM (27 ml) and 1M HOBt-NMP solution (12.0 ml, 12.0 mmol). To the resulting clear solution, DIC (1.872 ml, 12.0 mmol) was quickly added and the solution was shaken immediately thereafter. The solution was left to stand in a closed vial for 30 min. 30 ml (6.0 mmol HOBt ester) of this solution was added to each reactor and the resin was shaken for 2½ hours. The liquids were filtered off and the resin was washed with NMP/DCM 1:1 (4x 30 ml).

In a similar manner, the following amino acids were successively attached to the resin: Fmoc-Arg(Pbf)-OH, Fmoc-D-Phe-OH, Fmoc-Hyp(tBu)-OH and Fmoc-Glu(2-phenylisopropoxy)-OH. Coupling with Fmoc-Glu(2-phenylisopropoxy)-OH was performed by using HOAT instead of HOBt, and DIPEA (2.0 mmol per reactor added after HOAt ester formation). The resulting Fmoc-protected resin was extensively washed with DCM.
Selective side-chain deprotection of Lys and Glu: The resin was shaken with a solution of 2% TFA and 3% triisopropylsilane in DCM (30 ml) for 10 min and the liquid was filtered off. This procedure was repeated another eight times. The resin was washed with DCM (4x 30 ml), 10% DIPEA in DCM (2x 30 ml) and DCM (2x 30 ml).

Side-chain cyclisation of Lys with Glu: In a separate glass vial, PyBOP (6.246 g = 12.0 mmol) was mixed with 1M HOBt-NMP solution (12.0 ml = 12.0 mmol), DCM (30 ml) and NMP (18 ml). 30 ml (containing 6.0 mmol PyBOP/HOBt) of this solution was added to each reactor, followed by DIPEA (2.054 ml = 12.0 mmol). The resin was shaken for 18 h. The liquids were filtered off and the resin was washed with NMP/DCM 1:1 (4x 30 ml).

Capping of non-acylated amino groups: Each resin was shaken with a solution of Boc anhydride (12 mmol per reactor) in DCM (30 ml per reactor) for 1 h. The liquids were filtered off and the resin was washed with DCM (3x 30 ml), DCM/MeOH 2:1 (2x 30 ml), THF (4x 30 ml) and DCM (3x 30 ml).

This afforded 13.92 g of resin, corresponding to a supposed maximum loading of 0.29 mmol/g if complete reactions are assumed.

Step B for example 1: i 6-tetrazol-S-yhexadecanoyl-Gly-Thr-Gln-His-Ser-Nle-cIGlu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

A 10 ml Teflon reactor with frit was charged with resin Fmoc-c[Glu-Hyp(tBu)-D-Phe-Arg(Pbf)-Trp-Lys]-NH-Rink linker-poly(styrene) (0.345 g, theoretically 0.100 mmol, available by Step A described above). The resin was washed with DCM (3 ml).

Removal of Fmoc: The resin was shaken with a solution of 20 % piperidine in NMP (3.5 ml) for 20 min and then washed with NMP/DCM 1:1 (6x 4 ml).

Acylation with Fmoc-Nle-OH: In a separate glass vial, the Fmoc-amino acid (0.5 mmol) was mixed with NMP (0.65 ml), DCM (1.15 ml) and 1M HOBt-NMP solution (0.5 ml, 0.5 mmol). To the resulting clear solution, DIC (0.156 ml, 0.5 mmol) was quickly added and the solution was shaken immediately thereafter. The solution was left to stand in a closed vial for 30 min and then added to the resin. The mixture was shaken for 105 min. The liquids were filtered off and the resin was washed with NMP/DCM 1:1 (4x 4 ml).
In a similar manner, the following carboxylic acids were successively attached to the resin: Fmoc-Ser(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH and 16-(tetrazol-5-yl)hexadecanoic acid (available by the synthetic procedure described below). Finally, the resin was washed with NMP/DCM 1:1 (6x 3 ml), DCiWMeOH 2:1 (2x 3 ml), THF (2x 3 ml) and DCM (3x 3ml).

Cleavage from the resin: The resin was shaken with a premixed solution (4 ml) containing TFA (95 vol-%), triisopropylsilane (2.5 vol-%) and water (2.5 vol-%) for 2 h. The mixture was filtered and the filtrate was collected in a glass vial. The resin was washed with 2x 3 ml DCM/TFA 2:1 and the filtrates were collected. The combined filtrate solution was concentrated to give a red oil.

Precipitation with ether: The oily residue was treated with diethyl ether (30 ml) to give a solid precipitate. The ether phase was removed after centrifugation. The solid residue was washed again with diethyl ether (30 ml). After centrifugation and removal of the ether phase, the solid residue was left to stand overnight in order to remove remaining diethyl ether.

Purification: The crude product precipitated from diethyl ether was dissolved in a mixture of acetonitrile (5.5 ml), acetic acid (0.5 ml) and water to give a total volume of about 21 ml. The resulting liquid was filtered and then injected into a Gilson preparative HPLC device. Elution was performed with water/acetonitrile containing 0.1 % TFA with a gradient from 29 % to 41% acetonitrile. The eluate was collected as fractions of 5 ml (peak fractions) or 12 ml (non-peak fractions), respectively. Relevant fractions were checked by analytical HPLC. Fractions containing the pure target peptide were mixed and concentrated under reduced pressure to give a colourless solution. This was diluted with de-ionised water and treated with 1M aqueous HCl (0.6 ml). The resulting clear solution was dispensed into glass vials. The vials were capped with Millipore glassfibre prefilters. Freeze-drying for three days afforded the peptide hydrochloride (27.8 mg, 16 % yield) as a white solid.

Analytical HPLC (Waters Symmetry300 C18, 5 µm, 3.9 x 150 mm; 42 °C; water/acetonitrile containing 0.05 % TFA; gradient: 5 % → 95 % acetonitrile from 0 to 15 min; flow 1 ml/min): \( t_R = 8.32 \text{ min} \) (100 % purity by UV 214 nm)

LCMS (system 1): \( R_t = 3.29 \text{ min} \); \( ((m+2)/2) = 896 \)
Examples of further compounds of the invention which may be obtained in a manner analogous to the compound of Example 1 are the compounds of Examples 2-23, below:

**Example 2**

16-(T$^\theta$razol-5-yl)h$^\theta$xad$^\theta$canoyl-Gly-Thr-Gln-Dap-S$^\theta$r-N$l$θ-c[Glu-Hyp-D-Ph$^\theta$-Arg-T$^\phi$-Lys]-NH$_2$

LCMS (system 1): Rt = 3.28 min; ((m+2)/2) = 870

**Example 3**

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Thr-Gln-Dap-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

LCMS (system 1): Rt = 3.38 min; ((m+2)/2) = 943

This compound was prepared using the commercially available building block Fmoc-NH-CH$_2$-CH$_2$-O-CH$_2$-O-CH$_2$-CO$_2$H.
Example 4

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino) θthoxy]ethoxy]acetyl-Gly-Ser-Gln-His-Ser-Nle-
c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 2): Rt = 9.49 min; ((m+2)/2) = 947

Example 5

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino) θthoxy]ethoxy]acetyl-Gly-Ser-Gln-His-Dap-Nle-
c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 2): Rt = 9.56 min; ((m+2)/2) = 961

Example 6

4-{1 β-Trazol- 5-θOhθxadθcanoylsulfamoylbutanoyl-Gly-S  θr-D-Gln-His-Dap-Nl  θ-clGlu-Hyp-
D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 2): Rt = 10.36 min; ((m+2)/2) = 962
This compound was prepared using the building block 4-(N-(16-(tetrazol-5-yl)hexadecanoyl)-
sulfamoyl)butyric acid. The synthesis of the building block is described below.

