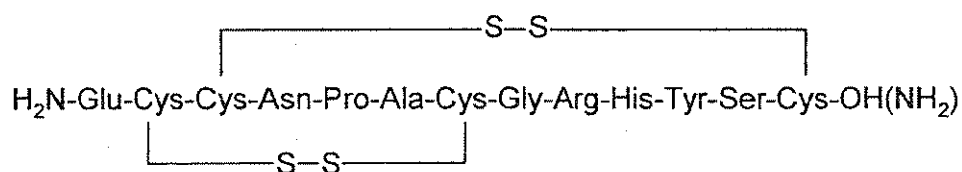


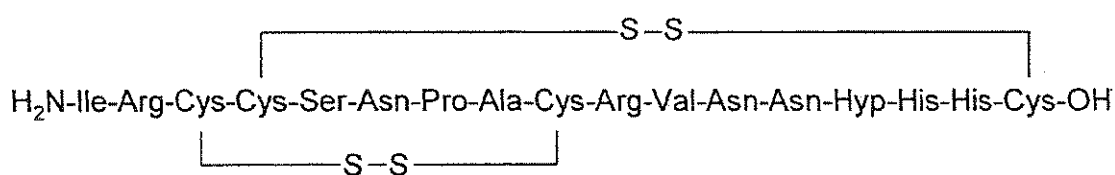
Highly bridged peptides from *Actinomadura namibiensis*

Several highly bridged peptides are known in the literature, for example conopeptides isolated from cone snails (for a review see e.g. Terlau & Olivera, *Physiol. Rev.* 2004, 84, 41–68) or the so-called lantibiotics (Chatterjee et al., *Chem. Rev.* 2005, 105, 633–683) from Gram-positive bacteria source. The said peptides have various utilities. The lantibiotic nisin, for example, is used as a food preservative since many years.

The conopeptides are useful for the treatment of pain, diabetes, multiple sclerosis and cardiovascular diseases and currently undergo preclinical or clinical development. Examples of conopeptides are α -GI (sequence: ECCNPACGRHYSC*, *amidated, connectivity: 1-3,2-4) and α -GID (sequence: IR γ CCSNPACRVNNOHVVC, connectivity: 1-3,2-4), wherein O/Hyp is hydroxyproline and the connectivity indicates the position of the cysteine involved in each specific disulphide bonds, for example, first to third and second to fourth as in α -GID:

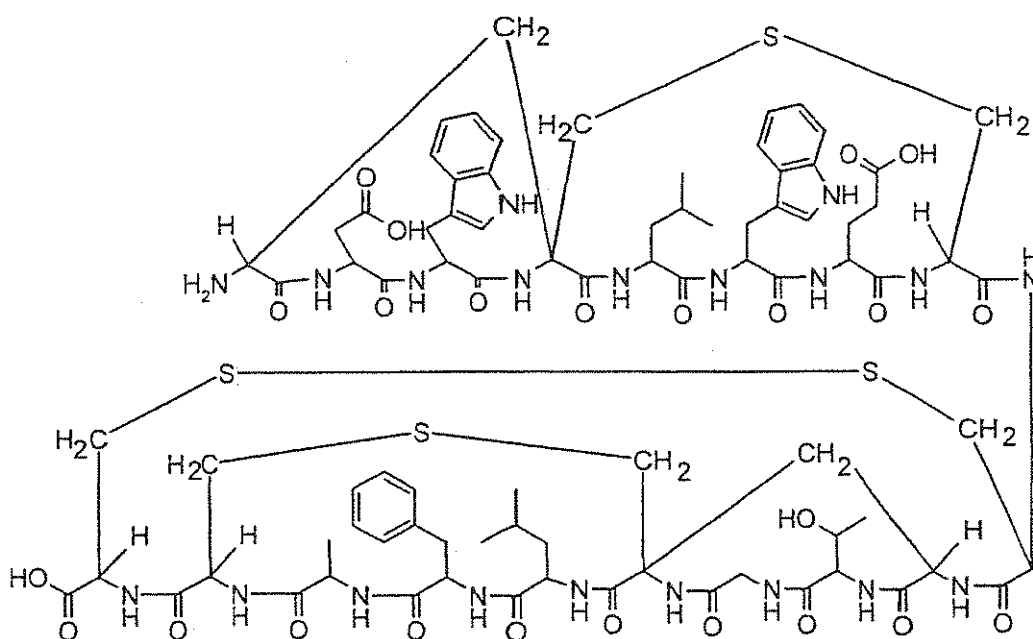


(α -GI),



(α -GID).

A novel group of highly bridged peptides named Labyrinthopeptins has been discovered recently (European patent application EP06020980.6). The so-called Labyrinthopeptins exhibit a unique bridging motif across their peptide chain, as illustrated by the compound in formula below:

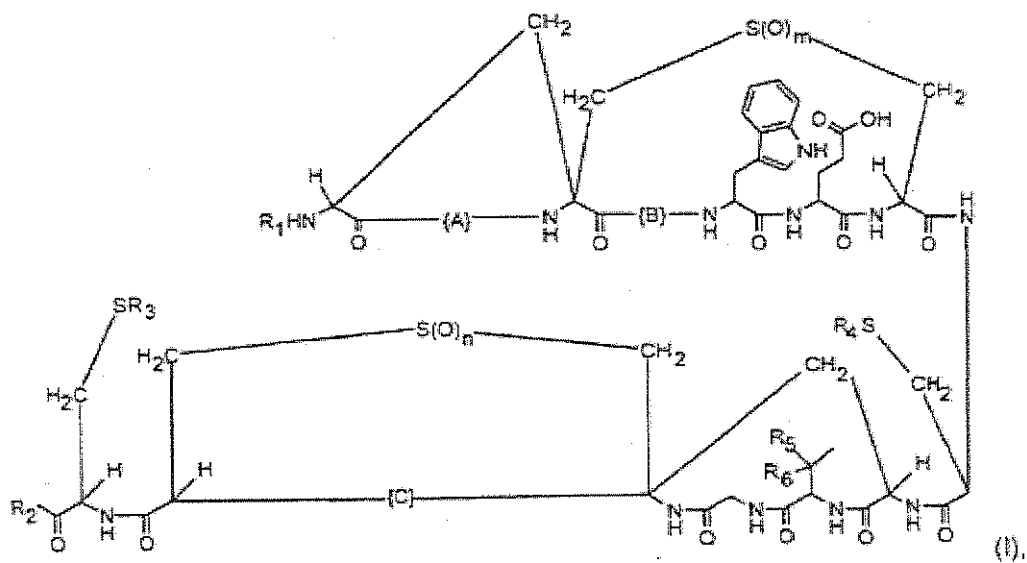


It has now been found that further highly bridged peptides of the Labyrinthopeptin class can be isolated from microorganism strain *Actinomadura namibiensis* (DSM 6313). The compounds are distinctly different from the Labyrinthopeptin derivatives as

5 described in patent application EP06020980.6.

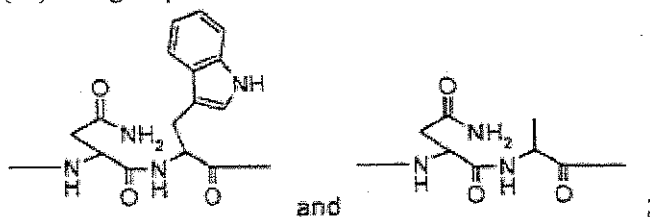
SUMMARY

In a first aspect, there is provided a compound of the formula (I)

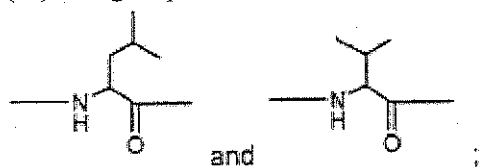


wherein

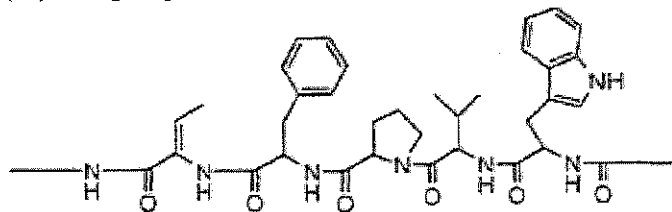
{A} is a group selected from



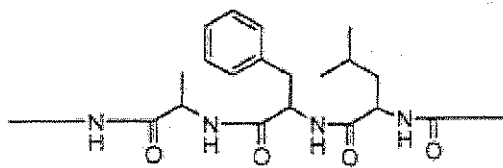
$\{B\}$ is a group selected from



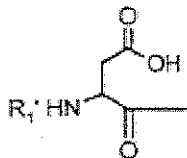
$\{C\}$ is a group selected from



and



R_1 is a group R_1' or a group



wherein R_1' is H, $C(O)-(C_1-C_6)alkyl$ or $C(O)-O-(C_1-C_6)alkyl$;

R_2 is OH, NH_2 , $NH-(C_1-C_6)alkyl$, $NH-(C_1-C_4)alkylene-phenyl$ or $NH-(C_1-C_4)alkylene-pyridyl$;

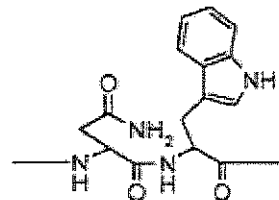
R_3 and R_4 are independently of each other H, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylene-C(O)NH_2$, $(C_1-C_6)alkylene-C(O)NH(C_1-C_4)alkyl$ or $(C_1-C_6)alkylene-C(O)N[(C_1-C_4)alkyl]_2$, or R_3 and R_4 together with the S atoms to which they are attached form a disulfide group S-S;

R_5 and R_6 are independently of each other H or OH, or R_5 and R_6 together are $=O$;

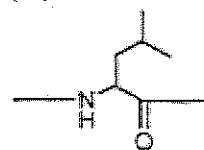
m and n are independently of one another 0, 1 or 2;

with the proviso that if

{A} is

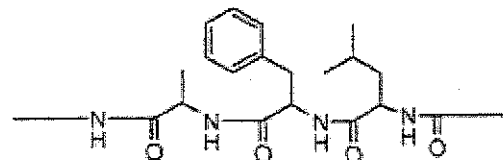


{B} is

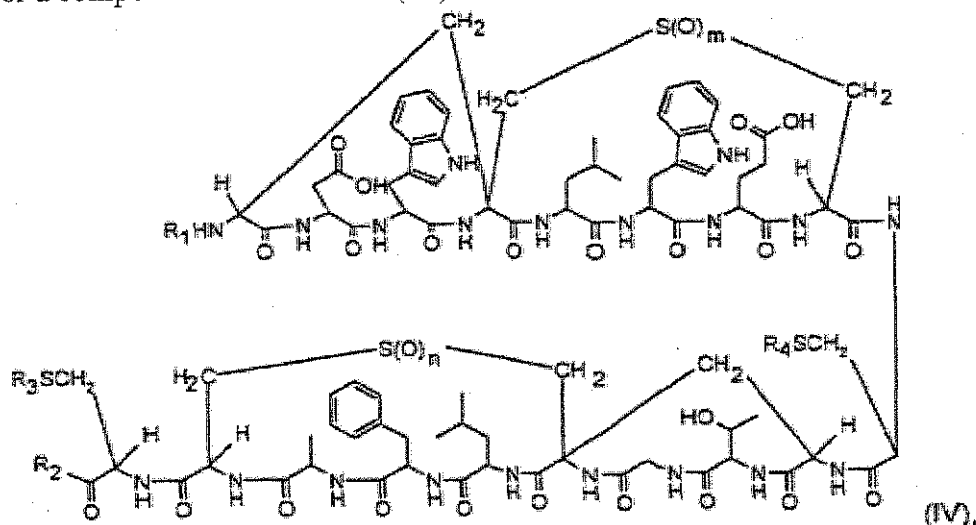


and

{C} is



R_3 and R_4 may not form a disulfide group S-S together with the S atoms to which they are attached;
or a compound of the formula (IV)



wherein

R_1 is H, $C(O)-(C_1-C_6)$ alkyl or $C(O)-O-(C_1-C_6)$ alkyl;

R_2 is OH, NH_2 , $NH-(C_1-C_6)$ -alkyl, $N[(C_1-C_6)$ -alkyl] $_2$, $NH-(C_1-C_4)$ -alkylene-phenyl or $NH-(C_1-C_4)$ -alkylene-pyridyl;

R_3 and R_4 are independently from each other H, (C_1-C_6) alkyl or (C_1-C_4) -alkylene- $C(O)NH_2$;

m and n are independently of one another 0, 1 or 2;

in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt thereof.

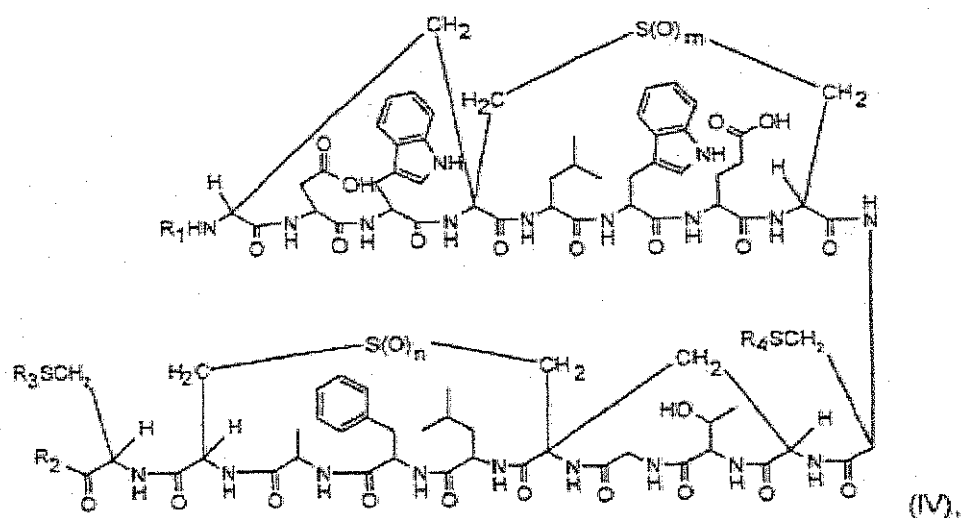
In a second aspect, there is provided a process for preparing a compound of the formula (I) as defined above, comprising

a) fermenting the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, under suitable conditions in a culture medium until one or more of the compounds of the formula (I) accrue(s) in the culture medium,

b) isolating a compound of the formula (I) from the culture medium, and

c) derivatizing, where appropriate, the compound isolated in step b) and/or, where appropriate, converting the compound isolated in step b) or the derivative of the compound isolated in step b) into a physiologically tolerated salt.

In a third aspect, there is provided a process for preparing a compound as defined above, wherein the compound is **characterized** by the formula (IV),



wherein

m and n are both 0,

R₁ is H,

R₂ is OH, and

R₃ and R₄ are independently of each other H, (C₁-C₆)alkyl, (C₁-C₆)alkylene-C(O)NH₂, (C₁-C₆)alkylene-C(O)NH(C₁-C₄)alkyl or (C₁-C₆)alkylene-C(O)N[(C₁-C₄)alkyl]₂, comprising

- a) fermenting the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, under suitable conditions in a culture medium until the compound of formula (IV, i.e.) Labyrinthopeptin A2, accrues in the culture medium,
- b) isolating the compound Labyrinthopeptin A2 from the culture medium, and
- c) derivatizing the compound isolated in step b) and, where appropriate, converting the derivative of the compound isolated in step b) into a physiologically tolerated salt.

In a fourth aspect, there is provided the use of a compound as defined above for the preparation of a medicament for the treatment of bacterial infections, viral infections and/or pain.

In a fifth aspect, there is provided a pharmaceutical composition comprising at least one compound as defined above and at least one pharmaceutically acceptable carrier.

In a sixth aspect, there is provided DNA coding for prepro-Labyrinthopeptin A2 having the nucleic acid sequence as shown in SEQ ID NO: 13.

In a seventh aspect, there is provided Prepro-Labyrinthopeptin A2 having the amino acid sequence as shown in SEQ ID NO: 14.

In an eighth aspect, there is provided Pro-Labyrinthopeptin A2 having the amino acid sequence as shown in SEQ. ID NO: 15.

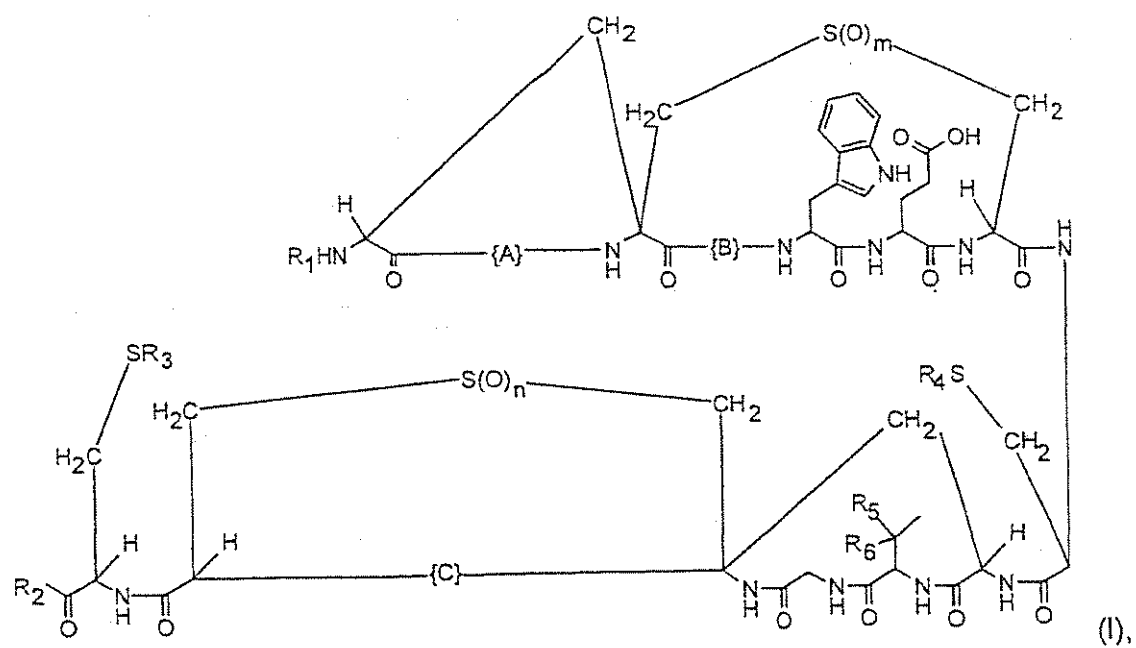
In a ninth aspect, there is provided DNA coding for prepro-Labyrinthopeptin A1 having the nucleic acid sequence as shown in SEQ. ID NO: 17.

