

ANTIBACTERIAL AND ANTIVIRAL PEPTIDES FROM ACTINOMADURA NAMIBIENSIS

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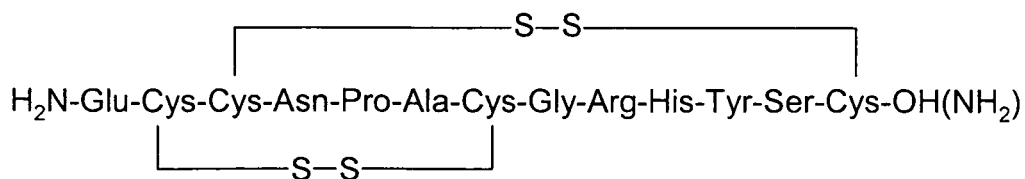
Field of invention

The present invention relates to the novel peptides isolated from *Actinomadura namibiensis* (DSM 6313), a method for its preparation and its use in the manufacture
 10 of a medicament for the treatment of bacterial infections, for the treatment of viral infections and/or for the treatment of pain.

Background of the Invention

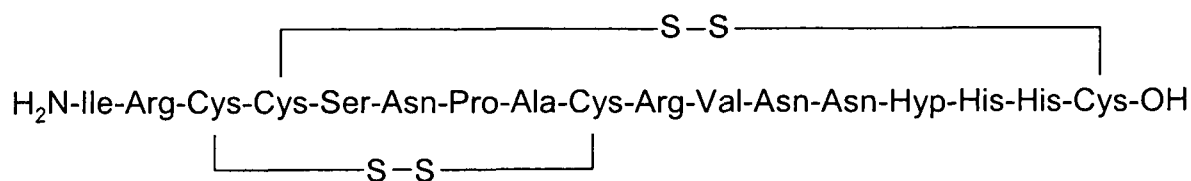
15 Several highly bridged peptides are known in the literature, for example conopeptides isolated from cone snails (for a review see e.g. Terlau & Olivera, *Physiol. Rev.* 2004, 84, 41–68) or the so-called lantibiotics (Chatterjee et al., *Chem. Rev.* 2005, 105, 633-683) from Gram-positive bacteria source.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

The conopeptides are e.g. 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*,
 25 *amidated, connectivity: 1-3,2-4) and α -GID (sequence: IR_γCCSNPACRVNNOHVC, 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:



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(α -GI)



(α -GID).