Example 7

\[
\]

LCMS (system 2): Rt = 11.60 min; \(((m+2)/2) = 772\)

Example 8

\[
(2-[2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy]ethoxy)acetyl-His-
Nle-c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH}_2
\]

LCMS (system 2): Rt = 10.93 min; \(((m+2)/2) = 842\)
Example 9


LCMS (system 2): Rt = 11.12 min; ((m+2)/2) = 815

This compound was prepared using the building block hexadecanedioic acid mono-tert-butyl ester. The synthesis of the building block is outlined below.

Example 10


LCMS (system 2): Rt = 12.20 min; ((m+2)/2) = 766

Example 11
Example 12

Example 13

Example 14
Example 15

Example 16

Example 17

(2-{2-{2-[(R)-4-Carboxy-2-(1H-tetrazol-5-yl)hexadecanoylamino]butanoylamo}no]ethox]y)ethoxy)ethoxy)ethoxy)acetyl-Ser-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

LCMS (system 2): Rt = 11.40 min; ((m+2)/2) = 937
Example 18


LCMS (system 2): $R_t = 10.06$ min; $((m+2)/2) = 941$

Example 19

Example 20

Example 21

The building block 16-(3-carboxy-propane-1-sulfonylamino)-16-oxo-hexadecanoic acid tert-butyl ester is a suitable starting point for the preparation of this compound. The synthesis of the building block is outlined below.

Example 22

Example 23
Example 24
i 5-Carboxypentadecanoyl-Gly-Ser-Ser-Tyr-Thr-Nle-ctGlu-Hyp-D-Phe-Arg-Trp-Lysl-NHg

LCMS (system 2): Rt = 11.4 min; ((m+2)/2) = 869

Example 25

LCMS (system 2): Rt = 11.77 min; ((m+2)/2) = 875

Example 26
{2-[2-(15-Carboxypentadecanoylamino)ethoxy]ethoxy}acetyl-Asn-Asn-Pro-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 2): Rt = 11.71 min; ((m+2)/2) = 857
Example 27

\((2-{2-[(R)-4-Carboxy-2-(16-(t θ trazol-5-yl)h θ xadecanoylamino)butanoylamino] θthoxy})ethoxy)\)ac θtyl-Gly-Ser-Gln-His-Dap-Nle-c[Gl u-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 2): \(Rt = 9.79\) min; \((m+2)/2\) = 1025

Example 28

\((2-[2-(2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy)acetylamino] θthoxy)ethoxy)ac θtyl-Ser-Gln-His-Dap-Nle-c[Gl u-Hyp-D-Ph θ-Arg-Trp]-NH₂\)

LCMS (system 2): \(Rt = 10.09\) min; \((m+2)/2\) = 1004
Example 29

(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]θthoxy}ethoxy)acetyl-Arg-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

LCMS (system 2): $R_t = 9.16$ min; $((m+2)/2) = 908$

Example 30

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]θthoxy}acθtyl-Gly-Ser-Gln-Dap-Sθr-Nθ-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

LCMS (system 3): $R_t = 2.88$ min; $((m+2)/2) = 936$

Example 31

(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

LCMS (system 3): $R_t = 3.25$ min; $((m+2)/2) = 899$
Example 32

{2-[2-{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂}

LCMS (system 3): Rt = 3.36 min; ((m+2)/2) = 828

Example 33

(2-[2-4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy)ethoxy)acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phε-Arg-Trp-Lys]-NH₂

LCMS (system 3): Rt = 3.24 min; ((m+2)/2) = 1035

Example 34

4-(1β-Trazol-S-γ-Ohδ canoylsulfamoylbutanoyl-Gly-S εr-Gln-His-Dap-Nl δ-cGlu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 3): Rt = 3.42 min; ((m+2)/2) = 963
**Example 35**

\[
\{2-[2-(1\text{-}6-(\text{Tetrazol}-5\text{-}yl)\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-D-S\thetar-His-His-Nle-c[Gluc-Hyp-D-Phe-Arg-Trp-Lys]-OH}
\]

**Example 36**

\[
(2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[1\text{-}6-(\text{Tetrazol}-5\text{-}yl)\text{hexadecanoylamino})\text{thoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Gluc-Hyp-D-Phe-Arg-Trp-Lys]-NH}_2}
\]

LCMS (system 3): \(R_t = 3.34\) min; \((m+3)/3) = 689

**Example 37**

\[
(2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[1\text{-}6-(\text{Tetrazol}-5\text{-}yl)\text{hexadecanoylamino})\text{ethoxy}\}\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Gluc-Hyp-D-Phe-Arg-Trp-Lys]-NH}_2}
\]

LCMS (system 3): \(R_t = 3.13\) min; \((m+3)/3) = 786
Example 38

\[
\{2-[2-(16-(\text{Tetrazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-His-Nle-} \\
\text{c}\{\text{Glu-Hyp-D-Phe-Arg-Trp-Lys}\}-\text{NH}_2
\]

LCMS (system 3): $R_t = 3.30$ min; $((m+2)/2) = 986$

Example 39

\[
\{2-[2-(16-(\text{Tetrazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Ser-Nle-} \\
\text{c}\{\text{Glu-Hyp-D-Phe-Arg-Trp-Lys}\}-\text{NH}_2
\]

LCMS (system 3): $R_t = 3.28$ min; $((m+2)/2) = 961$

Example 40

\[
\{2-[2-(16-(\text{Tetrazol-5-yl})\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}\}\text{acetyl-Gly-Ser-Gln-His-Nle-c}\{\text{Glu-Hyp-D-Phe-Arg-Trp-Lys}\}-\text{NH}_2
\]

LCMS (system 3): $R_t = 3.47$ min; $((m+3)/3) = 612$
Example 41

4-(15-Carboxypentadecanoylsulfamoyl)butanoyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

LCMS (system 3): Rt = 3.42 min; ((m+2)/2) = 943

Example 42

(2-{2-{(S)-4-Carboxy-4-(17-carboxyheptadecanoylamino)butanoylamino}ethoxy}ethoxy)-acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

MALDI-MS: m/z = 2040.1

Example 43


MALDI-MS: m/z = 2098.21
Example 44

\[
(2-{2-[\{(S)-3-Carboxy-3-(1\text{-}7\text{-}carboxyheptadecanoylamino}propanoylamino\}ethoxy}ethoxy)\text{-}acetyl-Gly-Ser-Gln-His-Dap-Nle-c[\text{Glu-Hyp-D-Phe-Arg-Trp-Lys}]-\text{NH}_2
\]

MALDI-MS: m/z = 2025.84

Example 45

\[
\{2-[2-(1\text{-}6-(Tetrazol-5-yl)hexadecanoylamino)\text{ }\theta\text{thoxy}ethoxy)\text{acyethyl-Gly-Ser-Gln-His-}\text{Thr-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys}]-\text{NH}_2
\]