In a tenth aspect, there is provided Prepro-Labyrinthopeptin A1 having the amino acid sequence as shown in SEQ ID NO: 18.

In an eleventh aspect, there is provided Pro-Labyrinthopeptin A1 having the amino acid sequence as shown in SEQ. ID NO: 19.

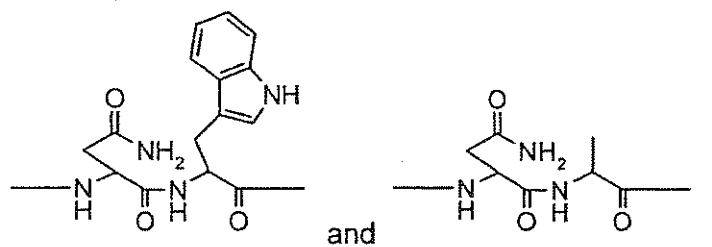
DETAILED DESCRIPTION

An embodiment of the present invention is a compound of the formula (I)



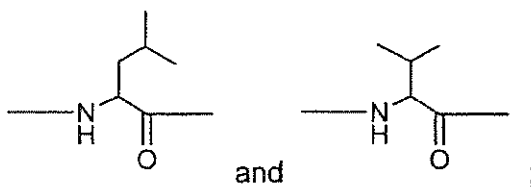
wherein

{A} is a group selected from

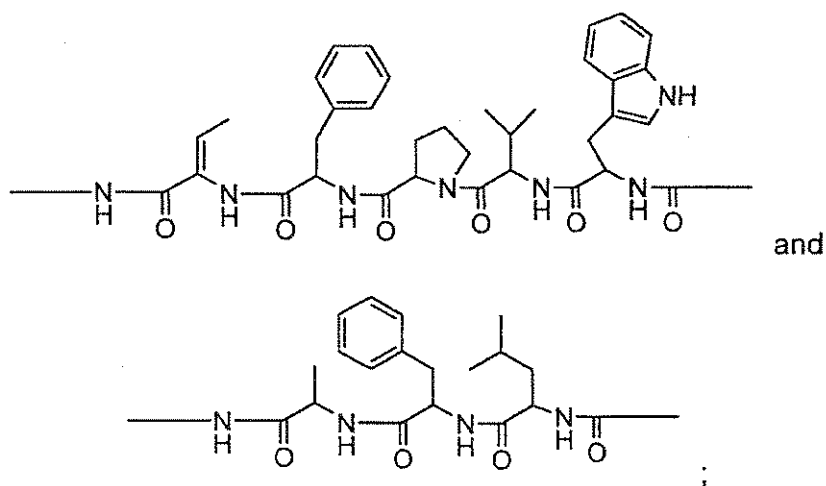


5

{B} is a group selected from

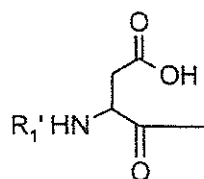


{C} is a group selected from



10

R_1 is a group R_1' or a group



wherein R_1' is H, $C(O)-(C_1-C_6)alkyl$ or $C(O)-O-(C_1-C_6)alkyl$;

R_2 is OH, NH_2 , $NH-(C_1-C_6)alkyl$, $NH-(C_1-C_4)alkylene-phenyl$ or $NH-(C_1-C_4)alkylene-pyridyl$;

5

R_3 and R_4 are independently of each other H, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylene-C(O)NH_2$, $(C_1-C_6)alkylene-C(O)NH(C_1-C_4)alkyl$ or $(C_1-C_6)alkylene-C(O)N[(C_1-C_4)alkyl]_2$, or R_3 and R_4 together with the S atoms to which they are attached form a disulfide group S-S;

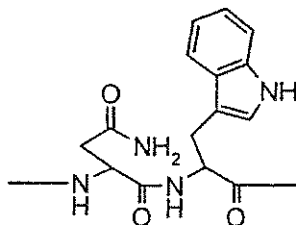
10

R_5 and R_6 are independently of each other H or OH, or R_5 and R_6 together are $=O$;

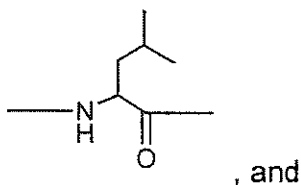
m and n are independently of one another 0, 1 or 2;

15 with the proviso that if

{A} is

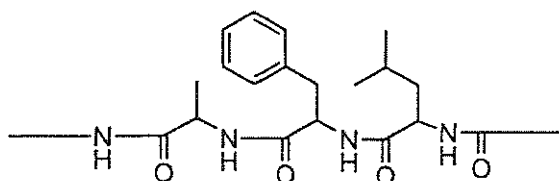


20 {B} is



{C} is

5



R₃ and R₄ may not form a disulfide group S-S together with the S atoms to which they are attached;

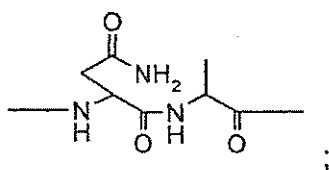
5

in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt thereof.

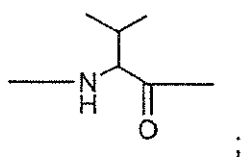
Preferably,

10

{A} is

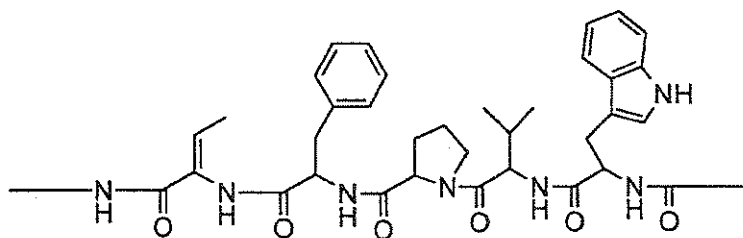


{B} is



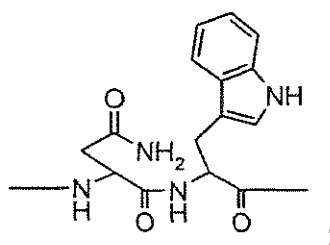
15

{C} is

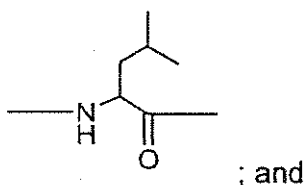


20 Further preferred,

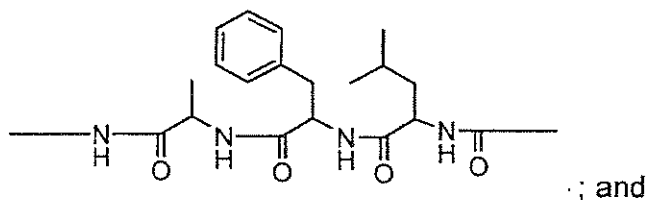
{A} is



5. {B} is



{C} is



10

R_1 is preferably a group R_1' .

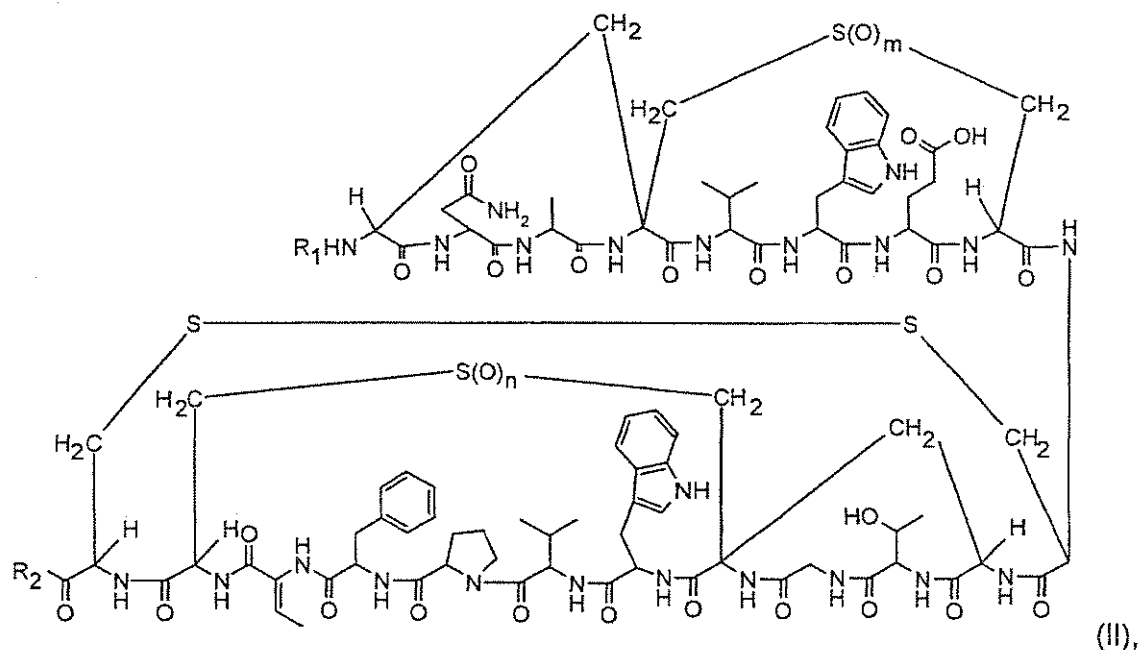
R_1' is preferably H.

15 R_2 is preferably OH.

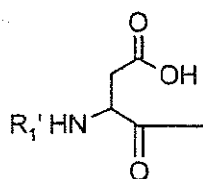
R_3 and R_4 are preferably independently of each other H, (C₁-C₆)alkyl, (C₁-C₆)alkylene-C(O)NH₂, or form a disulfide group S-S together with the S atoms to which they are attached. More preferred, R_3 and R_4 are H or form a disulfide group S-S together with the S atoms to which they are attached. Most preferred, R_3 and R_4 form a disulfide group S-S together with the S atoms to which they are attached.

R_5 and R_6 are preferably H or OH wherein if R_5 is OH then R_6 is H, and if R_5 is H then R_6 is OH, or R_5 and R_6 together are =O. More preferred, R_5 is OH and R_6 is H, or R_5 is H and R_6 is OH.

- 5 Preferably, compound (I) is characterized by a compound of the formula (II)



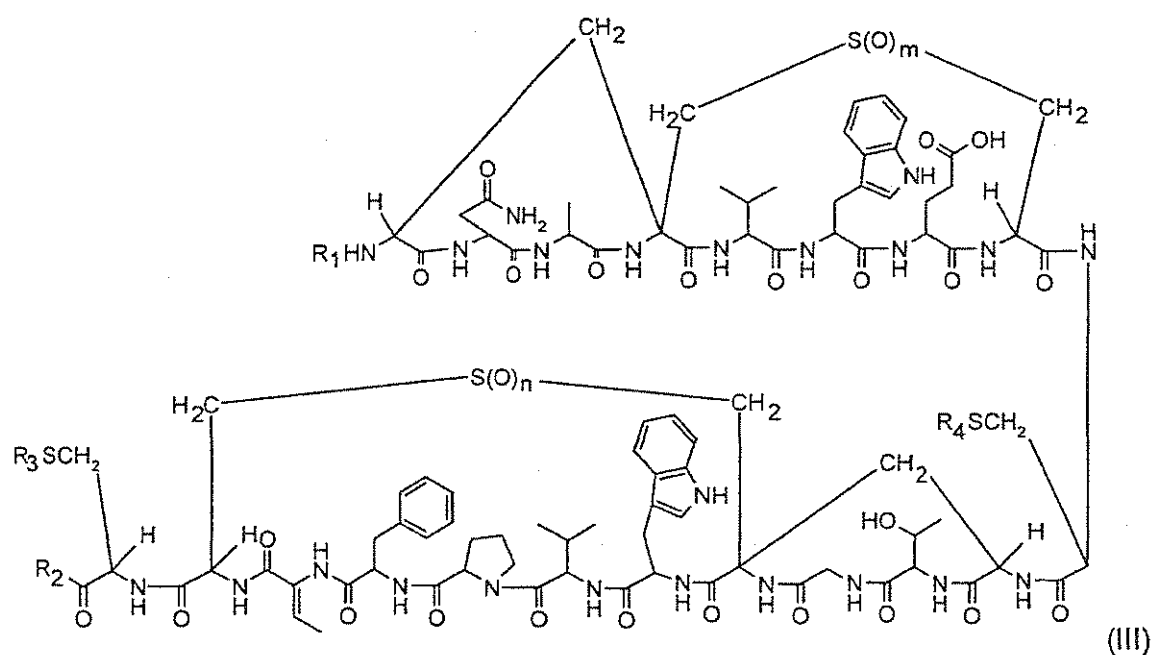
wherein R_1 is R_1' or a group



wherein R_1' is H, $C(O)-(C_1-C_6)alkyl$ or $C(O)-O-(C_1-C_6)alkyl$, preferably H.

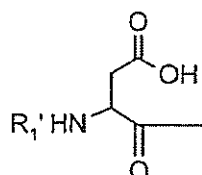
Further preferred, compound (I) is characterized by a compound of the formula (III)

8



wherein

- 5 R_1 is R_1' or a group



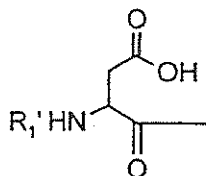
wherein R_1' is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl, preferably H;

R_2 is OH, NH₂, NH-(C₁-C₆)-alkyl, N[(C₁-C₆)-alkyl]₂, NH-(C₁-C₄)-alkylene-phenyl or
 10 NH-(C₁-C₄)-alkylene-pyridyl, preferably R_2 is H; and

R_3 and R_4 are independently from each other H, (C₁-C₆)alkyl or
 (C₁-C₄)-alkylene-C(O)NH₂.

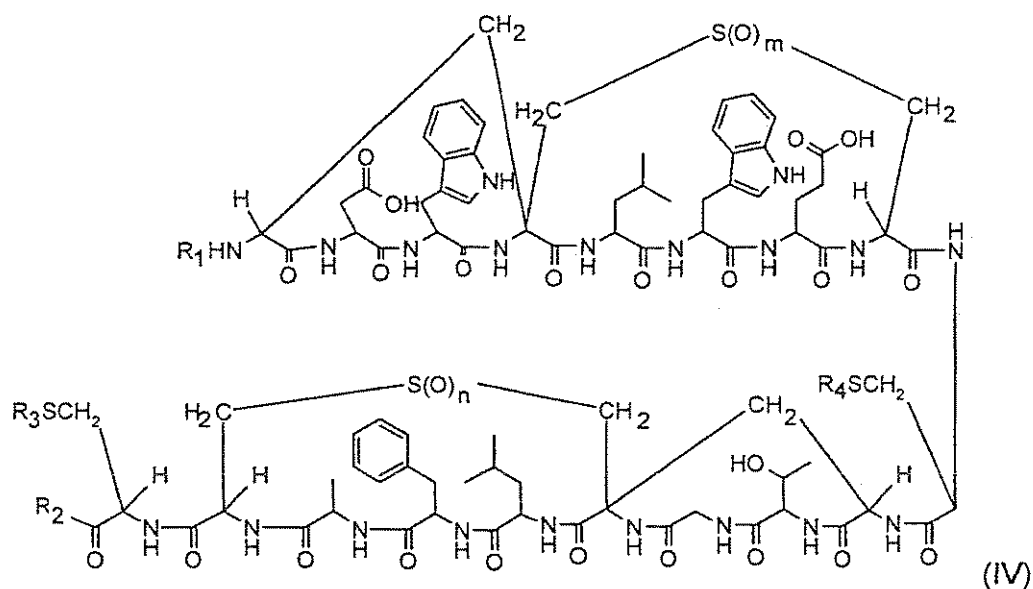
- 15 Compounds of the formulae (II) and (III) wherein R_1 is R_1' are subsequently named
 Labyrinthopeptins A1.

Compounds of the formulae (II) and (III) wherein R₁ is a group



are subsequently named Labyrinthopeptins A3.

5 Further preferred, compound (I) is characterized by a compound of the formula (IV)



wherein

10 R₁ is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl, and

R₂ is OH, NH₂, NH-(C₁-C₆)-alkyl, N[(C₁-C₆)-alkyl]₂, NH-(C₁-C₄)-alkylene-phenyl or NH-(C₁-C₄)-alkylene-pyridyl, and

15 R₃ and R₄ are independently from each other H, (C₁-C₆)alkyl or (C₁-C₄)-alkylene-C(O)NH₂.

Compounds of the formula (IV) are named Labyrinthopeptins A2.