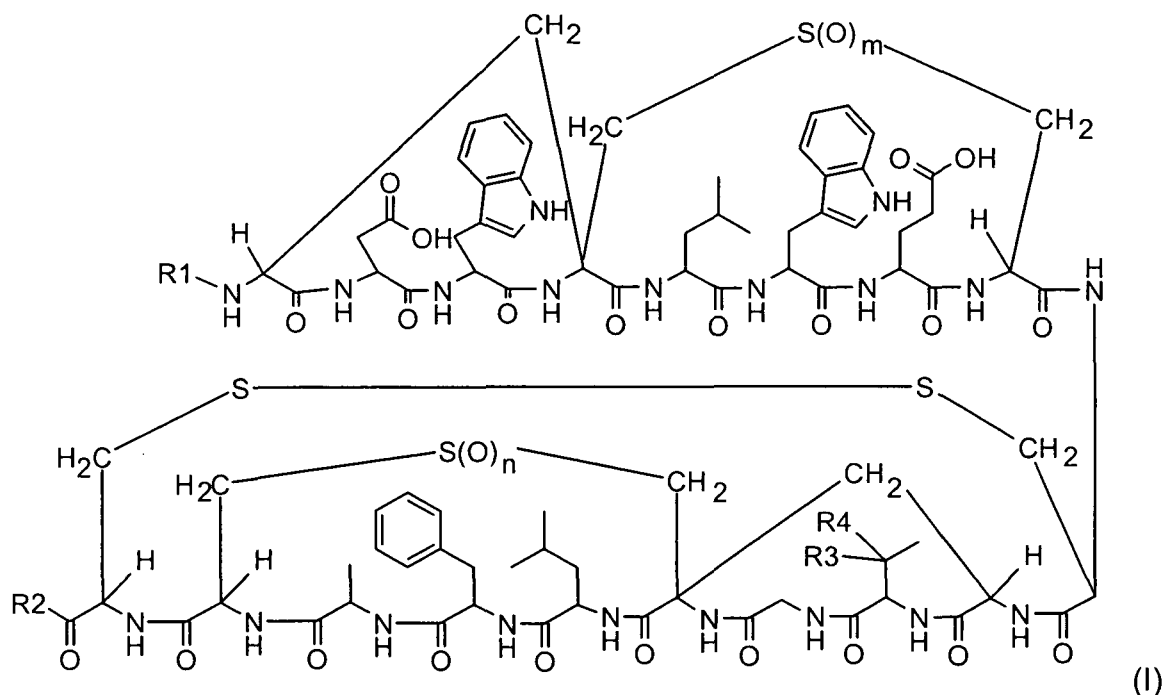
Summary of the invention

- 5 It has now surprisingly been found that highly bridged peptides can be isolated from microorganism strain *Actinomadura namibiensis* (DSM 6313) and are useful for the treatment of bacterial infections, viral infections and/or pain.

Detailed description of the invention

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



wherein

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

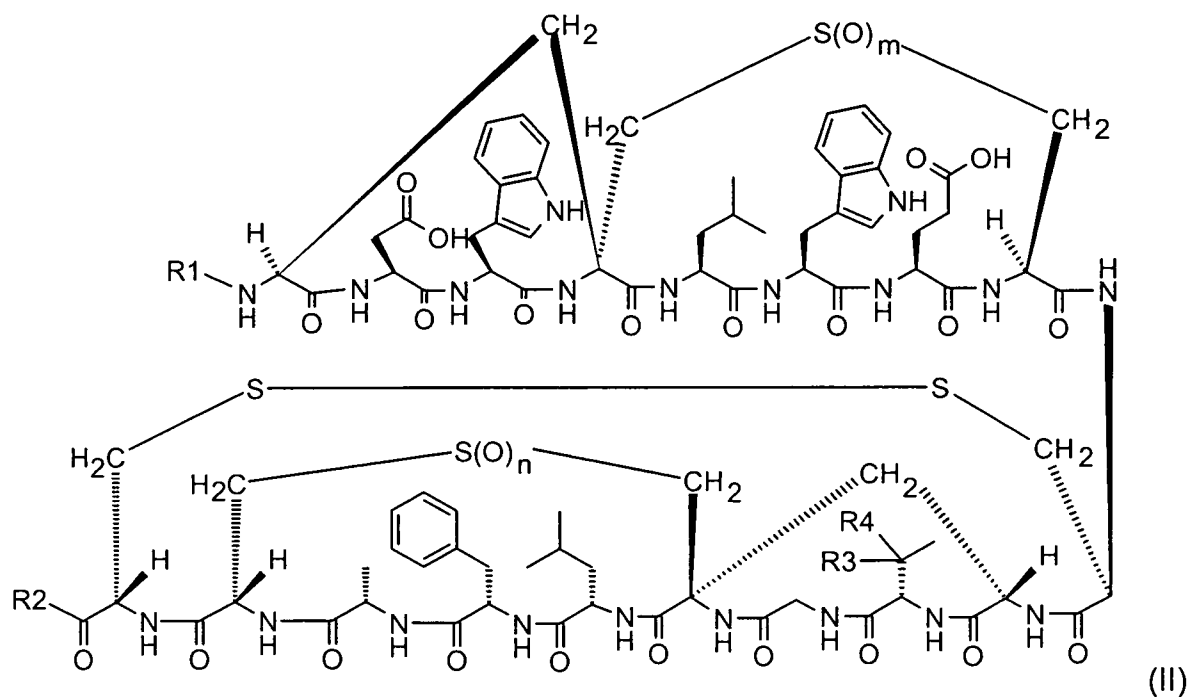
- 15 R2 is OH, NH₂, NH-(C₁-C₆)alkyl, NH-(C₁-C₄)alkylene-phenyl or NH-(C₁-C₄)alkylene-pyridyl;

R3 and R4 independently of each other H or OH, or R3 and R4 together are =O; and 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 further embodiment, the compound of the formula (I) is characterized by a compound of the formula (II)



wherein R1, R2, R3, R4, m and n are as defined above.