MALDI-MS: m/z = 1931.83

Example 46

\[
\{2-[2-(1\text{-}6-(Tetrazol-5-yl)hexadecanoylamino)\text{ }\theta\text{thoxy}ethoxy)\text{acyethyl-Gly-Ser-Gln-His-Dab-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys}]-\text{NH}_2
\]

MALDI-MS: m/z = 1934.06
Example 47

\[
{2-[2-(16-(T^\theta\text{trazol}-5-yl)\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}}\text{acetyl-Gly-Ser-Gln-His-homoS} \theta r-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-\text{NH}_2
\]

MALDI-MS: \(m/z = 1935.15\)

Example 48

\[
{2-[2-(16-(T^\theta\text{trazol}-5-yl)\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}}\text{acetyl-Gly-Ser-Gln-His-Orn-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH}_2
\]

MALDI-MS: \(m/z = 1948.27\)

Example 49

\[
{2-[2-(16-(T^\theta\text{trazol}-5-yl)\text{hexadecanoylamino})\text{ethoxy}]\text{ethoxy}}\text{acetyl-Gly-Ser-Gln-His-Lys-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH}_2
\]
Example 50

{2-[2-(16-(T$^\theta$trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Arg-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

Example 51

{2-[2-(16-(T$^\theta$trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-2-PyAla-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

Example 52

{2-[2-(16-(T$^\theta$trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-4-PyAla-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$
Preparation of 16-(tetrazol-5-yl)hexadecanoic acid

16-Bromohexadecanoic acid (26.83 g, 80 mmol) was suspended in a mixture of methanol (160 ml) and toluene (30 ml). Polymer-bound arenesulfonic acid (1.5 g; macroporous polystyrene beads; "Amberlyst 15"; Fluka 06423) and trimethylorthoformate (17.5 ml, 160 mmol) were added and the mixture was refluxed for 6 h at 90 °C oil bath temperature. The reaction mixture was left to stand overnight at room temperature and then filtered. The resulting filtrate was concentrated under reduced pressure to give crude 16-bromohexadecanoic acid methylester as a brownish liquid.

To the crude methyl ester (80 mmol), NMP (140 ml) and sodium cyanide (9.41 g, 192 mmol) were added. The resulting suspension was stirred at 155 °C for 2 h. After being cooled to room temperature, the resulting dark brown suspension was treated with water (550 ml). Concentrated 37% aqueous HCl (5 ml, approx. 60 mmol, caution, can give deadly HCN gas!) and ice were added to give a suspension of pH 9. The suspension was left to stand for 40 min and then filtered. The resulting filter cake was washed with water (2x 125 ml) and dried for 20 h on tissue paper to give a brownish solid mainly consisting of the desired nitrile, but still containing the corresponding alkyl bromide (approx. 20% by 1H NMR in deuterochloroform). For repeating the reaction, the residue was mixed with freshly powdered sodium cyanide (6.27 g, 128 mmol) and NMP (100 ml). The resulting dark brown suspension was stirred at 110 °C oil bath temperature for 5 h and then left to stand overnight at room temperature. The mixture was treated with a mixture of water (400 ml) and concentrated 37%
aqueous HCI (2.5 ml, approx. 30 mmol, caution, can give deadly HCN gas!), resulting in a suspension of pH 11. Ice was added and the suspension was left to stand for 45 min and then filtered. The resulting filter cake was washed with water (2x 125 ml) and dried overnight on tissue paper to give an off-white, pasty residue. According to LCMS and $^1$H NMR, this product was mainly the desired 16-cyanohexadecanoic acid methyl ester, along with minor amounts of 16-cyanohexadecanoic acid, water and NMP.

The crude nitrile, freshly powdered sodium azide (20.80 g, 320 mmol) and triethylamine hydrochloride (22.19 g, 160 mmol) were suspended in NMP (200 ml) and stirred at 150 °C oil bath temperature for 18 h. The reaction mixture was left to cool down to room temperature and then poured into a beaker. Water (500 ml) and 37 % aqueous HCl (42 ml, approx. 500 mmol) were added. The resulting suspension was stirred, left to stand for 40 min and then filtered. The resulting filter cake was washed with water (250 ml) and dried on the filter for three days to give an off-white pasty residue.

This product was suspended in a mixture of MeOH (180 ml) and aqueous NaOH (11.2 g, 280 mmol, dissolved in 50 ml water). The mixture was stirred at 85 °C oil bath temperature for 3 $^1$2 h. The oil bath was removed. To the warm solution, water (50 ml) was added. The resulting dim liquid was poured into a beaker and stirred with a mixture of water (400 ml) and 37 % aqueous HCl (30 ml, approx. 360 mmol). After addition of ice, the resulting suspension (approx. 800 ml) was left to stand for 50 min and then filtered. The resulting filter cake was washed with water (500 ml) to give a white wet solid.

This product (still wet) was recrystallized from MeCN (550 ml, crystallization overnight). The resulting precipitate was collected by filtration, washed with MeCN (2x 100 ml) and petroleum ether (100 ml) and dried on tissue paper for 24 h to give the title compound as a yellowish solid. The resulting filtrate was filtered again and the resulting solid was washed with MeCN (2x 100 ml) and dried on tissue paper for 23 h to give the title compound as a brownish solid. 19.71 g (76 % yield) of 16-(tetrazol-5-yl)hexadecanoic acid was obtained.

$^1$H NMR (DMSO-d6) $\delta =$ 1.23 (m, 22H), 1.47 (m, 2H), 1.67 (m, 2H), 2.18 (t, J = 7 Hz, 2H), 2.85 (t, J = 7 Hz, 2H).
Preparation of 4-(6-tetrazol-5-yl-hexadecanoylsulfamoyl)butyric acid

16-(Tetrazol-5-yl)hexadecanoic acid (6.49 g, 20.0 mmol) and carbonyldiimidazole (3.34 g, 20.6 mmol) were mixed. DMF (110 ml) was added and the resulting milky mixture was stirred for 2 h. Then, a solution of (4-sulfamoyl)butyric acid methyl ester (3.62 g, 20.0 mmol) in DMF (20 ml) was added, followed by addition of DBU (6.57 ml, 44.0 mmol). The resulting solution was stirred for 18 h and then poured into 0.1 M aqueous HCl (870 ml) to give a white precipitate. Residual material was washed from the reaction flask into the acidic suspension with MeOH (5 ml). The resulting suspension of pH 4-5 was left to stand for 2v2 h and then filtered. The filter cake was washed with 0.01 M aqueous HCl (170 ml) and water (280 ml) to give an off-white wet solid. This product (still wet) was recrystallized from MeCN (300 ml, crystallization overnight). The resulting precipitate was collected by filtration, washed with MeCN (80 ml) and dried on tissue paper to give 5.95 g (61 % yield) of 4-(16-tetrazol-5-yl-hexadecanoylsulfamoyl)butyric acid methyl ester as an off-white solid.

1H NMR (DMSO-d6) δ = 1.23 (m, 22H), 1.49 (m, 2H), 1.67 (m, 2H), 1.88 (m, 2H), 2.25 (t, J = 7 Hz, 2H), 2.48 (t, J = 7 Hz, 2H), 2.85 (t, J = 7 Hz, 2H), 3.39 (m, 2H), 3.59 (s, 3H).