Preferably, in the compounds of the formula (I), m and n are both 0, or m and n are both 2, or m is 0 and n is 2, or m is 2 and n is 0. Most preferred, m and n are both 0.

The present invention furthermore relates to all obvious chemical equivalents of the compounds of the formula (I) according to the invention. These equivalents are compounds which exhibit only a slight chemical difference, and have the same pharmacological effect, or which are converted into the compounds according to the invention under mild conditions. Said equivalents also include, for example, salts, reduction products, oxidation products, partial hydrolytic processes esters, ethers, acetals or amides of the compounds of the formula (I) as well as equivalents which the skilled person can prepare using standard methods and, in addition to this, all the optical antipodes and diastereomers and all the stereoisomeric forms.

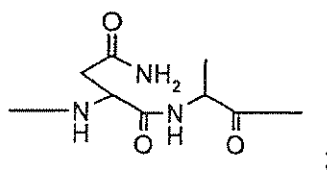
Unless otherwise indicated, the chiral centers in the compounds of the formula (I) can be present in the R configuration or in the S configuration. The invention relates both to the optically pure compounds and to stereoisomeric mixtures, such as enantiomeric mixtures and diastereomeric mixtures.

Physiologically tolerated salts of compounds of the formula (I) are understood as being both their organic salts and their inorganic salts, as are described in Remington's Pharmaceutical Sciences (17th edition, page 1418 (1985)). Because of their physical and chemical stability and their solubility, sodium, potassium, calcium and ammonium salts are preferred, inter alia, for acid groups; salts of hydrochloric acid, sulfuric acid or phosphoric acid, or of carboxylic acids or sulfonic acids, such as acetic acid, citric acid, benzoic acid, maleic acid, fumaric acid, tartaric acid and p-toluenesulfonic acid, are preferred, inter alia, for basic groups.

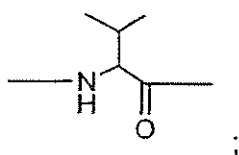
More preferred, the compounds of the formulae (I) to (IV) are characterized by the stereochemistry as shown for a compound of the formula (V), that is a compound of the formula (I), wherein

{A} is

11

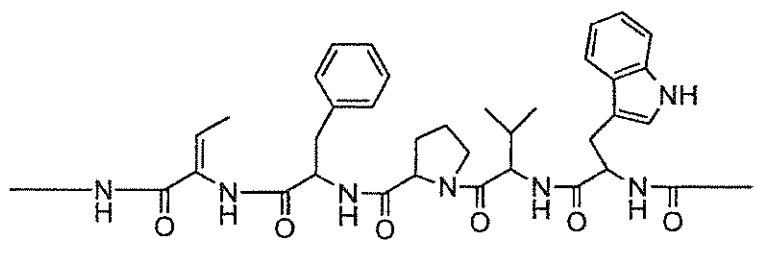
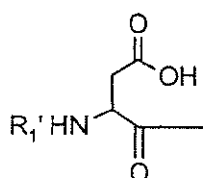


{B} is



5

{C} is

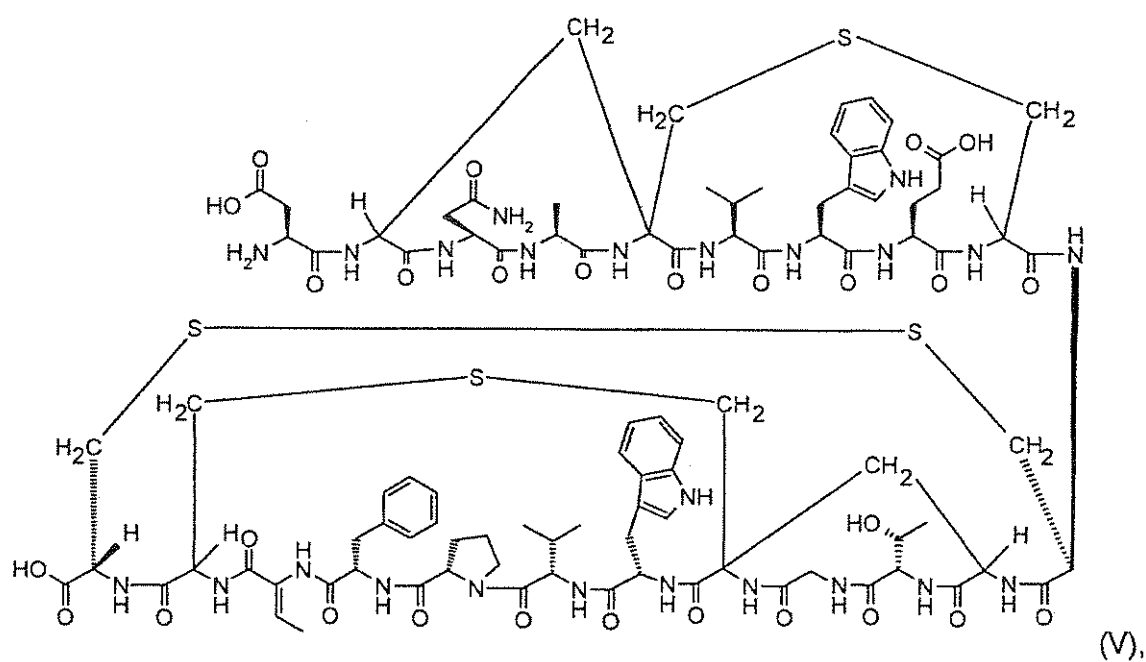
 R_1 is10 wherein R_1' is H; R_2 are H;

R_3 and R_4 together with the S atoms to which they are attached form a disulfide group S-S;

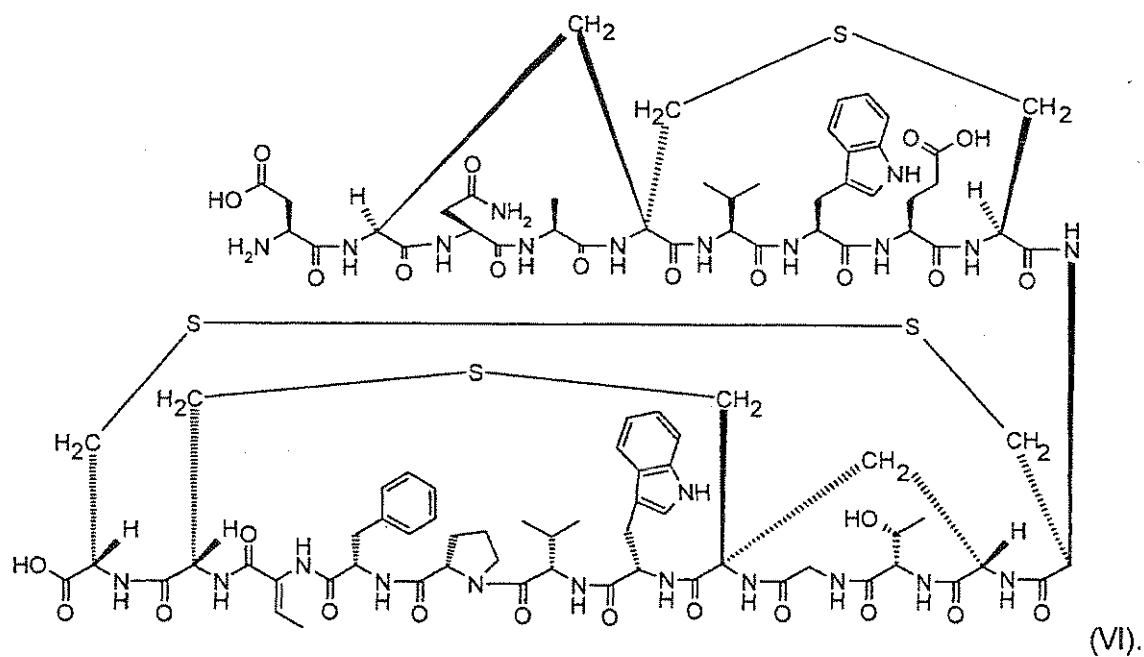
 R_5 is H;15 R_6 is OH; and

m and n are 0;

12



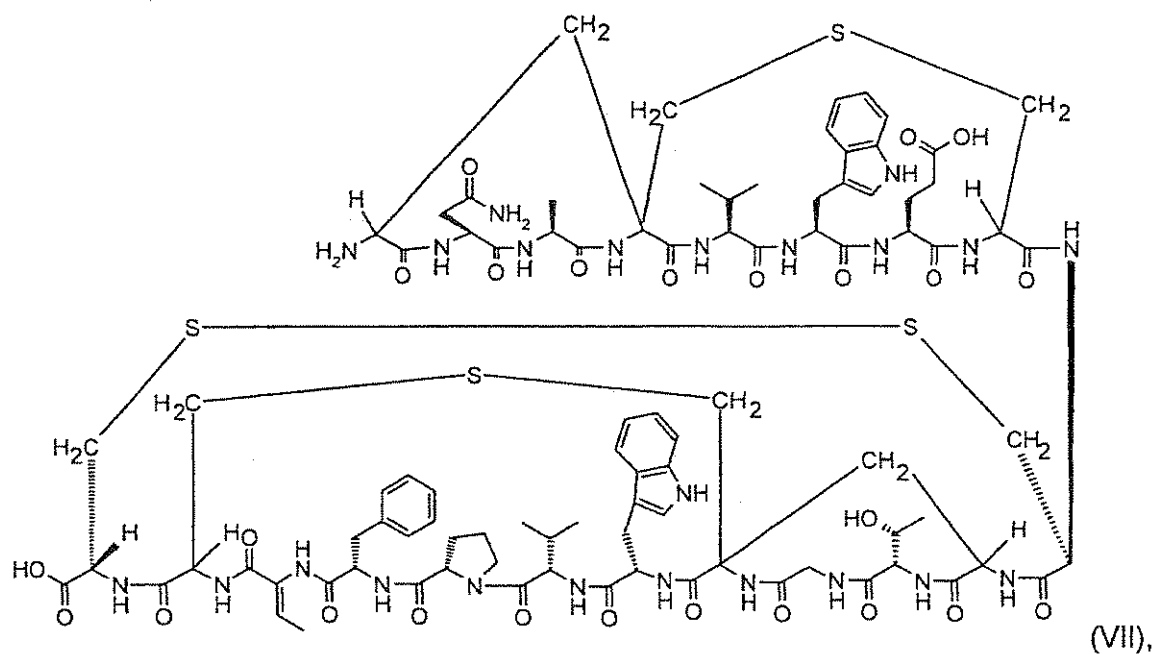
most preferred, as described in the formula (VI)



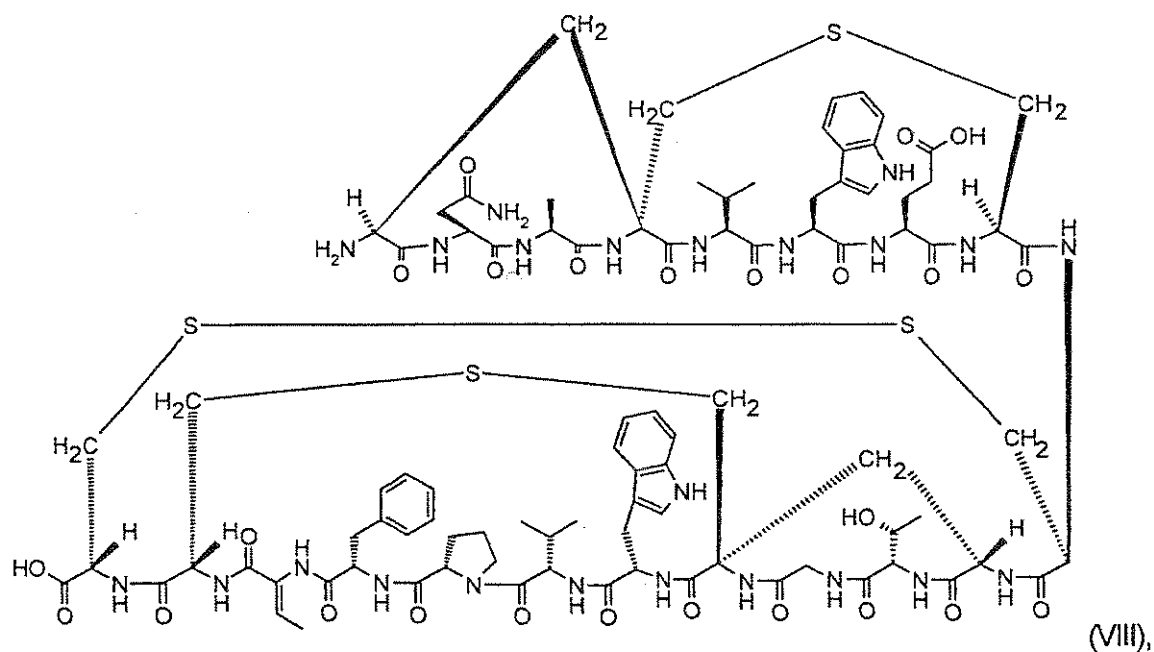
5

A further embodiment of the present invention is a compound of the formula (I), characterized by the formula (VII)

13



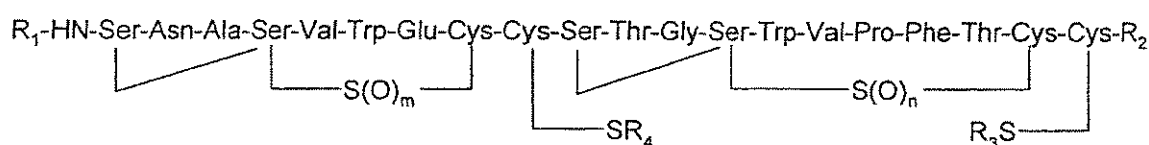
preferably by formula (VIII)



5

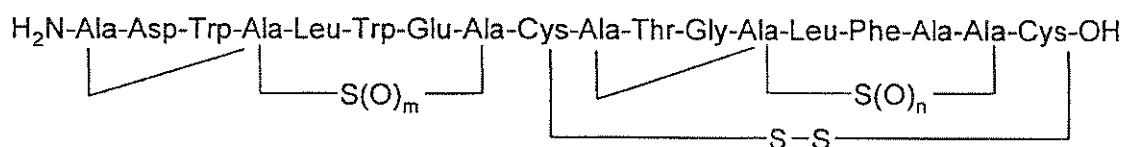
wherein the formulae (V) and (VI) refer to Labyrinthopeptin A3, and the formulae (VII) and (VIII) refer to Labyrinthopeptin A1.

For a further characterization of the compounds of the present invention, the peptide residues were converted back to their probable precursors from ribosomal peptide synthesis. The alpha, alpha-disubstituted amino acids in residues 4 and 13 are without precedence in the literature. The said amino acids may be described as a Ser residue, where the hydroxyl group at the beta-position is substituted, as shown below for the compounds of the formulae (II) and (III):



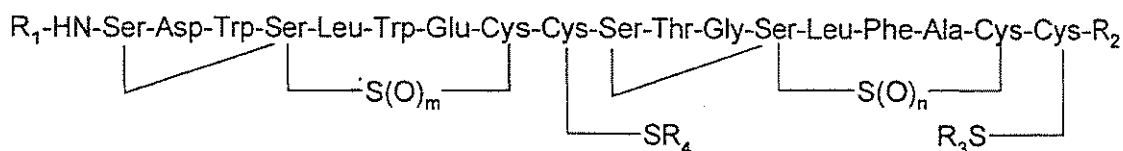
(SEQ ID NO: 1).

In a preceding European patent application for Labyrinthopeptin A2 (EP06020980.6), the following ribosomal precursor was assumed in the absence of knowledge on the biosynthesis:



(SEQ ID NO: 2).

On the basis of new insights in the biosynthesis of Labyrinthopeptins (see below), the biosynthetic precursor for compounds of the formula (IV) is described as follows:



(SEQ ID NO: 3).

The invention also relates to a process for preparing a compound of the formula (I) according to claim 1 comprising

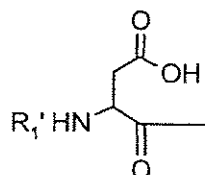
a) fermenting the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants

and/or mutants, under suitable conditions in a culture medium until one or more of the compounds of the formula (I) accrue(s) in the culture medium,

- b) isolating a compound of the formula (I) from the culture medium, and
 c) derivatizing, where appropriate, the compound isolated in step b) and/or, where
 5 appropriate, converting the compound isolated in step b) or the derivative of compound isolated in step b) into a physiologically tolerated salt.

Preferably, the compound isolated in step b) is characterized by formula (II) wherein m and n are both 0,

- 10 R_1 is R_1' or a group



wherein R_1' is H, and
 R_2 is OH.

- 15 Further preferred, the compound isolated in step b) is Labyrinthopeptin A2 which subsequently derivatized in step c) to a compound of the formula (IV) wherein m and n are both 0,
 R_1 is H,
 R_2 is OH, and
 20 R_3 and R_4 are independently of each other H, (C₁-C₆)alkyl, (C₁-C₆)alkylene-C(O)NH₂, (C₁-C₆)alkylene-C(O)NH(C₁-C₄)alkyl or (C₁-C₆)alkylene-C(O)N[(C₁-C₄)alkyl]₂.

- The culture medium is a nutrient solution or a solid medium containing at least one customary carbon source and at least one nitrogen source as well as one or more
 25 customary inorganic salts.

The process according to the invention can be used for fermenting on a laboratory scale (milliliter to liter scale) and for fermenting on an industrial scale (cubic meter scale).