The methyl ester (5.95 g, 12.2 mmol) was suspended in MeOH (50 ml). 1 M aqueous NaOH (43 ml, 43 mmol) was added and the resulting solution was stirred for 19 h. The solution was carefully acidified with 0.5 M aqueous HCl (100 ml, 50 mmol). Water (50 ml) was added. The resulting white suspension was left to stand for 45 min and then filtered. The filter cake was washed with water (200 ml) and then recrystallized from MeCN (200 ml, oil bath, yellowish solution when hot, crystallization overnight). The resulting precipitate was collected by filtration, washed with MeCN (100 ml) and dried on tissue paper to give the title compound as a
white solid. 5.10 g (54% yield over two steps) of 4-(16-tetrazol-5-yl-hexadecanoylsulfamoyl)butyric acid was obtained.

$^1$H NMR (DMSO-d$_6$) $\delta$ = 1.23 (m, 20H), 1.49 (m, 2H), 1.67 (m, 2H), 1.85 (m, 2H), 2.25 (t, $J$ = 7 Hz, 2H), 2.38 (t, $J$ = 7 Hz, 2H), 2.85 (t, $J$ = 7 Hz, 2H), 3.38 (m, partially overlapping with water peak at 3.35 ppm), 12.23 (broad s, 1H).

**Preparation of hexadecanedioic acid mono-tert-butyl ester**

This compound was prepared from hexadecanedioic acid and dimethylformamide-di-tert-butyl acetal according to the general procedure reported in the literature: U. Widmer, *Synthesis 1983*, 135.

**Preparation of 16-(3-carboxy-propane-1-sulfonylamino)-16-oxo-hexadecanoic acid tert-butyl ester**

Hexadecanedioic acid mono-tert-butyl ester (5.14 g, 15.0 mmol) was dissolved in DCM (30 ml) and MeCN (30 ml). Carbonyldiimidazole (2.51 g, 15.45 mmol) was added and the mixture was stirred for 2 h. A solution of (4-sulfamoyl)butyric acid methyl ester (2.72 g, 15.0 mmol) in DCM (30 ml) was added, followed by addition of DBU (2.69 ml, 18 mmol). The mixture was stirred overnight and then concentrated under reduced pressure. The resulting residue was treated with 0.2 M aqueous citrate buffer pH 4.5 (preparation of the buffer: 0.2 mol of citric acid and 0.35 mol of NaOH dissolved in one liter of water). After 20 min, the resulting precipitate was collected by filtration and washed with water (150 ml).
This product was dissolved in MeOH (70 ml) and THF (20 ml). 1 M aqueous NaOH (13 ml, 13 mmol) was slowly added and the mixture was stirred. After 40 min, a new portion of 1 M aqueous NaOH (14.3 ml, 14.3 mmol) was slowly added. The mixture was stirred overnight and then poured into a mixture of water (150 ml) and 0.2 M aqueous citrate buffer pH 4.5 (150 ml). After 1 h, the resulting precipitate was collected by filtration, washed with water (100 ml) and dried to give the crude title compound. Recrystallization from acetone (300 ml) afforded 2.44 g (33 % yield) of 16-(3-carboxy-propane-1-sulfonlamino)-16-oxo-hexadecanoic acid tert-butyl ester.

$^1$H NMR (DMSO-d6) $\delta = 1.23$ (m, 20H), 1.39 (s, 9H), 1.48 (m, 4H), 1.84 (m, 2H), 2.16 (t, $J = 7$ Hz, 2H), 2.24 (t, $J = 7$ Hz, 2H), 2.38 (t, $J = 7$ Hz, 2H), 3.37 (m, partially overlapping with water peak at 3.33 ppm).

PHARMACOLOGICAL METHODS

Assay (I) - Experimental protocol for efficacy testing on appetite with MC4 analogues, using an ad libitum fed rat model.

TAC:SPRD @mol rats or Wistar rats from M&B Breeding and Research Centre A/S, Denmark are used for the experiments. The rats have a body weight 200-250 g at the start of experiment. The rats arrive at least 10-14 days before start of experiment with a body weight of 180-200 g. Each dose of compound is tested in a group of 8 rats. A vehicle group of 8 rats is included in each set of testing.

When the animals arrive they are housed individually in a reversed light/dark phase (lights off 7:30 am, lights on 7:30 pm), meaning that lights are off during daytime and on during nighttime. Since rats normally initiate food intake when light is removed, and eat the major part of their daily food intake during the night, this set up results in an alteration of the initiation time for food intake to 7:30 am, when lights are switched off. During the acclimatization period of 10-14 days, the rats have free access to food and water. During this period the animals are handled at least 3 times. The experiment is conducted in the rats' home cages. Immediately before dosing the rats are randomised to the various treatment groups (n=8) by body weight. They are dosed according to body weight at between 7:00 am and 7:45 am, with a 1-3 mg/kg solution administered intraperitoneal (ip), orally (po) or subcutaneously (sc). The time of dosing is recorded for each group. After dosing, the rats are returned to their home cages,
where they then have access to food and water. The food consumption is recorded individually every hour for 7 hours, and then after 24 h and sometimes 48 h. At the end of the experimental session, the animals are euthanised.

The individual data are recorded in Microsoft excel sheets. Outliers are excluded after applying the Grubbs statistical evaluation test for outliers, and the result is presented graphically using the GraphPad Prism program.

**Assay (II) - Melanocortin receptor 3 and 5 (MC3 and MC5) cAMP functional assay using the AlphaScreen™ cAMP detection kit**

The cAMP assays for MC3 and MC5 receptors are performed on cells (either HEK293 or BHK cells) stably expressing the MC3 and MC5 receptors, respectively. The receptors are cloned from cDNA by PCR and inserted into the pcDNA 3 expression vector. Stable clones are selected using 1 mg/ml G418.

Cells at approx. 80-90% confluence are washed 3x with PBS, lifted from the plates with Versene and diluted in PBS. They are then centrifuged for 2 min at 1300 rpm, and the supernatant removed. The cells are washed twice with stimulation buffer (5mM HEPES, 0.1% ovalbumin, 0.005% Tween™ 20 and 0.5mM IBMX, pH 7.4), and then resuspended in stimulation buffer to a final concentration of 1x10^6 or 2x10^6 cells/ml. 25 μl of cell suspension is added to the microtiter plates containing 25 μl of test compound or reference compound (all diluted in stimulation buffer). The plates are incubated for 30 minutes at room temperature (RT) on a plate-shaker set to a low rate of shaking. The reaction is stopped by adding 25 μl of acceptor beads with anti-cAMP, and 2 min later 50 μl of donor beads per well with biotinylated cAMP in a lysis buffer. The plates are then sealed with plastic, shaken for 30 minutes and allowed to stand overnight, after which they are counted in an Alpha™ microplate reader.

EC₅₀ values are calculated by non-linear regression analysis of dose/response curves (6 points minimum) using the Windows™ program GraphPad™ Prism (GraphPad™ Software, USA). All results are expressed in nM.

For measuring antagonistic activity in the MC3 functional cAMP assay, the MC3 receptors are stimulated with 3 nM α-MSH, and inhibited by increasing the amount of potential antago-
nist. The IC_{50} value for the antagonist is defined as the concentration that inhibits MC3 stimulation by 50%.

**Assay (III) - Melanocortin receptor 4 (MC4) cAMP assay**

BHk cells expressing the MC4 receptor are stimulated with potential MC4 agonists, and the degree of stimulation of cAMP is measured using the Flash Plate® cAMP assay (NEN™ Life Science Products, cat. No. SMP004).

The MC4 receptor-expressing BHk cells are produced by transfecting the cDNA encoding MC4 receptor into BHKS70/KZ1 0-20-48, and selecting for stable clones expressing the MC4 receptor. The MC4 receptor cDNA, as well as a CHO cell line expressing the MC4 receptor, may be purchased from Euroscreentm. The cells are grown in DMEM, 10% FCS, 1 mg/ml G418, 250 nM MTX and 1% penicillin/streptomycin.

Cells at approx. 80-90% confluence are washed 3x with PBS, lifted from the plates with Versene and diluted in PBS. They are then centrifuged for 2 min at 1300 rpm, and the supernatant removed. The cells are washed twice with stimulation buffer, and resuspended in stimulation buffer to a final concentration of 0.75x10^6 cells/ml (consumption thereof: 7 ml per 96-well microtiter plate). 50 µl of cell suspension is added to the Flash Plate containing 50 µl of test compound or reference compound (all diluted in H_2O). The mixture is shaken for 5 minutes and then allowed to stand for 25 minutes at RT. The reaction is stopped by addition of 100 µl Detection Mix per well (Detection Mix = 11 ml Detection Buffer + 100 µl (~2µCi) cAMP [^{125}I tracer). The plates are then sealed with plastic, shaken for 30 minutes, and allowed to stand overnight (or for 2 hours) and then counted in the Topcounter (2 min/well).

The assay procedure and the buffers are generally as described in the Flash Plate kit-protocol (Flash Plate® cAMP assay (NEN™ Life Science Products, cat. No. SMP004)). However the cAMP standards are diluted in 0.1% HSA and 0.005% Tween™ 20 and not in stimulation buffer.

EC_{50} values are calculated by non-linear regression analysis of dose/response curves (6 points minimum) using the Windows™ program GraphPad™ Prism (GraphPad Software, USA). All results are expressed in nM.
Assay (IV) - Melanocortin receptor 1 (MC1) binding assay

The MC1 receptor binding assay is performed on BHK cell membranes stably expressing the MC1 receptor. The assay is performed in a total volume of 250 µl: 25 µl of \(^{125}\)I-NDP-α-MSH (22 pM in final concentration), 25 µl of test compound/control and 200 µl of cell membrane (35 µg/ml). Test compounds are dissolved in DMSO. Radioactively labeled ligand, membranes and test compounds are diluted in buffer: 25 mM HEPES, pH 7.0, 0.1 mM CaCl\(_2\), 1 mM MgSO\(_4\), 1 mM EDTA, 0.02% Bacitracin, 0.005% ovalbumin. The samples are incubated at 30°C for 90 min in Greiner micro-well plates, separated with GF/B filters that are pre-wetted for 60 min in 0.5% PEI, and washed 2-3 times with NaCl (0.9%) before separation of bound from unbound radiolabeled ligand by filtration. After filtration the filters are washed 10 times with ice-cold 0.9% NaCl. The filters are dried at 50°C for 30 min, sealed, and 30 µl of Microsint 0 (Packard, cat. No. 6013616) is added to each well. The plates are counted in a Topcounter (1 min/well).

The data are analysed by non-linear regression analysis of binding curves, using the Windows™ program GraphPad™ Prism (GraphPad Software, USA).

Assay (V) - Melanocortin receptor 4 (MC4) binding assay

In vitro \(^{125}\)I-NDP-Q-MSH binding to recombinant BHK cells expressing human MC4 receptor (filtration assay).

The assay is performed in 5 ml minisorb vials (Sarstedt No. 55.526) or in 96-well filterplates (Millipore MADVN 6550), and using BHK cells expressing the human MC4 receptor (obtained from Professer Wikberg, Uppsala, Sweden). The BHK cell membranes are kept at -80°C until assay, and the assay is run directly on a dilution of this cell membrane suspension, without further preparation. The suspension is diluted to give maximally 10% specific binding, i.e. to approx. 50-100 fold dilution. The assay is performed in a total volume of 200 µl: 50 µl of cell suspension, 50 µl of \(^{125}\)I-NDP-α-MSH (~79 pM in final concentration), 50 µl of test compound and 50 µl binding buffer (pH 7) mixed and incubated for 2 h at 25°C [binding buffer: 25 mM HEPES, pH 7.0, 1 mM CaCl\(_2\), 1 mM MgSO\(_4\), 1 mM EGTA, 0.02% Bacitracin, 0.005% Tween™ 20]. Alternatively, HSA may be substituted with ovalbumin. The samples are incubated at 30°C for 90 min in Greiner micro-well plates, separated with GF/B filters that are pre-wetted for 60 min in 0.5% PEI, and washed 2-3 times with NaCl (0.9%) before separation of bound from unbound radiolabeled ligand by filtration. After filtration the filters are washed 10 times with ice-cold 0.9% NaCl. The filters are dried at 50°C for 30 min, sealed, and 30 µl of Microsint 0 (Packard, cat. No. 6013616) is added to each well. The plates are counted in a Topcounter (1 min/well).
Tween™ 20 and 0.1% HSA or, alternatively, 0.1% ovalbumin (Sigma; catalogue No. A-5503)]. Test compounds are dissolved in DMSO and diluted in binding buffer. Radiolabeled ligand and membranes are diluted in binding buffer. The incubation is stopped by dilution with 5 ml ice-cold 0.9% NaCl, followed by rapid filtration through Whatman GF/C filters pre-treated for 1 hour with 0.5% polyethyleneimine. The filters are washed with 3 x 5 ml ice-cold NaCl. The radioactivity retained on the filters is counted using a Cobra II auto gamma counter.

The data are analysed by non-linear regression analysis of binding curves, using the Windows™ program GraphPad™ Prism (GraphPad Software, USA).

**Assay (VI) - Evaluation of energy expenditure**

TAC:SPRD rats or Wistar rats from M&B Breeding and Research Centre A/S, Denmark are used. After at least one week of acclimatization, rats are placed individually in metabolic chambers (Oxymax system, Columbus Instruments, Columbus, Ohio, USA; systems calibrated daily). During the measurements, animals have free access to water, but no food is provided to the chambers. Lightdark cycle is 12h:12h, with lights being switched on at 6:00. After the animals have spent approx. 2 hours in the chambers (i.e. when the baseline energy expenditure is reached), test compound or vehicle are administered (po, ip or sc), and recording is continued in order to establish the action time of the test compound. Data for each animal (oxygen consumption, carbon dioxide production and flow rate) are collected every 10-18 min for a total of 22 hours (2 hours of adaptation (baseline) and 20 hours of measurement). Correction for changes in O₂ and CO₂ content in the inflowing air is made in each 10-18 min cycle.

Data are calculated per metabolic weight [(kg body weight)⁰.⁷⁵] for oxygen consumption and carbon dioxide production, and per animal for heat. Oxygen consumption (VO₂) is regarded as the major energy expenditure parameter of interest.