- Suitable carbon sources for the fermentation are assimilable carbohydrates and sugar alcohols, such as glucose, lactose, sucrose or D-mannitol, as well as carbohydrate-containing natural products, such as malt extract or yeast extract. Examples of
- 5 nitrogen-containing nutrients are amino acids; peptides and proteins and also their breakdown products, for example casein, peptones or tryptones; meat extracts; yeast extracts; gluten; ground seeds, for example from corn, wheat, beans, soya or the cotton plant; distillation residues from producing alcohol; meat meals; yeast extracts; ammonium salts; nitrates. Preference is given to the nitrogen source being one or
- 10 more peptide(s) which has/have been obtained synthetically or biosynthetically. Examples of inorganic salts are chlorides, carbonates, sulfates or phosphates of the alkali metals, the alkaline earth metals, iron, zinc, cobalt and manganese. Examples of trace elements are cobalt and manganese.
- 15 Conditions which are especially suitable for forming the Labyrinthopeptins according to the invention are as follows: from 0.05 to 5%, preferably from 0.1 to 2.5%, yeast extract; from 0.2 to 5.0%, preferably from 0.1 to 2%, casitone; from 0.02 to 1.0%, preferably from 0.05 to 0.5%, $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$; from 0.02 to 1.5%, preferably from 0.05 to 0.7%, $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ and from 0.00001% to 0.001% cyanocobalamin. The
- 20 percentage values which are given are in each case based on the weight of the total nutrient solution.

- The microorganism is cultured aerobically, that is, for example, submerged while being shaken or stirred in shaking flasks or fermenters, or on solid medium, where
- 25 appropriate while air or oxygen is being passed in. The microorganism can be cultured in a temperature range of from about 18 to 35°C, preferably at from about 20 to 32°C, in particular at from 27 to 30°C. The pH range should be between 4 and 10, preferably between 6.5 and 7.5. The microorganism is generally cultured under these conditions for a period of from 2 to 10 days, preferably of from 72 to 168 hours. The micro-
- 30 organism is advantageously cultured in several steps, i.e. one or more preliminary cultures are initially prepared in a liquid nutrient medium, with these preliminary cultures then being inoculated into the actual production medium, i.e. the main culture,

for example in a ratio by volume of from 1:10 to 1:100. The preliminary culture is obtained, for example, by inoculating the strain, in the form of vegetative cells or spores, into a nutrient solution and allowing it to grow for from about 20 to 120 hours, preferably for from 48 to 96 hours. Vegetative cells and/or spores can be obtained, for
5 example, by allowing the strain to grow for from about 1 to 15 days, preferably for from 4 to 10 days, on a solid or liquid nutrient substrate, for example yeast agar.

The Labyrinthopeptin derivatives can be isolated and purified from the culture medium using known methods and taking account of the chemical, physical and biological
10 properties of the natural substances. HPLC was used to test the concentrations of the respective Labyrinthopeptin derivatives in the culture medium or in the individual isolation steps, with the quantity of the substance formed expediently being compared with a calibration solution.

15 For the isolation, the culture broth or the culture together with the solid medium is optionally lyophilized, and the Labyrinthopeptin derivatives are extracted from the lyophilizate using an organic solvent or a mixture of water and an organic solvent, preferably containing 50-90% organic solvent. Examples of organic solvents are methanol and 2-propanol. The organic solvent phase contains the natural substances
20 according to the invention; it is concentrated, where appropriate, in vacuo and subjected to further purification.

The further purification of one or more compounds according to the invention is effected by chromatography on suitable materials, preferably, for example, on
25 molecular sieves, on silica gel, on aluminum oxide, on ion exchangers or on adsorber resins or on reversed phases (RPs). This chromatography is used to separate the Labyrinthopeptin derivatives. The Labyrinthopeptin derivatives are chromatographed using buffered, basic or acidified aqueous solutions or mixtures of aqueous and organic solutions.

30

Mixtures of aqueous or organic solutions are understood as being all water-miscible organic solvents, preferably methanol, 2-propanol or acetonitrile, at a concentration of

from 5 to 99% organic solvent, preferably from 5 to 50% organic solvent, or else all buffered aqueous solutions which are miscible with organic solvents. The buffers which are to be used are the same as specified above.

- 5 The Labyrinthopeptin derivatives are separated, on the basis of their differing polarities, by means of reversed phase chromatography, for example on MCI (adsorber resin, Mitsubishi, Japan) or Amberlite XAD (TOSHAAS), or on other hydrophobic materials, for example on RP-8 or RP-18 phases. In addition, the separation can be effected by means of normal-phase chromatography, for example
10 on silica gel, aluminum oxide and the like.

Buffered, basic or acidified aqueous solutions are understood as being, for example, water, phosphate buffer, ammonium acetate and citrate buffer at a concentration of up to 0.5 M, as well as formic acid, acetic acid, trifluoroacetic acid, ammonia and
15 triethylamine, or all commercially available acids and bases known to the skilled person, preferably at a concentration of up to 1%. In the case of buffered aqueous solutions, particular preference is given to 0.1% ammonium acetate.

The chromatography can be carried out using a gradient which began with 100% water
20 and ended with 100% organic solvent; the chromatography was preferably run with a linear gradient of from 5 to 95% acetonitrile.

Alternatively, it is also possible to carry out a gel chromatography or chromatography on hydrophobic phases. The gel chromatography can e.g. be carried out on
25 polyacrylamide gels or copolymer gels. The sequence of the abovementioned chromatographic steps can be reversed.

Insofar as Labyrinthopeptins are present as stereoisomers, they can be separated using known methods, for example by means of separation using a chiral column.

30

The derivatization of the OH group to an ester or ether derivative is effected using methods which are known per se (J. March, Advanced Organic Chemistry, John Wiley

& Sons, 4th edition, 1992), for example by means of reaction with an acid anhydride or by reaction with an di-alkyl carbonate or di-alkyl sulfate. Derivatization of the COOH group to an ester or amid derivative is effected using methods which are known per se (J. March, Advanced Organic Chemistry, John Wiley & Sons, 4th edition, 1992), for

5 example by means of reaction with ammonia to the respective CONH₂ group, or with an optionally activated alkyl compound to the respective alkyl ester. Oxidation of -CH₂-S-CH₂- groups to a -CH₂-S(O)-CH₂- or a -CH₂-S(O)₂-CH₂- group can be achieved upon exposing the respective Labyrinthopeptin derivative to oxygen or air. Reduction of disulfides, optionally followed by alkylation of free SH groups, is effected using

10 methods which are known per se (A. Henschen, Analysis of cyst(e)ine residues, disulfide bridges, and sulfhydryl groups in proteins, in: B. Wittmann-Liebold, J. Salnikow, V.A. Erdman (Eds.), Advanced Methods in Protein Microsequence Analysis, Springer, Berlin, 1986, pp. 244–255), for example the reduction by means of dithiothreitol, and the alkylation using alkyl iodides. Sulfide reduction to a compound of

15 the formula (I) wherein R₃ and R₄ are H, (C₁-C₆)alkyl, (C₁-C₆)alkylene-C(O)NH₂, (C₁-C₆)alkylene-C(O)NH(C₁-C₄)alkyl or (C₁-C₆)alkylene-C(O)N[(C₁-C₄)alkyl]₂ can be achieved by reacting a compound of the formula (I) wherein R₃ and R₄ form a disulfide group S-S together with the S atoms to which they are attached with an (C₁-C₆)alkyl-halogenide or halogen-(C₁-C₆)alkylene-C(O)NH₂, halogen-

20 (C₁-C₆)alkylene-C(O)NH(C₁-C₄)alkyl or halogen-(C₁-C₆)alkylene-C(O)N[(C₁-C₄)alkyl]₂ in the presence of dithiothreitol (general literature). Halogen is F, Cl, Br or I.

An isolate of the microorganism strain *Actinomadura namibiensis* was deposited by Hoechst AG, Frankfurt, Germany, under identification reference FH-A 1198 in the

25 Deutsche Sammlung von Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] GmbH (DSMZ), Mascheroder Weg 1B (as of 2008: Inhoffenstr. 7 B), 38124 Braunschweig, Germany, in accordance with the rules of the Budapest treaty, on 23.01.1991 under the following number: DSM 6313.

Microorganism strain *Actinomadura namibiensis* is further described by Wink et al. in

30 International Journal of Systematic and Evolutionary Microbiology 2003, 53, 721-724.

Instead of the strain *Actinomadura namibiensis* (DSM 6313), it is also possible to use its mutants and/or variants which synthesize one or more of the compounds according to the invention.

5 A mutant is a microorganism in which one or more genes in the genome has/have been modified, with the gene, or the genes, which is/are responsible for the ability of the organism to produce the compound according to the invention remaining functional and heritable.

10 Such mutants can be produced, in a manner known per se, using physical means, for example irradiation, as with ultraviolet rays or X-rays, or chemical mutagens, such as ethyl methanesulfonate (EMS); 2-hydroxy-4-methoxybenzophenone (MOB) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), or as described by Brock et al. in "Biology of Microorganisms", Prentice Hall, pages 238-247 (1984).

15

A variant is a phenotype of the microorganism. Microorganisms have the ability to adapt to their environment and therefore exhibit highly developed physiological flexibility. All the cells of the microorganism are involved in the phenotypic adaptation, with the nature of the change not being genetically conditioned and being reversible
20 under altered conditions (H. Stolp, Microbial ecology: organism, habitats, activities. Cambridge University Press, Cambridge, GB, page 180, 1988).

Screening for mutants and/or variants which synthesize one or more of the compounds according to the invention is achieved by optionally lyophilizing the fermentation
25 medium and extracting the lyophilizate or the fermentation broth with an organic solvent or a mixture of water and an organic solvent as defined above, and analyzing by means of HPLC or TLC or by testing the biological activity.

The fermentation conditions may be applied to *Actinomadura namibiensis* (DSM 6313)
30 and for mutants and/or variants thereof.

A further embodiment of the present invention is the use of a compound of the formula

(I), as defined above, for the treatment of bacterial infections, especially bacterial infections caused by Gram-positive bacteria, for the treatment of viral infections and/or for the treatment of pain, especially neuropathic pain or inflammatory triggered pain.

- 5 The above described medicament (also referred to as pharmaceutical preparation or pharmaceutical composition) contains an effective amount of at least one compound of the formula (I), in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt or chemical equivalent thereof, as described above, and at least one pharmaceutically acceptable carrier, preferably one
10 or more pharmaceutically acceptable carrier substances (or vehicles) and/or additives (or excipients).

The medicament can be administered orally, for example in the form of pills, tablets, lacquered tablets, coated tablets, granules, hard and soft gelatine capsules, solutions,
15 syrups, emulsions, suspensions or aerosol mixtures. Administration, however, can also be carried out rectally, for example in the form of suppositories, or parenterally, for example intravenously, intramuscularly or subcutaneously, in the form of injection solutions or infusion solutions, microcapsules, implants or rods, or percutaneously or topically, for example in the form of ointments, solutions or tinctures, or in other ways,
20 for example in the form of aerosols or nasal sprays.

The medicaments according to the invention are prepared in a manner known per se and familiar to one skilled in the art, pharmaceutically acceptable inert inorganic and/or organic carrier substances and/or additives being used in addition to the compound(s)
25 of the formula (I) in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt or chemical equivalent thereof, as described above. For the production of pills, tablets, coated tablets and hard gelatine capsules it is possible to use, for example, lactose, corn starch or derivatives thereof, talc, stearic acid or its salts, etc. Carrier substances for soft gelatine capsules and
30 suppositories are, for example, fats, waxes, semisolid and liquid polyols, natural or hardened oils, etc. Suitable carrier substances for the production of solutions, for example injection solutions, or of emulsions or syrups are, for example, water, saline,

alcohols, glycerol, polyols, sucrose, invert sugar, glucose, vegetable oils, etc. Suitable carrier substances for microcapsules, implants or rods are, for example, copolymers of glycolic acid and lactic acid. The pharmaceutical preparations normally contain about 0.5 to about 90 % by weight of a compound of the formula (I) and/or their

5 physiologically acceptable salts and/or their prodrugs. The amount of the active ingredient of the formula (I) in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt or chemical equivalent thereof, as described above, in the medicaments normally is from about 0.5 to about 1000 mg, preferably from about 1 to about 500 mg.

10

In addition to the active ingredients of the formula (I) in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt or chemical equivalent thereof, as described above, and to carrier substances, the pharmaceutical preparations can contain one or more additives such as, for example,

15

fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, preservatives, sweeteners, colorants, flavorings, aromatizers, thickeners, diluents, buffer substances, solvents, solubilizers, agents for achieving a depot effect, salts for altering the osmotic pressure, coating agents or antioxidants. They can also contain two or more compounds of the formula (I) in any stereochemical form, or a mixture of

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any stereochemical forms in any ratio, or a physiologically tolerable salt or chemical equivalent thereof. In case a pharmaceutical preparation contains two or more compounds of the formula (I), the selection of the individual compounds can aim at a specific overall pharmacological profile of the pharmaceutical preparation. For example, a highly potent compound with a shorter duration of action may be combined

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with a long-acting compound of lower potency. The flexibility permitted with respect to the choice of substituents in the compounds of the formula (I) allows a great deal of control over the biological and physico-chemical properties of the compounds and thus allows the selection of such desired compounds. Furthermore, in addition to at least one compound of the formula (I), the pharmaceutical preparations can also contain

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one or more other therapeutically or prophylactically active ingredients.

When using the compounds of the formula (I) the dose can vary within wide limits and,

as is customary and is known to the physician, is to be suited to the individual conditions in each individual case. It depends, for example, on the specific compound employed, on the nature and severity of the disease to be treated, on the mode and the schedule of administration, or on whether an acute or chronic condition is treated or whether prophylaxis is carried out. An appropriate dosage can be established using clinical approaches well known in the medical art. In general, the daily dose for achieving the desired results in an adult weighing about 75 kg is from about 0.01 to about 100 mg/kg, preferably from about 0.1 to about 50 mg/kg, in particular from about 0.1 to about 10 mg/kg, (in each case in mg per kg of body weight). The daily dose can be divided, in particular in the case of the administration of relatively large amounts, into several, for example 2, 3 or 4, part administrations. As usual, depending on individual behaviour it may be necessary to deviate upwards or downwards from the daily dose indicated.

Example 1: Preparation of a cryoculture of *Actinomadura namibiensis* (DSM 6313)

100 ml culture medium (10 g starch, 2 g yeast extract, 10 g glucose, 10 g glycerine, 2.5 g cornsteep powder, 2 g peptone, 1 g NaCl, 3g CaCO₃ in 1 l tap water, pH 7.2 before sterilization) were seeded with the strain *Actinomadura namibiensis* (DSM 6313) in a sterile 500 ml Erlenmeyer flask and incubated for 72 hours at 27°C and 120 rpm on a shaker. Subsequently, 1 ml of the culture and 1 ml sterile conservation solution (20 g glycerine, 10 g saccharose, 70 ml de-ionised water) were mixed and stored at -80°C. Alternatively, small pieces of a well-grown culture on agar were transferred into Cryotubes® (Vangard International) with 1.5 ml 50% sterile glycerine solution and stored at -196°C in liquid nitrogen.

Example 2: Preparation of Labyrinthopeptins

A sterile 500 ml Erlenmeyer flask containing 100 ml of the culture medium described in Example 1 was seeded with a culture of *Actinomadura namibiensis* (DSM 6313) which was grown on an agar plate and was incubated at 27°C and 120 rpm on a shaker. After 72 hours, further Erlenmeyer flasks containing the same culture medium in the

same amount were seeded with 2 ml of this pre-culture each and incubated under identical conditions for 168 hours. Alternatively, a 300 ml Erlenmeyer flask containing 100 ml of the culture medium described in Example 1 was seeded with a culture of *Actinomadura namibiensis* (DSM 6313) and incubated at 25°C and 180 rpm. After 72 hours, further Erlenmeyer flasks containing the same culture medium in the same amount were seeded with 5 ml of this pre-culture each and incubated under identical conditions for 168 hours.

Example 3: Solid phase extraction of Labyrinthopeptins

After completion of a 40 L-fermentation of *Actinomadura namibiensis* (DSM 6313) the culture broth has been filtered. The culture filtrate (ca. 30 L) has been loaded onto a column (dimension: 160 x 200 mm) filled ca. 3 L of CHP-20P material. Compounds were eluted at a flow rate of 250 ml/min using a gradient from 5% to 95% of isopropanol in water. Fractions have been collected every 4 min over a period of 45 min. Fractions containing the Labyrinthopeptins have been pooled and freeze-dried (Fraction 8: MW = 2190 Da; Fraction 9: MW = 2190 and 2074 Da; Fraction 10-12: MW = 2074 Da).

Example 4: Pre-purification of Labyrinthopeptin A1 using RP-18 Chromatography

Fraction 10-12 (670 mg) from Example 3 has been dissolved in 500 ml methanol and loaded onto a Phenomenex Luna® 10µ C18 (2) column (dimension: 50 mm x 250 mm) with a Phenomenex Luna® 10µ C18 (2) pre-column (dimension: 21.2 mm x 60 mm). Compounds were eluted with a gradient from 5% to 75% acetonitrile in water over a period of 40 min at a flow rate of 190 ml/min (buffer: 0.1 % ammonium acetate, pH 9.0, adjusted using a 30% aqueous ammonia solution). Fractions were collected every minute. Fractions 21-22 contained the desired Labyrinthopeptin (MW = 2074 Da). After freeze-drying, 322 mg crude product was obtained.