**Assay (VII) - Evaluation of binding to albumin**

Test compounds are tested in a functional assay (Assay III) and a binding assay (Assay V), wherein Assay III contains HSA, and Assay V contains ovalbumin. EC₅₀ values are determined from Assay III, and Ki values from Assay V. The ratio EC₅₀/Ki is then calculated.
In the event of no albumin binding the ratio $EC_{50}/Ki$ will be 1 or below. The stronger the binding to albumin, the higher will be the ratio; for albumin-binding test compounds, the ratio $EC_{50}/Ki$ will thus be $\geq 1$, such as $\geq 10$, e.g. $\geq 100$. 
CLAIMS

1. A compound according to formula I:

$$\text{T-A-L-P}$$  

wherein

T represents tetrazol-5-yl;

A represents a straight-chain, branched and/or cyclic $\text{C}_6\text{H}_{15-30}$alkyl, $\text{C}_6\text{H}_{15-30}$alkenyl or $\text{C}_6\text{H}_{15-30}$alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxy and aryl;

L is a bond or a chemical structure covalently linking A and P; and

P represents a peptide structure comprising at least six $\alpha$-amino acid residues.

2. A compound according to claim 1, wherein T-A represents 10- (tetrazol-5-yl)decyl, 11- (tetrazol-5-yl)undecyl, 12-(tetrazol-5-yl)dodecyl, 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecyl, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl, 17-(tetrazol-5-yl)heptadecyl; 18-(tetrazol-5-yl)octadecyl or 19-(tetrazol-5-yl)nonadecyl.

3. A compound according to formula II:

$$\text{R}^1\text{R}^2\text{C}=(\text{O})\text{R}^3\text{S}^1\text{Z}^1\text{Z}^2\text{Z}^3\text{Z}^4\text{Z}^5\text{Z}^6\text{c}[\text{X}^1\text{X}^2\text{X}^3\text{X}^4\text{X}^5]\text{R}^4$$  

wherein

$\text{R}^1$ represents tetrazol-5-yl or carboxy;

$\text{R}^2$ represents a straight-chain, branched and/or cyclic $\text{C}_6\text{H}_{15-30}$alkyl, $\text{C}_6\text{H}_{15-30}$alkenyl or $\text{C}_6\text{H}_{15-30}$alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxy and aryl;

$\text{R}^3$ is absent or represents $\text{-NH-S}(\text{=O})\text{R}^2\text{(CH}_2\text{)}_{3-5}\text{C}(\text{=O})\text{-}$ or a peptide fragment comprising one or two amino acid residues and containing at least one carboxy group;

$\text{S}^1$ is absent or represents a 4-aminobutyric acid residue, Gly, $\beta$-Ala, or a glycolether-based structure according to one of the formulas IIa-IIlg:

$$\text{-HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{C}(\text{=O})\text{-}$$  

$$\text{-[HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{C}(\text{=O})\text{]}_2\text{-}$$  

$$\text{-[HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{C}(\text{=O})\text{]}_3\text{-}$$  

$$\text{-[HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{NH-C}(\text{=O})\text{-CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{=O})\text{]}_1\text{-3-}$$  

$$\text{-[HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{NH-C}(\text{=O})\text{-CH}_2\text{O-CH}_2\text{C}(\text{=O})\text{]}_1\text{-3-}$$  

$$\text{-[HN-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{O-CH}_2\text{O-CH}_2\text{O-CH}_2\text{CH}_2\text{C}(\text{=O})\text{]}_1\text{-3-}$$  

$$\text{-HN-CH}_2\text{CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{C}(\text{=O})\text{-}$$  

$$\text{-HN-CH}_2\text{CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{C}(\text{=O})\text{-}$$
Z1 is absent or represents Gly, β-Ala, Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Gln, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;
Z2 is absent or represents Gly, β-Ala, Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Gln, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;
Z3 represents Ser, D-Ser, Thr, D-Thr, His, D-His, Asn, D-Asn, Glu, D-Gln, Glu, D-Glu, Asp, D-Asp, Ala, D-Ala, Pro, D-Pro, Hyp or D-Hyp;
Z4 represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Tyr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab, Dap or Orn;
Z5 represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab, Dap or Orn;
Z6 represents Ala, D-Ala, Val, D-Val, Leu, D-Leu, He, D-Ile, Met, D-Met, Nle or D-Nle;
X1 represents Glu, Asp, Lys, homoCys, Orn, Dab or Dap;
X2 represents His, Cit, Dab, Dap, Cgl, Cha, Val, He, tBuGly, Leu, Tyr, Glu, Ala, Nle, Met, Met(O), Met(O2), Gln, Gln(alkyl), Gln(aryl), Asn, Asn(alkyl), Asn(aryl), Ser, Thr, Cys, Pro, Hyp, Tic, 2-PyAla, 3-PyAla, 4-PyAla, (2-thienyl)alanine, 3-(thienyl)alanine, (4-thiazolyl)Ala, (2-furyl)alanine, (3-furyl)alanine or Phe, wherein one or more hydrogens on the phenyl moiety of said Phe may optionally and independently be substituted by a substituent selected among halogen, hydroxy, alkoxy, nitro, benzoyl, methyl, trifluoromethyl, amino and cyano;
X3 represents D-Phe, wherein one or more hydrogens on the phenyl moiety in D-Phe may optionally and independently be substituted by a substituent selected among halogen, hydroxy, alkoxy, nitro, methyl, trifluoromethyl and cyano;
X4 represents Trp, 2-Nal, (3-benzo[b]thienyl)alanine or (S)-2,3,4,9-tetrahydro-1 H-β-carboline-3-carboxylic acid;
X5 represents Glu, Asp, Cys, homoCys, Lys, Orn, Dab or Dap;
wherein X1 and X5 are joined, rendering the compound of formula II cyclic, either via a disulfide bridge deriving from X1 and X5 both independently being Cys or homoCys, or via an amide bond formed between a carboxylic acid in the side-chain of X1 and an amino group in the side-chain of X5, or between a carboxylic acid in the side-chain of X5 and an amino group in the side-chain of X1;
R4 represents OR’ or N(R’)2, wherein each R’ independently represents hydrogen or represents C1-alkyl, C2-alkenyl or C2-alkynyl which may optionally be substituted with one or more amino or hydroxy;
with the proviso that said compound of formula II is not 15-carboxypentadecanoyl-Gly-Ser-
Gln-His-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH2 or
2-[2-(15-carboxypentadecanoylamino)ethoxy]ethoxyacetyl-Ser-Gln-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$;
and pharmaceutically acceptable salts, prodrugs and solvates thereof.

4. A compound according to claim 3, wherein $S^1$ is absent.

5. A compound according to claim 3, wherein $S^1$ represents a structure according to formula IIa.

6. A compound according to claim 3, wherein $S^1$ represents a structure according to formula INb.

7. A compound according to claim 3, wherein $S^1$ represents a structure according to formula INc.

8. A compound according to any one of claims 3-7, wherein $Z^1$ is absent.

9. A compound according to any one of claims 3-7, wherein $Z^1$ represents Gly.

10. A compound according to any one of claims 3-9, wherein $Z^2$ represents Ser, Thr, Gln, Gly or His.

11. A compound according to any one of claims 3-10, wherein $Z^2$ represents Ser or Thr.

12. A compound according to any one of claims 3-11, wherein $Z^3$ represents Gln, D-Gln, Asn, D-Asn, Ser or D-Ser.

13. A compound according to formula IVa, IVb or IVc:

   \[
   R^1-R^2-C(=O)-R^3-S^2-Z^4-Z^5-Z^6-c[X^1-X^2-X^3-Arg-X^4-X^5]R^4 \quad \text{[IVa]}
   \]

   \[
   R^1-R^2-C(=O)-R^3-S^2-Z^5-Z^6-c[X^1-X^2-X^3-Arg-X^4-X^5]R^4 \quad \text{[IVb]}
   \]

   \[
   R^1-R^2-C(=O)-R^3-S^2-Z^6-c[X^1-X^2-X^3-Arg-X^4-X^5]R^4 \quad \text{[IVc]}
   \]

   wherein

   $R^1$ represents tetrazol-5-yl or carboxy;
R² represents a straight-chain, branched and/or cyclic C₆₂₀alkyl, C₆₂₀alkenyl or C₆₂₀alkynyl which may optionally be substituted with one or more substituents selected from halogen, hydroxyl and aryl;

R³ is absent or represents -NH-S(=O)₂(CH₂)₅-C(=O)- or a peptide fragment comprising one or two amino acid residues and containing at least one carboxy group;

S² represents a glycolether-based structure according to one of the formulas Ilia-Ilg:

- [HN-CH₂-CH₃-O-CH₂-CH₂-O-CH₂-C(=O)]²-
  - [HN-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-C(=O)]²⁻
  - [HN-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-C(=O)]³⁻

Z⁴ represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Tyr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, homoArg, Arg, Lys, Dab, Dap or Orn;

Z⁵ represents Gly, Ala, Pro, Hyp, Ser, homoSer, Thr, Gln, Asn, 2-PyAla, 3-PyAla, 4-PyAla, His, HomoArg, Arg, Lys, Dab, Dap or Orn;

Z⁶ represents Ala, D-Ala, Val, D-Val, Leu, D-Leu, He, D-Ile, Met, D-Met, Nle or D-Nle;

X¹ represents Glu, Asp, Cys, homoCys, Lys, Orn, Dab or Dap;

X² represents His, Cit, Dab, Dap, Cgl, Cha, Val, He, tBuGly, Leu, Tyr, Glu, Ala, Nle, Met, Met(O), Met(O₂), Gln, Gln(alkyl), Gln(ary), Asn, Asn(alkyl), Asn(ary), Ser, Thr, Cys, Pro, Hyp, Tic, 2-PyAla, 3-PyAla, 4-PyAla, (2-thienyl)alanine, (3-thienyl)alanine, (4-thiazolyl)Ala, (2-furyl)alanine, (3-furyl)alanine or Phe, wherein one or more hydrogens on the phenyl moiety of said Phe may optionally and independently be substituted by a substituent selected among halogen, hydroxy, alkoxy, nitro, benzoyl, methyl, trifluoromethyl, amino and cyano;

X³ represents D-Phe, wherein one or more hydrogens on the phenyl moiety in D-Phe may optionally and independently be substituted by a substituent selected among halogen, hydroxy, alkoxy, nitro, methyl, trifluoromethyl and cyano;

X⁴ represents Trp, 2-Nal, (3-benzo[b]thienyl)alanine or (S)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid;

X⁵ represents Glu, Asp, Cys, homoCys, Lys, Orn, Dab or Dap;

wherein X¹ and X⁵ are joined, rendering the compound of formula IVa, IVb or IVc cyclic, either via a disulfide bridge deriving from X¹ and X⁵ both independently being Cys or homoCys, or via an amide bond formed between a carboxylic acid in the side-chain of X¹ and an amino acid residue X⁵.
group in the side-chain of X⁵, or between a carboxylic acid in the side-chain of X⁵ and an amino group in the side-chain of X¹;

R⁴ represents OR’ or N(R’), wherein each R’ independently represents hydrogen or represents Cᵦ-alkyl, C^ealkenyl or C^ealkynyl which may optionally be substituted with one or more amino or hydroxy;

with the proviso that said compound of formula IVa, IVb or IVc is not 2-[2-(15-carboxypentadecanoylamino)ethoxy]ethoxyacetyl-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂; and pharmaceutically acceptable salts, prodrugs and solvates thereof.

14. A compound according to claim 13, wherein S² represents a structure according to formula IIIa or INb.

15. A compound according to any one of claims 3-14, wherein R¹-R² represents 10-(tetrazol-5-yl)decyl, 11-(tetrazol-5-yl)undecyl, 12-(tetrazol-5-yl)dodecyl, 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecyl, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl, 17-(tetrazol-5-yl)heptadecyl; 18-(tetrazol-5-yl)octadecyl or 19-(tetrazol-5-yl)nonadecyl.

16. A compound according to any one of claims 3-14, wherein R¹-R² represents 13-(tetrazol-5-yl)tridecyl, 14-(tetrazol-5-yl)tetradecyl, 15-(tetrazol-5-yl)pentadecyl, 16-(tetrazol-5-yl)hexadecyl or 17-(tetrazol-5-yl)heptadecyl.

17. A compound according to any one of claims 3-14, wherein R¹-R² represents 15-(tetrazol-5-yl)pentadecyl.

18. A compound according to any one of claims 3-14, wherein R¹-R² represents 12-carboxydodecyl, 13-carboxytridecyl, 14-carboxytetradecyl, 15-carboxypentadecyl, 16-carboxyhexadecyl, 17-carboxyheptadecyl, 18-carboxyoctadecyl or 19-carboxynonadecyl.

19. A compound according to any one of claims 3-14, wherein R¹-R² represents 14-carboxytetradecyl.

20. A compound according to any one of claims 3-14, wherein R¹-R² represents 16-carboxyhexadecyl.

21. A compound according to any one of claims 3-20, wherein R³ is absent.
22. A compound according to any one of claims 3-20, wherein R\textsubscript{3} represents -NH-S(=O)\textsubscript{2}(CH\textsubscript{2})\textsubscript{3}C(=O)-, Glu, D-Glu, γ-Glu, D-γ-Glu, Asp, D-Asp, β-Asp, D-β-Asp or Gly-Y-Glu.

23. A compound according to any one of claims 3-20, wherein R\textsubscript{3} represents -NH-S(=O)\textsubscript{2}(CH\textsubscript{2})\textsubscript{3}C(=O)-.

24. A compound according to any one of claims 3-20, wherein R\textsubscript{3} represents D-Glu, γ-Glu, β-Asp or Gly-γ-Glu.

25. A compound according to any one of claims 3-24, wherein Z\textsubscript{4} represents Ser, homoSer, Gln, Asn, Tyr, His, Arg, homoArg, Lys, Orn, Dab or Dap.

26. A compound according to any one of claims 3-25, wherein Z\textsubscript{4} represents Ser, His, Arg or Dap.

27. A compound according to any one of claims 3-26, wherein Z\textsubscript{5} represents Ser, homoSer, Thr, Pro, Hyp, His, Lys, Orn, Dab or Dap.

28. A compound according to any one of claims 3-27, wherein Z\textsubscript{5} represents Ser, His or Dap.

29. A compound according to any one of claims 3-28, wherein Z\textsubscript{6} represents Ala, Val, Leu, He, Met or Nle.

30. A compound according to any one of claims 3-29, wherein Z\textsubscript{6} represents Nle.

31. A compound according to any one of claims 3-30, wherein X\textsubscript{2} represents Ser, Hyp, Cit, Dap, Asn, Gln or (4-thiazolyl)Ala.