Example 5: Final purification of Labyrinthopeptin A1

Fractions 21-22 from Example 4 (60 mg) have been dissolved in 50 ml methanol and loaded onto a Phenomenex Luna® 5 μ C18 (2) Axia column (dimension: 30 mm x 100 mm) with a Waters XTerra® Prep MS C18 10 μ pre-column (dimension: 19 x 10 mm). Compounds were eluted with a gradient from 5% to 75% acetonitrile in water over a period of 40 min at a flow rate of 70 ml/min (buffer: 0.1 % ammonium acetate, pH 4.6, adjusted using aqueous acetic acid). The eluents have been collected in 10 ml-fractions using UV-triggering. Labyrinthopeptin-containing fractions (f. 9-12) have been pooled. After freeze-drying, 17 mg of Labyrinthopeptin A1 have been obtained.

10 Example 6: Pre-purification of Labyrinthopeptin A3 using RP-18 chromatography

Fraction 8 (~ 850 mg) from example 3 has been dissolved in 500 ml methanol and loaded onto a Phenomenex Luna® 10 μ C18 (2) column (dimension: 50 mm x 250 mm) with a Phenomenex Luna® 10 μ C18 (2) pre-column (dimension: 21.2 mm x 60 mm). Compounds were eluted with a gradient from 5% to 75% acetonitrile in water over a period of 40 min (buffer: 0.1 % ammonium acetate, pH 7.0) at a flow rate of 190 ml/min. Fractions were collected every minute. Fraction 19 contained the desired Labyrinthopeptin (MW = 2190 Da). After freeze-drying, 48 mg crude product was obtained.

20

Example 7: Final purification of Labyrinthopeptin A3

Fraction 19 from example 6 (48 mg) has been dissolved in 50 ml methanol and loaded onto a Phenomenex Luna® 5 μ C18 (2) Axia column (dimension: 30 mm x 100 mm) with a Waters XTerra® Prep MS C18 10 μ pre-column (dimension: 19 mm x 10 mm). Compounds were eluted with a gradient from 5% to 75% acetonitrile in water over a period of 40 min at a flow rate of 70 ml/min (buffer: 0.1 % ammonium acetate, pH 9.0, adjusted using a 30% aqueous ammonia solution). The eluents have been collected in fractions using UV-triggering. Labyrinthopeptin-containing fractions (F9-12) have been pooled. After freeze-drying, 12 mg of Labyrinthopeptin A3 have been obtained.

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Example 8: Characterisation of Labyrinthopeptins A1 and A3 by high performance liquid chromatography with diode-array and mass spectrometry detection (HPLC-DAD-MS)

- 5 Labyrinthopeptins A1 and A3 were analyzed on a Waters Acquity UPLC System with Sample Manager, Binary Solvent Manager and PDA (Photodiode Array Detector). As UPLC column a Waters Acquity UPLC BEH C18 (1.7 μ ; 2.1x100 mm) was used and eluted at a flow rate of 0.6 ml/min with a gradient of water:acetonitrile (9:1) within 15 min to 100 % acetonitrile, all solvents buffered with 6.5mM ammonium acetate to pH
- 10 4.6. UV spectra were recorded by the PDA detector at wavelengths between 200 and 600 nm. Mass spectra were recorded with a Bruker μ TOF LC MS using an orthogonal electrospray ionisation, a sampling-rate of 0.5 Hz and a detection-limit of 150-1500 atomic mass units.

15 Example 9: Characterisation of Labyrinthopeptins A1

Labyrinthopeptin A1 eluted at 5.46 min (PDA). The UV spectrum is featured by λ_{max} of 218 nm (sh) and 279 nm.

- 20 Doubly-charged molecular ions were observed at m/z (I): 1035.87 (4539), 1036.37 (5566), 1036.87 (4086), 1037.37 (2296), 1037.87 (1034) and 1038.37 (280) in the negative mode. In positive mode doubly-charged molecular ions of m/z (I): 1037.88 (2925), 1038.38 (3252), 1038.88 (2492), 1039.38 (1396), and 1039.88 (623) were observed.

25

Characterisation of Labyrinthopeptin A1 by high resolution ESI-FTICR-mass spectrometry: A solution of Labyrinthopeptin A1 in methanol ($c = 0.2$ mg/ml) was admitted through a syringe pump at a flow rate of 2 μ l/min to a Bruker Apex III FTICR MS (7T magnet) equipped with an electrospray source. Spectra were recorded in the

30 positive mode using an external calibration.

m/z observed in Da ($z=2$, $M+2Na^+$ ion)	1059.8693
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Exact, mono-isotopic mass of neutral [M]	2073.7592
Theoretical mass [M] for C ₉₂ H ₁₁₉ N ₂₃ O ₂₅ S ₄	2073.7630
Molecular formula	C ₉₂ H ₁₁₉ N ₂₃ O ₂₅ S ₄

Example 10: Characterisation of Labyrinthopeptin A3

Labyrinthopeptin A3 eluted at 4.79 min (PDA). The UV spectrum is featured by $\lambda_{\max}$ of
 5 218 nm (sh) and 274 nm (sh).

Doubly-charged molecular ions were observed at m/z (I): 1093.38 (1262), 1093.88 (1587), 1094.39 (1201), 1094.89 (686) and 1095.38 (195) in the negative mode. In
 10 positive mode, doubly-charged molecular ions of m/z (I): 1095.40 (365), 1095.91 (433) and 1096.41 (294) were observed.

Characterisation of Labyrinthopeptin A3 by high resolution ESI-FTICR-mass spectrometry (method as described in Example 9):

m/z observed in Da (z=2, M+2Na ⁺ ion)	1117.3847
Exact, mono-isotopic mass of neutral [M]	2188.7900
Theoretical mass [M] for C ₉₆ H ₁₂₄ N ₂₄ O ₂₈ S ₄	2188.7900
Molecular formula	C ₉₆ H ₁₂₄ N ₂₄ O ₂₈ S ₄

15 Example 11: Amino acid analysis of Labyrinthopeptin A1

Hydrolysis: Labyrinthopeptin A1 (0.05 mg) was hydrolyzed in nitrogen atmosphere with
 6 N HCl, 5% phenole at 110°C for 24 h. The hydrolysate was dried in a stream of
 20 nitrogen.

Achiral GC-MS: The hydrolysate was heated with bis-(trimethylsilyl)trifluoroacetamide (BSTFA)/acetonitrile (1:1) at 150 °C for 4 h. For GC-MS experiments a DB5-fused-silica-capillary (l = 15 m × 0.25 µm fused silica coated with dimethyl-(5%-

phenylmethyl)-polysiloxane, $d_f = 0.10 \mu\text{m}$; temperature programme: $T = 65^\circ/3'/6/280^\circ\text{C}$) was used.

Chiral GC-MS: The hydrolysate was esterified with 200 μl 2 N HCl in ethanol at 110°C for 30 min and dried. Subsequently, the mixture was acylated with 25 μl trifluoroacetic acid anhydride (TFAA) in 100 μl dichloromethane at 110°C 10 min for and dried. For GC-MS a fused-silica-capillary was used ($l = 22 \text{ m} \times 0.25 \mu\text{m}$ fused silica coated with chirasil-S-Val (Machery-Nagel), $d_f = 0.13 \mu\text{m}$; temperature programme: $T = 55^\circ/3'/3,2/180^\circ\text{C}$).

10

		configuration
Amino acids	1 Ala, 1 Thr, 1 Asx, 2 Cys, 1 Phe, 1 Glx, 2 Trp, 1 Gly, 2 Val, 1 Pro = 13 AS	all S-amino acids, except for 2 Cys in the R configuration

Example 12: Identification of the structural genes for Labyrinthopeptins A1 and A3

A cosmid bank of the microorganism *Actinomadura namibiensis* (DSM 6313) was generated by Agowa GmbH, Berlin, based on the pWEB-cosmid vector (Epicentre Biotechnologies, Madison, USA). Filters were prepared by RZPD GmbH, Berlin, applying a methodology described in: Zehetner & Schäfer, Methods Mol. Biol. 2001, 175, 169-188.

Based on the known structure of Labyrinthopeptin A2, elongated degenerated primers were deduced from the N-terminal and C-terminal end (Fw: 5'-CAGGAAACAGCTATGACCGAYTGGWSNYTNTGGG-3' (SEQ ID NO: 4); Rev: 5'-TGTAACGACGGCCAGTRCANGANGCRAANARRC-3' (SEQ ID NO: 5); Dabard et al., Appl. Environ. Microbiol. 2001, 4111-4118.). The 5'-elongation of the primers was to enhance the expected PCR-product size for better detection and handling (PCR-conditions: 3 min 95°C ; 30 x (60 s 95°C ; 30 s 50°C ; 60 s 72°C) 7 min 72°C ; Taq-polymerase). The PCR-product was gel-purified and cloned into the vector pDrive

(Qiagen). Sequencing resulted in a 18 nucleotide length sequence from the middle of the A2 gene (AGTGCTGTAGCACGGGAA, SEQ ID NO: 6). Based on this 18 nucleotide long known sequence, a two-step PCR rendered to more sequence information. In the first step, a single-specific primer-PCR was performed with a
5 degenerated reversed(rev)-primer of the C-terminal end of A2
(5'-RCARCANGCRAANARRCTTCC-3', SEQ ID NO: 7) and an unspecific forward(fw)-
primer (5'-CACGGTACCTAGACTAGTGACCAAGTGCGCCGGTC-3', SEQ ID NO: 8)
(PCR-conditions: 3 min 95°C; 10 x (45 s 95°C; 45 s 38°C; 3.5 min 72°C); 30 x (45 s
95°C; 45 s 52°C; 3.5 min 72°C) 5 min 72°C; Taq-polymerase). After an exonuclease-I
10 digest in order to digest the primers (5 µl PCR-sample + 0.5 µl exonuclease-I (20U/µl);
15 min 37°C; 15 min 80°C heat inactivation), the PCR-sample was used as template
for a second PCR (PCR-conditions: 3 min 95°C; 30 x (45 s 95°C; 45 s 56°C; 3.5 min
72°C) 5 min 72°C; Taq-polymerase). The second PCR was performed in a nested-
PCR manner with a primer pair consisting of the unspecific fw-primer from the first
15 PCR and a specific rev-primer, including the known 18 nucleotides
(5'-CTTCCCGTGCTACAGCACTCCC-3', SEQ ID NO: 9). The 0.4 kbp product was
gel-purified and cloned into pDrive. Sequencing showed the expected amino acid
sequence of the C-terminal end of A2. Out of this 0.4 kbp sequence, a Dig-labelled
probe was constructed by PCR (Fw: 5'-ATGGACCTCGCCACGGGCTC-3', SEQ ID
20 NO: 10; 5'-CTTCCCGTGCTACAGCACTCCC-3', SEQ ID NO: 11). This Dig-labelled
probe was used to screen the filters by hybridization and detection via anti-Dig-
antibody labeled with alkaline phosphatase. In this manner, one positive cosmid was
obtained and sequenced.

25 Sequence data were analyzed by local blast and frameplot. The analysis yielded the
following open reading frame (orf) that included the structural gene of Labyrinthopeptin
A2:

30 TGACGCCCCGCACACCGTTCCACCGATGAGAGGTGACAGTCCCATGGCGTCGATC
CTGGAACCTCCAGAACCTGGACGTCGAGCACGCCCGCGGCGAGAACCGCTCCGA
CTGGAGCCTGTGGGAGTGCTGTAGCACGGGAAGCCTGTTGCGCTGCTGCTGA
(SEQ ID NO: 12)

Within this orf, the following sequence represents the structural gene of prepro-Labyrinthopeptin A2 (leader sequence followed by propeptide-encoding sequence followed by stop-codon TGA):

5

ATGGCGTCGATCCTGGAACCTCCAGAACCTGGACGTCGAGCACGCCCCGCGGCGA
GAACCGCTCCGACTGGAGCCTGTGGGAGTGCTGTAGCACGGGAAGCCTGTTCGC
CTGCTGCTGA (SEQ ID NO: 13)

- 10 Translation of the DNA sequence as shown in SEQ ID NO: 13 gave the following amino acid sequence of prepro-Labyrinthopeptin A2 (SEQ ID NO: 14) and of pro-Labyrinthopeptin A2 (SEQ ID NO: 15):

MASILELQNLDVEHARGENR SDWSLWECCSTGSLFACC (SEQ ID NO: 14)

15

SDWSLWECCSTGSLFACC (SEQ ID NO: 15)

- The propeptide sequence is transformed into Labyrinthopeptin A2 by posttranslational modifications by enzymes of the microorganism *Actinomadura namibiensis* (DSM
20 6313).

Example 13: Structure determination of Labyrinthopeptins A1 and A3:

- The upstream region of the A2 gene displays another small orf with high homology to
25 the structural gene of Labyrinthopeptin A2. This open reading frame (orf) included the structural gene of Labyrinthopeptin A1 and A3. The orf for Labyrinthopeptin A1 has the following gene sequence:

- TGAACATCCACCATGGCATCCATCCTTGAGCTCCAGGACCTGGAGGTCGAGCGC
30 GCCAGCTCGGCCGCCGACAGCAACGCCAGCGTCTGGGAGTGCTGCAGCACGGG
CAGCTGGGTTCCTTCACCTGCTGCTGA (SEQ ID NO: 16)

Within this orf, the following sequence represents the structural gene of prepro-Labyrinthopeptins A1 and A3 (leader sequence followed by propeptide-encoding sequence followed by stop-codon TGA):

5 ATGGCATCCATCCTTGAGCTCCAGGACCTGGAGGTCGAGCGCGCCAGCTCGGCC
GCCGACAGCAACGCCAGCGTCTGGGAGTGCTGCAGCACGGGCAGCTGGGTTCC
CTTCACCTGCTGCTGA (SEQ ID NO: 17)

Translation of the DNA sequence as shown in SEQ ID NO: 17 gave the following
10 amino acid sequence of prepro-Labyrinthopeptin A1 (SEQ ID NO: 18) and of pro-Labyrinthopeptin A1 (SEQ ID NO: 19):

MASILELQDLEVERASSAADSNASWVECCSTGSWVPFTCC (SEQ ID NO: 18)

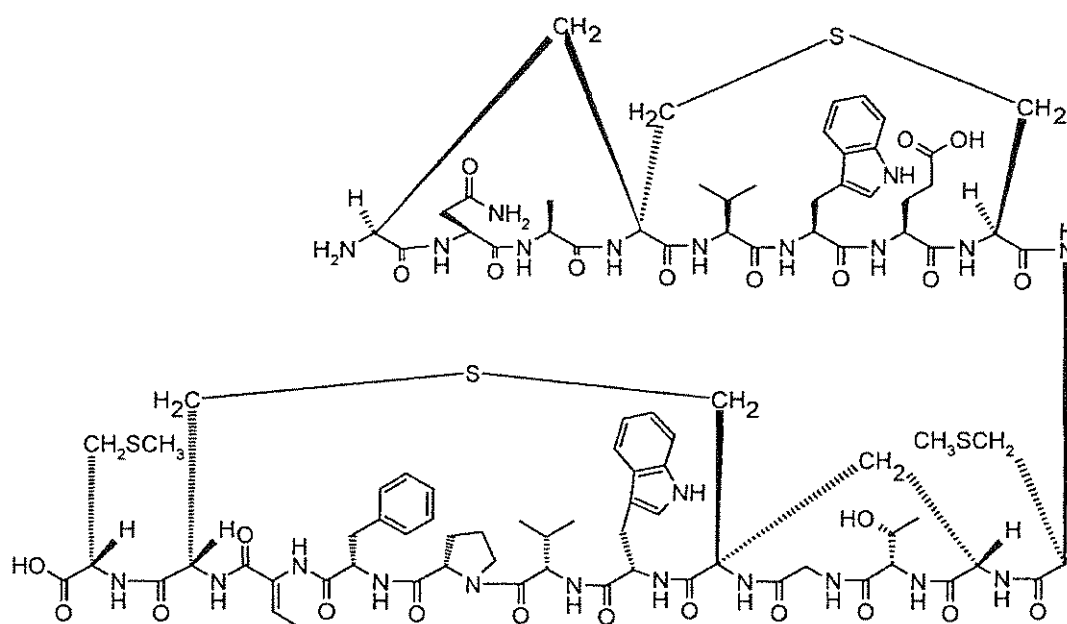
15 SNASWVECCSTGSWVPFTCC (SEQ ID NO: 19)

This amino acid sequence was in agreement with the expected amino acid composition of Labyrinthopeptin A1 based on results of amino acid- and MS-analysis of Labyrinthopeptin A1 (vide supra). Posttranslational modifications of side chains were
20 deduced that are analogous to those of Labyrinthopeptin A2. The stereochemistry of amino acids has been taken from the amino acid analysis (Example 11). Finally, a dehydration of the threonine (Thr) residue to give dehydrobutyric acid was deduced to match the empirical molecular formula calculated from high-resolution MS. On the basis of the stereochemistry of the posttranslationally modified amino acid of the
25 analogous Labyrinthopeptin A2, formula (VIII) is derived for Labyrinthopeptin A1.

Previous mass analysis suggested an Asp as the difference between Labyrinthopeptins A1 and A3. This was confirmed by the coded sequence, which included an Asp in position -1 ahead the protease cleavage side of Labyrinthopeptin
30 A1. Under the assumption that that Labyrinthopeptins A1 and A3 are encoded by the same gene, differing only in the protease cleavage of the leader sequence, the additional Asp is at the N-terminus of Labyrinthopeptin A3. In this manner, formula (V)

was derived for Labyrinthopeptin A3. On the basis of the stereochemistry of the posttranslationally modified amino acid of the analogous Labyrinthopeptin A2, formula (VI) is derived for Labyrinthopeptin A3.

- 5 Example 14: Cleavage of the disulfide-bridge of Labyrinthopeptin A1 and subsequent alkylation with methyl iodide



- Labyrinthopeptin A1 (50 mg, 0.024 mmol) was dissolved in methanol (3 ml) and a
- 10 dithiothreitol solution was added at room temperature (1 ml, freshly prepared from 75 mg dithiothreitol in a solution of 40 mg NaHCO₃ in 1 ml water). The mixture was stirred for 1 h at 60 °C. Afterwards it was cooled down to room temperature and methyl iodide (50 µl, 0.80 mmol) was added. After 4 h at room temperature the mixture was filtered and purified by reversed phase HPLC using a Phenomenex Luna® Axia 5 µm C18 (2)
- 15 column (dimension: 100 mm x 30 mm) with a Waters XTerra® Prep MS C18 10 µm pre-column (dimension: 19 mm x 10 mm). The gradient was running from 5% to 95% acetonitrile in water within 30 minutes (buffer: pH 2.0, adjusted with formic acid). The flow was 60 ml/min and the peaks were fractionated by UV. Fractions 12 and 13 were combined and yielded 23.1 mg (45.5 %) of the desired compound after lyophilization.
- 20 The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

$RT_{\min} = 5.46$ min (PDA; LC-method as in Example 8)

UV ($\lambda_{\max}$): 217 nm (sh), 279 nm

ESI-MS (neg): $[M-2H]^{2-} = 1050.894$

5 Experimental neutral monoisotopic mass, $[M] = 2103.802$

Neutral monoisotopic mass calculated for $C_{94}H_{125}N_{23}O_{25}S_4$: 2103.810

Molecular formula: $C_{94}H_{125}N_{23}O_{25}S_4$

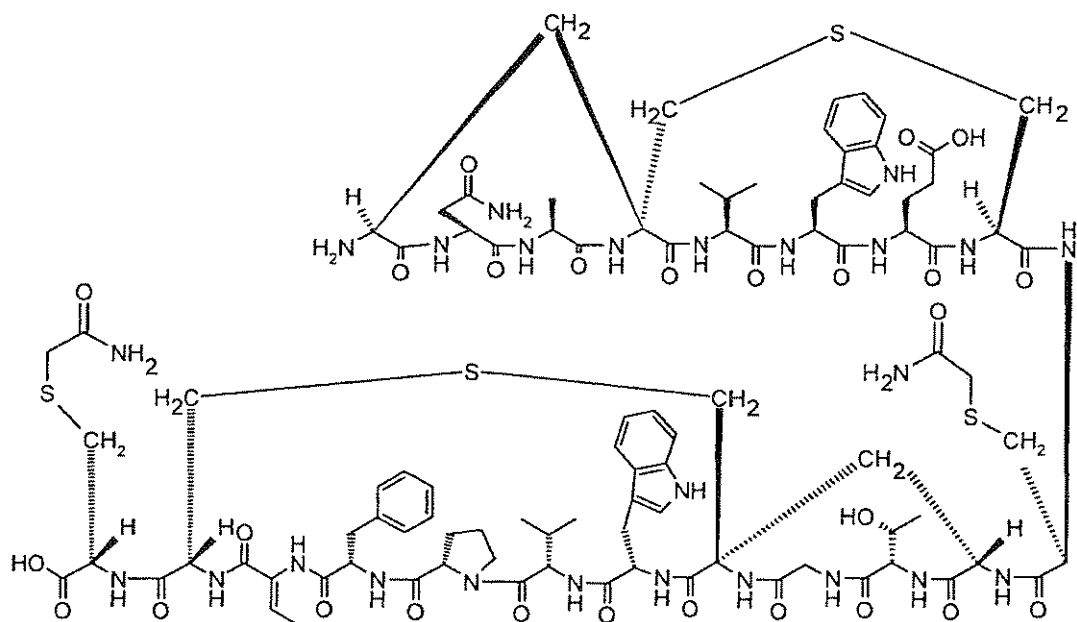
Chemical molecular weight = 2105.44.

10 Example 15: Cleavage of the disulfide-bridge of Labyrinthopeptin A1 and subsequent alkylation with iodo-acetamide

Labyrinthopeptin A1 (50 mg, 0.024 mmol) was dissolved in methanol (3 ml) and a dithiothreitol solution was added at room temperature (1 ml, freshly prepared from 70
15 mg dithiothreitol in a solution of 40 mg $NaHCO_3$ in 1 ml water). The mixture was stirred for 1 h at 60°C. Afterwards it was cooled down to room temperature and iodo-acetamide (40 mg, 0.216 mmol) was added. The mixture was stirred over night at room temperature. The solution was filtered and purified by reversed-phase HPLC using a Phenomenex Luna® Axia 5 μ m C18 (2) column (dimension: 100 mm x 30
20 mm) with a Waters XTerra® Prep MS C18 10 μ m pre-column (dimension: 19 mm x 10 mm). The gradient was running from 5% to 95% acetonitrile in water within 30 minutes (buffer: 0.1% ammonium acetate, pH 4.6, adjusted with acetic acid). The flow was 60 ml/min and the peaks were fractionated by UV. The following compounds were obtained:

25

Bis-acetamided Labyrinthopeptin A1:



Fractions 7 and 8 were combined and yielded 13.3 mg (25.2 %) of the desired compound after lyophilization. The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

$RT_{min} = 5.09$ min (PDA; LC-method as in Example 8)

UV (λ_{max}): 218 nm (sh), 280 nm

ESI-MS (neg): $[M-2H]^{2-} = 1093.9022$

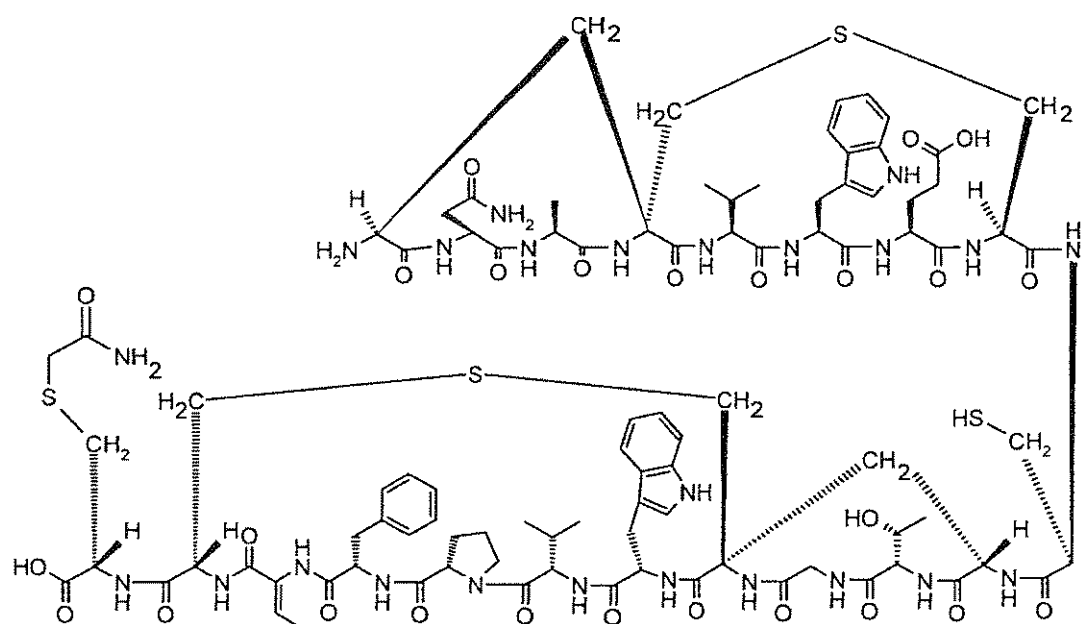
Experimental neutral monoisotopic mass, $[M] = 2189.819$

Neutral monoisotopic mass calculated for $C_{96}H_{127}N_{25}O_{27}S_4$: 2189.822

Molecular formula: $C_{96}H_{127}N_{25}O_{27}S_4$

Chemical molecular weight = 2191.49.

Mono-acetamided Labyrinthopeptin A1:



Fractions 10 and 11 were combined and yielded 5.3 mg (10.3 %) of the desired compound after lyophilization. The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

$RT_{min} = 5.31$ min (PDA; LC-method as in Example 8)

UV (λ_{max}): 217 nm (sh), 280 nm

ESI-MS (neg): $[M-2H]^{2-} = 1065.390$

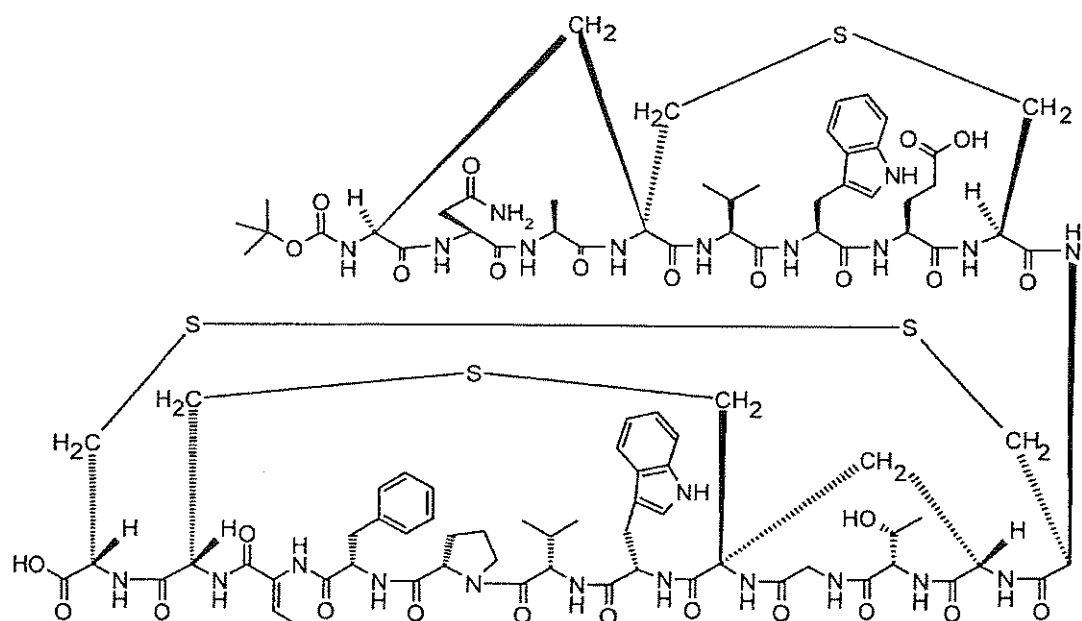
Experimental neutral monoisotopic mass, $[M] = 2132.794$

Neutral monoisotopic mass calculated for $C_{94}H_{124}N_{24}O_{26}S_4$: 2132.800

Molecular formula: $C_{94}H_{124}N_{24}O_{26}S_4$

Chemical molecular weight = 2134.44.

Example 16: Synthesis of a Boc-protected Labyrinthopeptin A1



To a solution of Labyrinthopeptin A1 (50 mg, 0.024 mmol) in dimethylformamide (3 ml), di-tert-butyl-dicarbonate (11 mg, 0.048 mmol) and n-ethyldiisopropylamine (6 mg, 0.048 mmol) were added at room temperature. The mixture was stirred for 2 h at room temperature. Afterwards it was purified by reversed-phase HPLC using a Phenomenex Luna® Axia 5 μ m C18 (2) column (dimension: 100 mm x 30 mm) with a Waters XTerra® Prep MS C18 10 μ m pre-column (dimension: 19 mm x 10 mm). The gradient was running from 5% to 95% acetonitrile in water within 30 minutes (buffer: 0.1% ammonium acetate, pH 7.0). The flow was 60 ml/min and the peaks were fractionated by UV. Fractions 4-7 were combined and yielded 21.4 mg (40.8%) of the desired compound after lyophilization. The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

RT_{min} = 5.30 min (PDA; LC-method as in Example 8)

UV (λ_{max}): 219 nm (sh), 278 nm

ESI-MS (neg): $[M-2H]^{2-} = 1085.895$

Experimental neutral monoisotopic mass, $[M] = 2173.805$

Neutral monoisotopic mass calculated for C₉₇H₁₂₇N₂₃O₂₇S₄: 2173.815

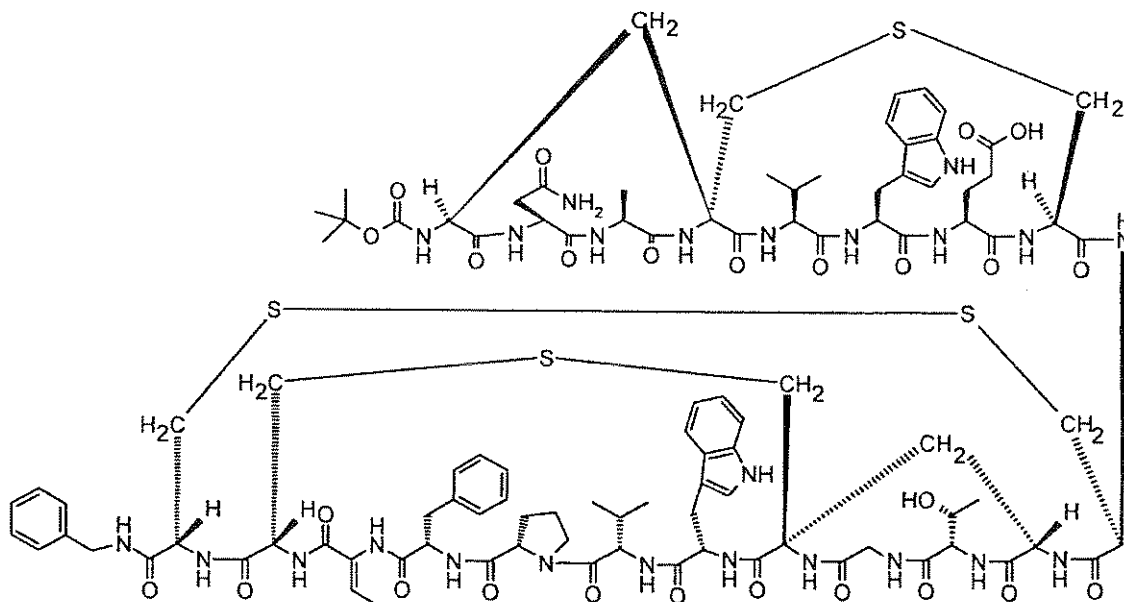
Molecular formula: C₉₇H₁₂₇N₂₃O₂₇S₄

Chemical molecular weight = 2174.49.

Example 17: Benzyl derivatives of Labyrinthopeptin A1

To a solution of Labyrinthopeptin A1 (50 mg, 0.024 mmol) in dimethylformamide (2 ml),
5 di-tert-butyl-dicarbonate (10 mg, 0.046 mmol) and n-ethyldiisopropylamine (7 mg,
0.054 mmol) were added at room temperature. After 1 h at room temperature,
Labyrinthopeptin A1 was completely disappeared. Benzylamine (6.8 mg, 0.063 mmol)
and n-propyl phosphonic acid anhydride (T3P®, 50 µl, 0.072 mmol, 50 % in DMF)
were added. The mixture was stirred for 2 h at room temperature. Afterwards it was
10 purified by reversed-phase HPLC using a Phenomenex Luna® Axia 5 µm C18 (2)
column (dimension: 100 mm x 30 mm) with a Waters XTerra® Prep MS C18 10 µm
pre-column (dimension: 19 mm x 10 mm). The gradient was running from 5% to 95%
acetonitrile in water within 30 minutes (buffer: 0.1 % ammonium acetate, pH 7.0). The
flow was 60 ml/ min and the peaks were fractionated by UV (220 nm). The following
15 two compounds were obtained:

Mono-benzyl derivative of Labyrinthopeptin A1:



Fractions 7 and 8 were combined and yielded 10.4 mg (19.1 %) of the desired compound after lyophilization. The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

5 $RT_{min} = 7.03$ min (PDA; LC-method as in Example 8)

UV (λ_{max}): 217 nm (sh), 275 nm

ESI-MS (neg): $[M-2H]^{2-} = 1130.427$

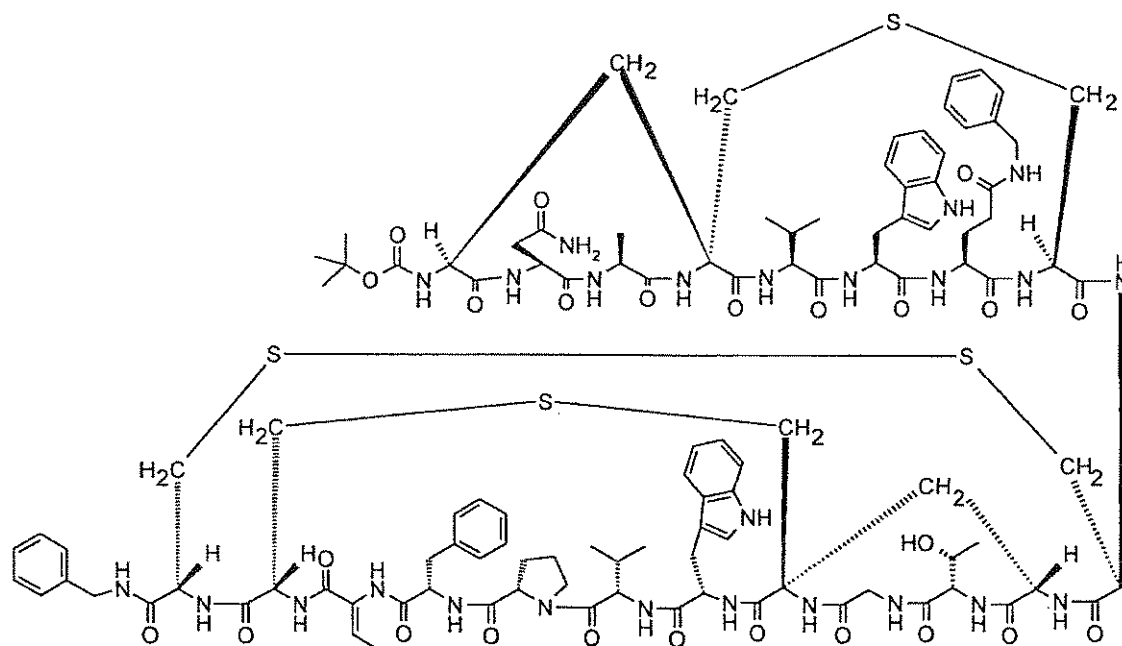
Experimental neutral monoisotopic mass, $[M] = 2262.868$

Neutral monoisotopic mass calculated for $C_{104}H_{134}N_{24}O_{26}S_4$: 2262.878

10 Molecular formula: $C_{104}H_{134}N_{24}O_{26}S_4$

Chemical molecular weight = 2264.63.