32. A compound according to any one of claims 3-31, wherein X\textsubscript{2} represents Hyp, Dap, Cit or Gln.

33. A compound according to any one of claims 3-32, wherein X\textsubscript{2} represents Hyp.
34. A compound according to any one of claims 3-33, wherein \( X^1 \) is Glu, \( X^3 \) is D-Phe, \( X^4 \) is Trp and \( X^5 \) is Lys.

35. A compound according to any one of claims 3-33, wherein \( X^1 \) is Asp, \( X^3 \) is D-Phe, \( X^4 \) is Trp and \( X^5 \) is Lys.

36. A compound according to any one of claims 3-35, wherein \( R^4 \) is \( \text{NH}_2 \).

37. A compound according to any one of claims 3-35, wherein \( R^4 \) is \( \text{OH} \).

38. A compound according to claim 3 or 13, selected from the group consisting of:

\[
16-(\text{Trazol-5-yl})\text{hθxadθcanoyl-Gly-Thr-Gln-His-Sθr-Nlθc[Glu-Hyp-D-Ph θ-Arg-Tφ-Lys]-NH}_2
\]

\[
16-(\text{Trazol-5-yl})\text{hθxadθcanoyl-Gly-Thr-Gln-Dap-Sθr-Nlθc[Glu-Hyp-D-Ph θ-Arg-Tφ-Lys]-NH}_2
\]
{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Thr-Gln-Dap-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Ser-Nle-c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH₂

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂
4-(16-(tetrazol-5-yl)hexadecanoylsulfamoyl)butanoyl-Gly-Ser-D-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂


10 (2-[2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy]ethoxy)acetyl-His-Nle-c[Glu-Dap-D-Phe-Arg-Trp-Lys]-NH₂
the compound:


the compound:


the compound:
the compound:

5

the compound:

10 the compound:
the compound:

(2-{2-{2-{2-{[(R)-4-Carboxy-2-(1 6-(1 H-tetrazol-5-yl)hexadecanoylamino)butanoylamino]-ethoxy}ethoxy}acetylamino}ethoxy)acetyl-Ser-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

the compound:
the compound:

{2-[2-(15-(Carboxy)pentadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Dap-Nle-
c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

the compound:

the compound:
the compound:

\[ \text{i}^5\text{-Carboxypentadecanoyl-Gly-Ser-S_\theta-Tyr-Thr-Nle-ctGlu-Hyp-D-Phe-Arg-Trp-Lysl-NHg} \]

\[ \{2-\text{[2-(15-Carboxypentadecanoylamino)ethoxy]ethoxy}acetyl-Ser-Tyr-Hyp-Nle-ct[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2 \} \]

\[ \{2-\text{[2-(15-Carboxypentadecanoylamino)ethoxy]ethoxy}acetyl-Asn-Asn-Pro-Nle-ct[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH_2 \} \]
(2-{2-[(R)-4-Carboxy-2-(16-(tetrAzol-5-yl)hexadecanoylamino)butanoylamino]ethoxy}-ethoxy)acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

{2-[2-{2-{2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy}ethoxy}acetylamino}ethoxy]ethoxyjacetyl-Ser-Gln-His-Dap-Nle-ctGlu-Hyp-D-Phe-Arg-Trp-Lysj-NHg

(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-Arg-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂
{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-θr-Gln-Dap-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

(2-{2-[4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoylamino]ethoxy}ethoxy)acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

4-(16-(Tetrazol-5-yl)hexadecanoylsulfamoyl)butanoyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH$_2$

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-D-Ser-His-His-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-OH
{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Ser-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

5


4-(15-Carboxypentadecanoylsulfamoyl)butanoyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂

10
(2-{2-{(S)-4-Carboxy-4-(17-carboxyheptadecanoylamino)butanoylamino}ethoxy}ethoxy)-acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂


(2-{2-{(S)-3-Carboxy-3-(17-carboxyheptadecanoylamino)propanoylamino}ethoxy}ethoxy)-acetyl-Gly-Ser-Gln-His-Dap-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH₂
{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Thr-Nle-c\[Glu-Hyp-D-Phe-Arg-Trp-Lys]\-NH\_2

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Dab-Nle-c\[Glu-Hyp-D-Phe-Arg-Trp-Lys]\-NH\_2

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-homoSer-Nle-c\[Glu-Hyp-D-Phe-Arg-Trp-Lys]\-NH\_2

{2-[2-(16-(Tetrazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Orn-Nle-c\[Glu-Hyp-D-Phe-Arg-Trp-Lys]\-NH\_2
\{2-[(16-(T\textsuperscript{5}trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Lys-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH\textsubscript{2}

and

\{2-[(16-(T\textsuperscript{5}trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-Arg-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH\textsubscript{2}

and

\{2-[(16-(T\textsuperscript{5}trazol-5-yl)hexadecanoylamino)ethoxy]ethoxy}acetyl-Gly-Ser-Gln-His-2-PyAla-Nle-c[Glu-Hyp-D-Phe-Arg-Trp-Lys]-NH\textsubscript{2}
39. A method of delaying the progression from IGT to type 2 diabetes, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

40. A method of delaying the progression from type 2 diabetes to insulin-requiring diabetes, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

41. A method of treating obesity or preventing overweight, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

42. A method of regulating appetite, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

43. A method of inducing satiety, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

44. A method of preventing weight gain after successfully having lost weight, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.
45. A method of increasing energy expenditure, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

46. A method of treating a disease or state related to overweight or obesity, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

47. A method of treating bulimia, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

48. A method of treating a disease or state selected from atherosclerosis, hypertension, diabetes, type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction and risk of premature death, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

49. A method of treating, in an obese patient, a disease or state selected from type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction and risk of premature death, comprising administering to an obese patient in need thereof an effective amount of a compound according to any one of claims 1-38, optionally in combination with one or more additional therapeutically active compounds.

50. A method according to any one of claims 39-49, wherein said additional therapeutically active compound is selected from antidiabetic agents, antihyperlipidemic agents, antiobesity agents, antihypertensive agents and agents for the treatment of complications resulting from, or associated with, diabetes.

51. A method according to any one of claims 39-49, wherein said compound according to any one of claims 1-38 is administered to said patient in a unit dosage form comprising from
about 0.05 mg to about 1000 mg of said compound.

52. A method of activating MC4 in a subject, the method comprising administering to said subject an effective amount of a compound according to any one of claims 1-38.

53. A method according to any one of claims 39-52, wherein said compound according to any of claims 1-38 is administered parenterally or sublingually.

54. A compound according to any one of claims 1-38 for use in therapy.

55. A pharmaceutical composition comprising a compound according to any one of claims 1-38.

56. The use of a compound according to any one of claims 1-38 in the manufacture of a medicament for: delaying the progression from IGT to type 2 diabetes; delaying the progression from type 2 diabetes to insulin-requiring diabetes; treating obesity or preventing overweight; regulating appetite; inducing satiety; preventing weight regain after successful weight loss; increasing energy expenditure; treating a disease or state related to overweight or obesity; treating bulimia; treating atherosclerosis, hypertension, type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction or risk of premature death; or treating, in an obese patient, a disease or state selected from type 2 diabetes, impaired glucose tolerance (IGT), dyslipidemia, coronary heart disease, gallbladder disease, gall stone, osteoarthritis, cancer, sexual dysfunction or risk of premature death.