Bis-benzyl derivative of Labyrinthopeptin A1:



15

Fractions 13 and 14 were combined and yielded 9.9 mg (17.5 %) of the desired compound after lyophilization. The product was characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

20 $RT_{min} = 8.31$ min (PDA; LC-method as in Example 8)

UV (λ_{max}): 214 nm (sh), 276 nm

ESI-MS (pos): $[M+2(\text{NH}_4)]^{2+} = 1194.002$

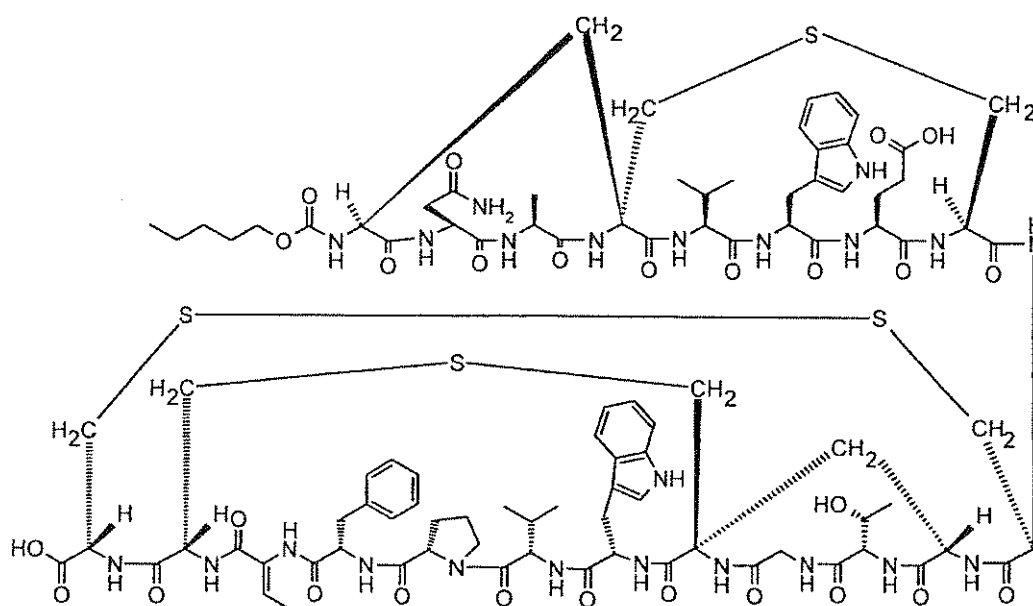
Experimental neutral monoisotopic mass, $[M] = 2351.937$

Neutral monoisotopic mass calculated for $\text{C}_{111}\text{H}_{141}\text{N}_{25}\text{O}_{25}\text{S}_4$: 2351.941

5 Molecular formula: $\text{C}_{111}\text{H}_{141}\text{N}_{25}\text{O}_{25}\text{S}_4$

Chemical molecular weight = 2353.77.

Example 18: Acylation reactions at the N-terminus of Labyrinthopeptin A1



10

To a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 5 mg, 0.028 mmol) in dimethylformamide (2 ml), n-methylmorpholine (8.6 mg, 0.085 mmol) was added at room temperature. After 1 h at room temperature n-hexanoic acid (3.3 mg, 0.028 mmol) was added. After stirring the mixture for 30 minutes Labyrinthopeptin A1 (50 mg, 0.024 mmol) was added followed by stirring for 2 h at room temperature. The mixture was purified by reversed-phase HPLC using a Waters XBridge Shield® 5 μm C18 column (dimension: 100 mm x 30 mm) with a Waters XBridge Shield® C18 10 μm pre-column (dimension: 19 mm x 10 mm). The gradient was running from 5% to 95% acetonitrile in water within 30 minutes (buffer: 0.1% ammonium acetate, pH 7.0). The flow was 60 ml/min and the peaks were fractionated by UV (220 nm). Fraction 39 yielded 2.0 mg (3.8%) of the desired compound after lyophilization. The product was

15

20

characterized by UV spectroscopy and mass spectrometry (Bruker Daltonics MicroTof).

$RT_{\min} = 5.41$ min (PDA; LC-method as in example 8)

5 UV ($\lambda_{\max}$): 216 nm (sh), 266 nm

ESI-MS (neg): $[M-2H]^{2-} = 1084.906$

Experimental neutral monoisotopic mass, $[M] = 2171.827$

Neutral monoisotopic mass calculated for $C_{98}H_{129}N_{23}O_{26}S_4$: 2171.836

Molecular formula: $C_{98}H_{129}N_{23}O_{26}S_4$

10 Chemical molecular weight = 2173.52.

Example 19: Antibacterial activity for Labyrinthopeptins and derivatives

The compounds were dissolved in water with 10 % MeOH to a final concentration of 1
15 mg/ml. For the bioassay sterile Nunc plates with a size of 24 x 24 cm were used. For one plate 200 ml of agar were used. The agar was cooled after autoclaving to 55°C and 2-4 ml of culture suspension of the test organism were added before plating. To each plate 64 filter plates with 6 mm in diameter were added.

To each filter 20 µl of the test solution were added and incubated for 1 to 3 days at 28
20 °C or 37 °C. The inhibition zone in mm was reported. For a detailed description of the methods, see Bauer et al., Amer. J. Clin. Pathol. 1966, 45, 493-496; Müller & Melchinger, Methoden in der Mikrobiologie, Franckhsche Verlagshandlung, Stuttgart (1964); Mueller & Hinton, Proc. Soc. Expt. Biol. Med. 1941, 48, 330-333.

Tested compound	Streptomyces murinus (DSM 40091) 28°C	Bacillus subtilis (ATCC 6633) 37°C
Labyrinthopeptin A1, Ex. 7	13	16,5
Labyrinthopeptin A1 derivative, Ex. 16	0	9
Labyrinthopeptin A1 derivative, Ex. 18	7	8
Labyrinthopeptin A1 Bis-benzyl derivative, Ex. 17	7	9
Tetracycline (control substance, 1 mg/ml)	24	32

Example 20: Neuropathic pain activity

Labyrinthopeptin A1 was studied in the spared nerve injury (SNI) mouse model of neuropathic pain in order to proof the activity on tactile allodynia. Under general anaesthesia, the two major branches of the sciatic nerve in adult male C57B6 mice (weight: 22.8g +/- 0.35 SEM) have been ligated and transected, with the sural nerve left intact. Tactile allodynia has been determined with the automatic von Frey test: using a dump needle stick, the plantar skin of hind paws was exposed to a pressure stimulus of increasing intensity up to 5 g. The force in grams at which the animal responded with hindpaw withdrawal was used as a read-out for tactile allodynia. The study was performed 7 days after nerve lesion over 6 hours with an additional measurement after 24 hours. Within two days after nerve transection, tactile allodynia developed completely and remained stable over at least two weeks. The compound was administered intravenous as a single application (3 mg/kg). As a vehicle for the intravenous application was the 1:1:18 (ethanol:solutol: phosphate buffered saline) vehicle chosen.

Paw withdrawal threshold (PWT) measurements have been used to calculate significant treatment effects, and for AUC calculations over a reference time period (6 hours) and subsequent % benefit calculations. For the statistical analysis the PWT values of the ipsilateral hind paws were used in two ways: first, with a 2-way ANOVA based on the PWT values for specific times (within a period of 24 hours) and second with a 1-way ANOVA on non-transformed delta AUC values [AUC1-6hour].

25

Two-way analysis of variance with repeated measures (Repeated factor: TIME, Analysis variable: PWT) followed by a Complementary Analysis (Effect of factor GROUP for each level of factor TIME (Winer analysis), Analysis variable: PWT) and a subsequent Dunnett's test for factor TREATMENT for each level of factor TIME (Two sided comparison vs level VEHICLE) revealed highly significant differences from the vehicle group from 1 to 6 hours after intravenous application for each compound. The effect was gone 24 hours after application. 1-way ANOVA using delta [AUC1-6hour] values revealed a p value of $p < 0.0001$. Dunnett analysis and gave significant

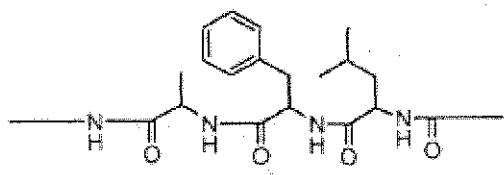
30

treatment effects for Labyrinthopeptin A1. The percent benefit of the treatment was evaluated using the |AUC1-6hour| values of the ipsilateral vehicle group (0% benefit) and all |AUC1-6hour| values of the contralateral sides of all three groups (100% benefit = maximal possible effect). Compared to these margins Labyrinthopeptin A1 achieved

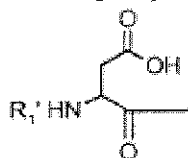
5 95% benefit.

In conclusion, the compounds of the formula (I) significantly reduce tactile allodynia in the SNI mouse model of neuropathic pain.

and



R_1 is a group R_1' or a group



wherein R_1' is H, $C(O)-(C_1-C_6)alkyl$ or $C(O)-O-(C_1-C_6)alkyl$;

R_2 is OH, NH_2 , $NH-(C_1-C_6)alkyl$, $NH-(C_1-C_4)alkylene-phenyl$ or $NH-(C_1-C_4)alkylene-pyridyl$;

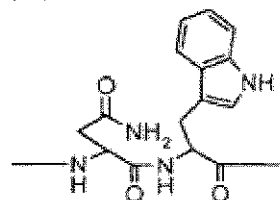
R_3 and R_4 are independently of each other H, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylene-C(O)NH_2$, $(C_1-C_6)alkylene-C(O)NH(C_1-C_4)alkyl$ or $(C_1-C_6)alkylene-C(O)N[(C_1-C_4)alkyl]_2$, or R_3 and R_4 together with the S atoms to which they are attached form a disulfide group S-S;

R_5 and R_6 are independently of each other H or OH, or R_5 and R_6 together are =O;

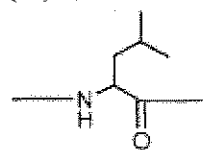
m and n are independently of one another 0, 1 or 2;

with the proviso that if

{A} is

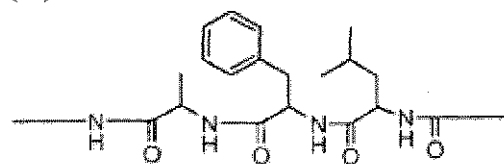


{B} is



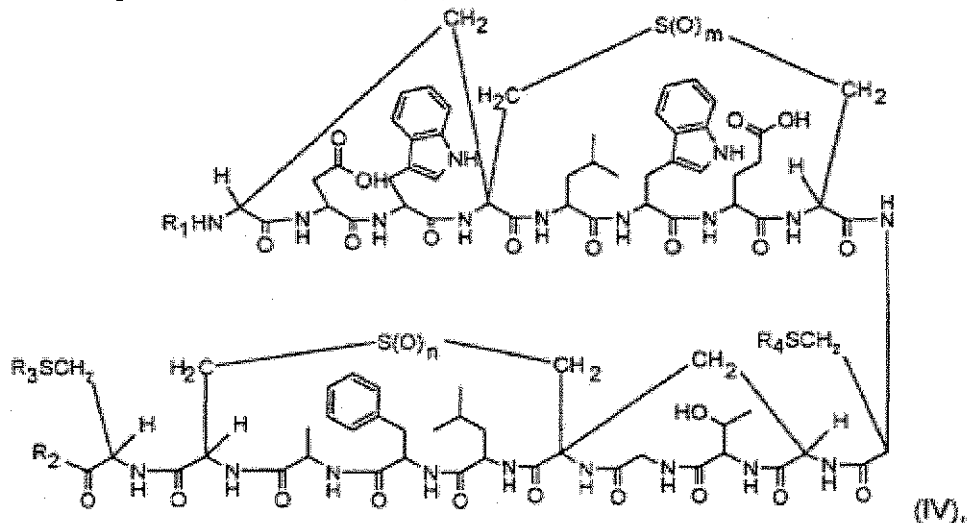
and

{C} is



R_3 and R_4 may not form a disulfide group S-S together with the S atoms to which they are attached;

or a compound of the formula (IV)



wherein

R_1 is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl;

R_2 is OH, NH₂, NH-(C₁-C₆)-alkyl, N[(C₁-C₆)-alkyl]₂, NH-(C₁-C₄)-alkylene-phenyl or NH-(C₁-C₄)-alkylene-pyridyl;

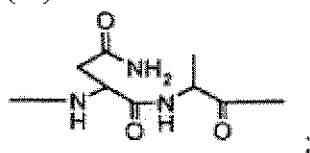
R_3 and R_4 are independently from each other H, (C₁-C₆)alkyl or (C₁-C₄)-alkylene-C(O)NH₂;

m and n are independently of one another 0, 1 or 2;

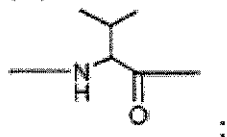
in any stereochemical form, or a mixture of any stereochemical forms in any ratio, or a physiologically tolerable salt thereof.

2. A compound of the formula (I) according to claim 1, wherein

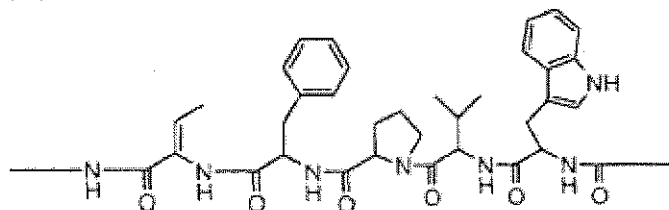
{A} is



{B} is

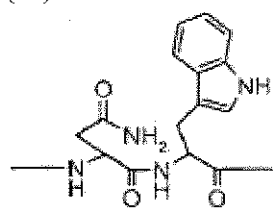


{C} is

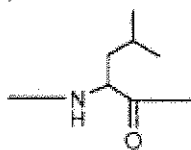


3. A compound of the formula (I) according to claim 1, wherein

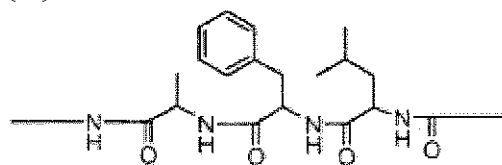
{A} is



{B} is



{C} is



and

R_1 is preferably a group R_1' .

4. A compound of the formula (I) according to any one of claims 1 to 3, wherein R_1' is H.

5. A compound of the formula (I) or formula (IV) according to any one of claims 1 to 4, wherein R_2 is OH.

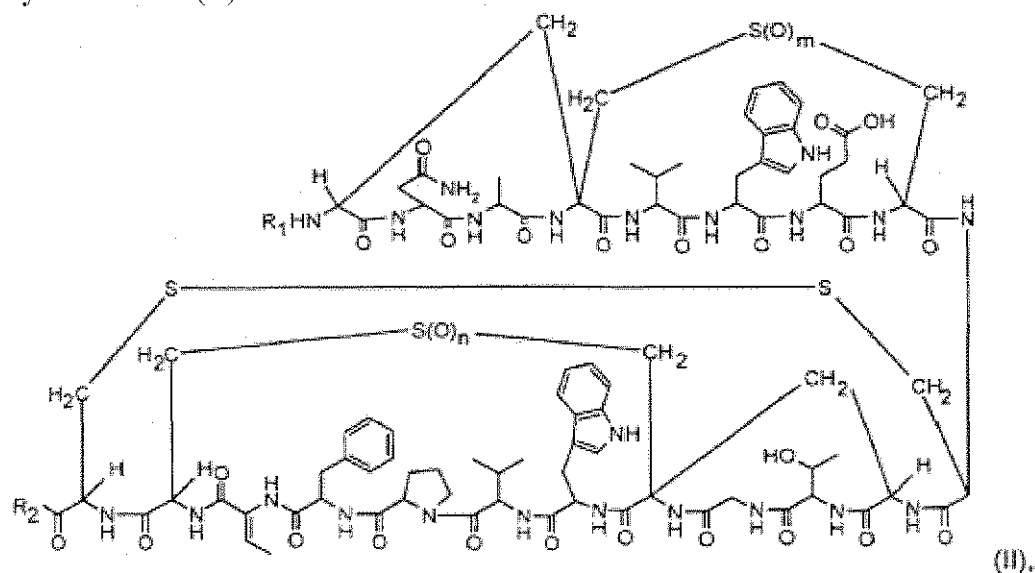
6. A compound of the formula (I) according to any one of claims 1 to 5, wherein R_3 and R_4 are independently of each other H, (C_1-C_6) alkyl, (C_1-C_6) alkylene- $C(O)NH_2$, or form a disulfide group S-S together with the S atoms to which they are attached.

7. A compound of the formula (I) according to any one of claims 1 to 6, wherein R_3 and R_4 are H or form a disulfide group S-S together with the S atoms to which they are attached.

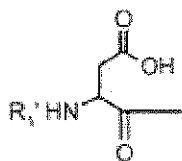
8. A compound of the formula (I) according to any one of claims 1 to 7, wherein R_5 and R_6 are H or OH wherein if R_5 is OH then R_6 is H, and if R_5 is H then R_6 is OH, or R_5 and R_6 together are =O.

9. A compound of the formula (I) according to any one of claims 1 to 8, wherein R_5 is OH and R_6 is H, or R_5 is H and R_6 is OH.

10. A compound of the formula (I) according to any one of claims 1 to 9, **characterized by the formula (II)**

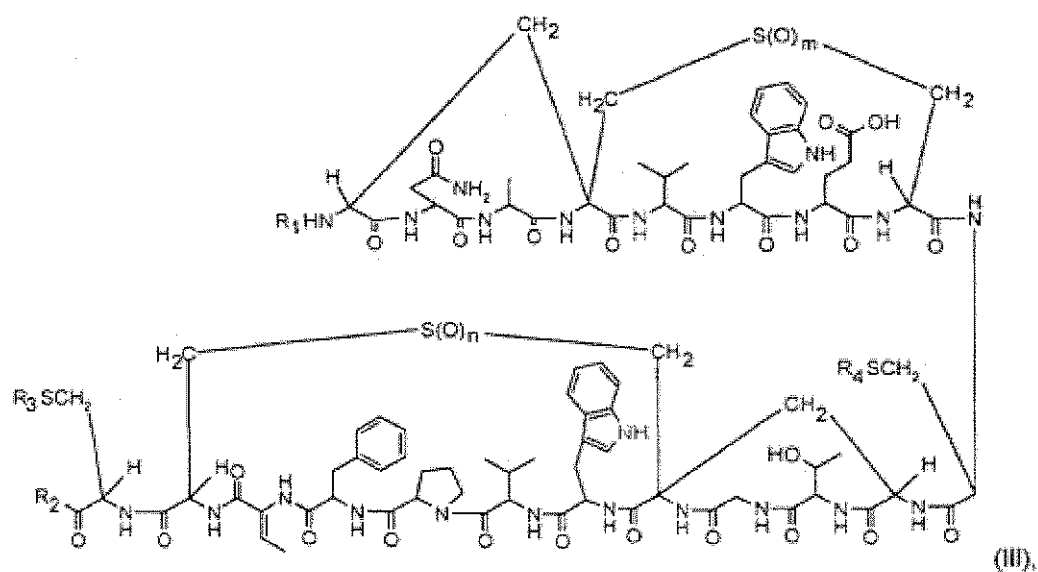


wherein R_1 is R_1' or a group



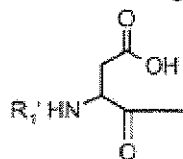
wherein R_1' is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl, preferably H.

11. A compound of the formula (I) according to any one of claims 1 to 9, **characterized by the formula (III)**



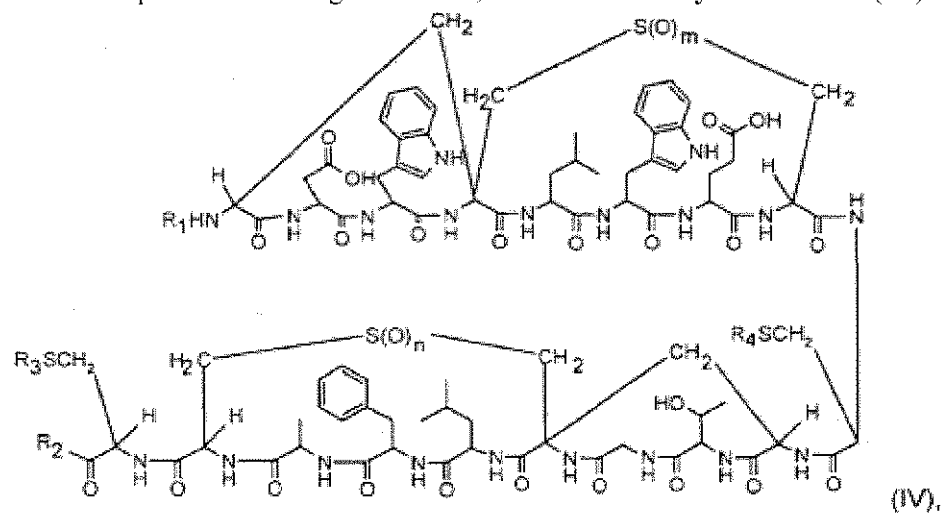
wherein

R_1 is R_1' or a group



wherein R_1' is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl, preferably H;
 R_2 is OH, NH₂, NH-(C₁-C₆)-alkyl, N[(C₁-C₆)-alkyl]₂, NH-(C₁-C₄)-alkylene-phenyl or NH-(C₁-C₄)-alkylene-pyridyl, preferably R_2 is OH; and
 R_3 and R_4 are independently from each other H, (C₁-C₆)alkyl or (C₁-C₄)-alkylene-C(O)NH₂.

12. A compound according to claim 1, **characterized by the formula (IV)**



wherein

R_1 is H, C(O)-(C₁-C₆)alkyl or C(O)-O-(C₁-C₆)alkyl,

R_2 is OH, NH₂, NH-(C₁-C₆)-alkyl, N[(C₁-C₆)-alkyl]₂, NH-(C₁-C₄)-alkylene-phenyl or NH-(C₁-C₄)-alkylene-pyridyl, and

R_3 and R_4 are independently from each other H, (C₁-C₆)alkyl or (C₁-C₄)-alkylene-C(O)NH₂.

13. A compound of the formula (I) or formula (IV) according to any one of claims 1 to 12, wherein m and n are 0; or m and n are 2; or m is 0 and n is 2; or m is 2 and n is 0.

14. A compound of the formula (I) or formula (IV) according to any one of claims 1 to 13, wherein m and n are 0.

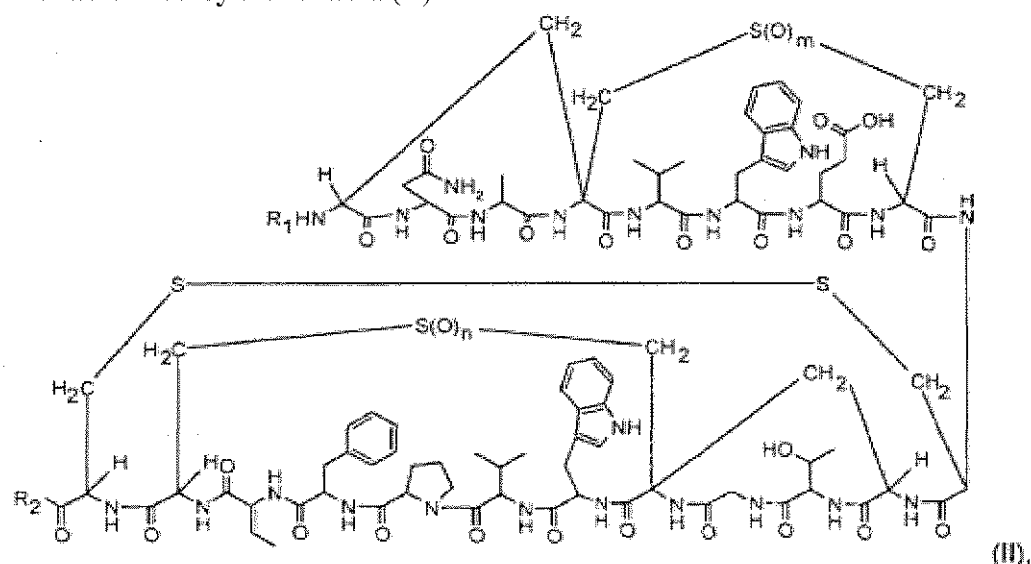
15. A process for preparing a compound of the formula (I) according to claim 1, comprising

a) fermenting the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, under suitable conditions in a culture medium until one or more of the compounds of the formula (I) accrue(s) in the culture medium,

b) isolating a compound of the formula (I) from the culture medium, and

c) derivatizing, where appropriate, the compound isolated in step b) and/or, where appropriate, converting the compound isolated in step b) or the derivative of the compound isolated in step b) into a physiologically tolerated salt.

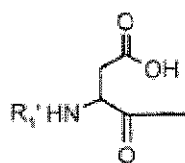
16. The process according to claim 15, wherein the compound isolated in step b) is **characterized** by the formula (II)



wherein

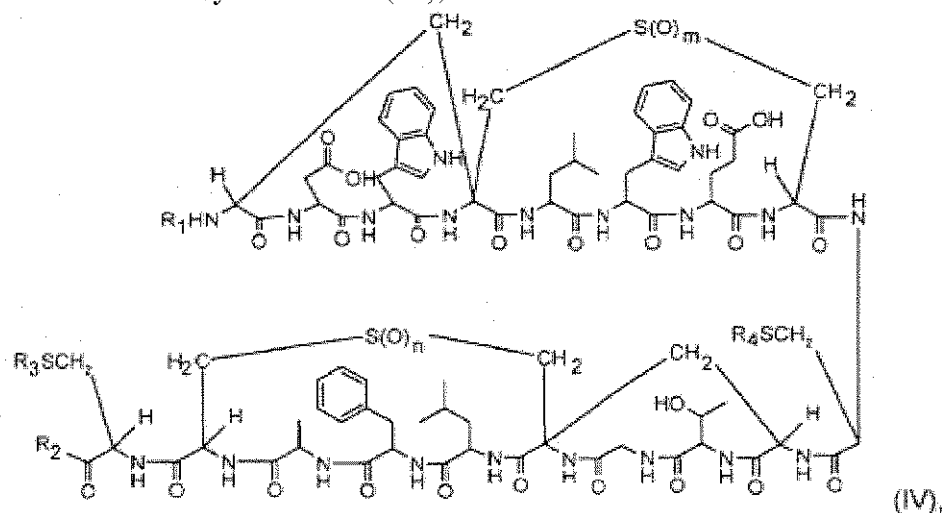
m and n are 0,

R_1 is R_1' or a group



wherein R_1' is H, and
 R_2 is OH.

17. A process for preparing a compound according to claim 1, wherein the compound is characterized by the formula (IV),



wherein

m and n are both 0,

R_1 is H,

R_2 is OH, and

R_3 and R_4 are independently of each other H, (C_1-C_6) alkyl, (C_1-C_6) alkylene- $C(O)NH_2$, (C_1-C_6) alkylene- $C(O)NH(C_1-C_4)$ alkyl or (C_1-C_6) alkylene- $C(O)N[(C_1-C_4)$ alkyl] $_2$, comprising

a) fermenting the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, under suitable conditions in a culture medium until the compound of formula (IV, i.e.) Labyrinthopeptin A2, accrues in the culture medium,

b) isolating the compound Labyrinthopeptin A2 from the culture medium, and

c) derivatizing the compound isolated in step b) and, where appropriate, converting the derivative of the compound isolated in step b) into a physiologically tolerated salt.

18. Use of a compound according to any one of claims 1 to 14 for the preparation of a medicament for the treatment of bacterial infections, viral infections and/or pain.

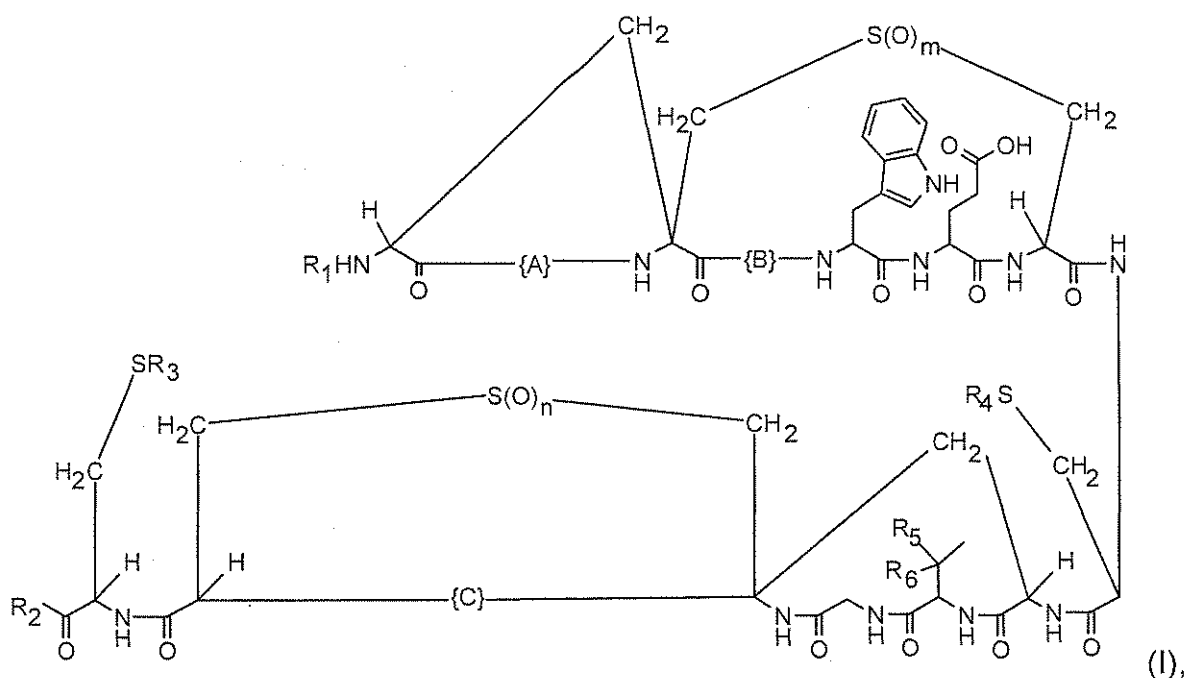
19. A pharmaceutical composition comprising at least one compound according to any one of claims 1 to 14 and at least one pharmaceutically acceptable carrier.
20. DNA coding for prepro-Labyrinthopeptin A2 having the nucleic acid sequence as shown in SEQ ID NO: 13.
21. Prepro-Labyrinthopeptin A2 having the amino acid sequence as shown in SEQ ID NO: 14.
22. Pro-Labyrinthopeptin A2 having the amino acid sequence as shown in SEQ. ID NO: 15.
23. DNA coding for prepro-Labyrinthopeptin A1 having the nucleic acid sequence as shown in SEQ. ID NO: 17.
24. Prepro-Labyrinthopeptin A1 having the amino acid sequence as shown in SEQ ID NO: 18.
25. Pro-Labyrinthopeptin A1 having the amino acid sequence as shown in SEQ. ID NO: 19.

Highly bridged peptides from *Actinomadura namibiensis*

Abstract

5

The invention refers to so-called Labyrinthopeptin derivatives of the formula (I)



- 10 wherein $\{A\}$, $\{B\}$, $\{C\}$, R_1 - R_6 , m and n are as defined herein, obtainable from microorganism strain *Actinomadura namibiensis* (DSM 6313), its use for the treatment of bacterial infections, viral infections and/or pain, a pharmaceutical composition comprising it, prepro-Labyrinthopeptin, pro-Labyrinthopeptin, and DNA coding for prepro-Labyrinthopeptin and pro-Labyrinthopeptin.

15

(No suitable figure)

DE2008-027.txt
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<170> PatentIn version 3.3
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Ser4 bridged with Ser1 via ch2
Ser4 bridged with Cys8 via S(O)m wherein m is 0, 1 or 2
Ser4 bridged with Ser4 via S(O)m wherein m is 0, 1 or 2
Cys9 optionally bridged with Cys20 via S-S
Ser10 bridged with Ser13 via CH2
Ser13 bridged with Ser10 via CH2
Ser13 bridged with Cys19 via S(O)n wherein n is 0, 1 or 2
Cys19 bridged with Ser13 via S(O)n wherein n is 0, 1 or 2
Cys20 optionally bridged with Cys9 via S-S

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Ala8 bridged with Ala4 via S(O)m wherein m is 0, 1 or 2
Cys9 bridged with Cys18 via S-S
Ala10 bridged with Ala13 via CH2
Ala13 bridged with Ala10 via CH2
Ala13 bridged with Ala17 via S(O)n wherein n is 0, 1 or 2
Ala19 bridged with Ala13 via S(O)n wherein n is 0, 1 or 2
Cys18 optionally bridged with Cys9 via S-S

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 Ser4 bridged with Ser1 via CH2
 Ser4 bridged with Cys8 via S(O)m wherein m is 0, 1 or 2
 Cys8 bridged with Ser4 via S(O)m wherein m is 0, 1 or 2
 Ser10 bridged with Ser13 via CH2
 Ser13 bridged with Ser10 via CH2
 Ser13 bridged with Ser17 via S(O)n wherein n is 0, 1 or 2
 Ser19 bridged with Ser13 via S(O)n wherein n is 0, 1 or 2

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35

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22

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Cys Cys

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Phe Thr Cys Cys
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