R1 is preferably H. R2 is preferably OH. R3 and R4 are preferably H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH, or R3 and R4 together are =O. More preferred, R3 and R4 are H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH.

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Preferably, 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

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A further embodiment of the present invention is a compound of the formula (I) or of the formula (II) wherein

R1 is H;

R2 is OH;

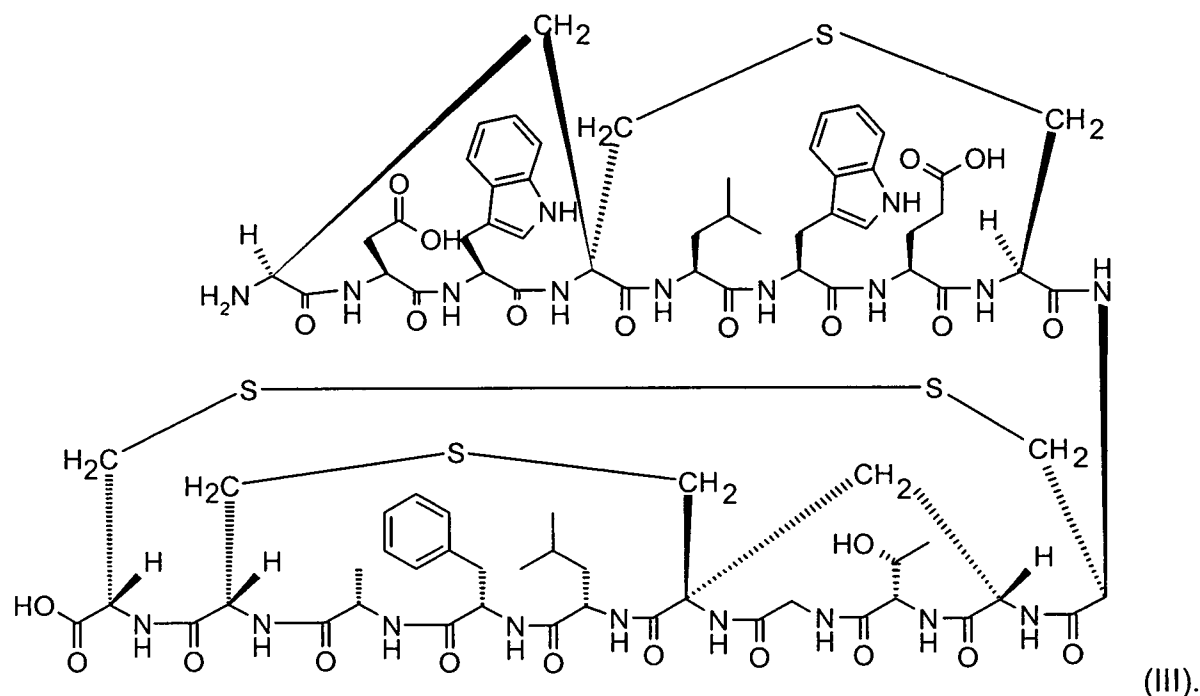
R3 and R4 are H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH; and

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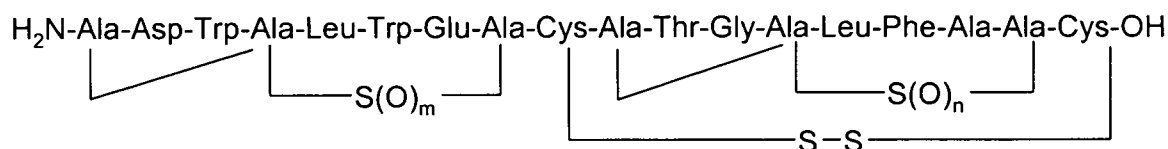
m and n are independently of one another 0, 1 or 2, preferably m and n are both 0,

or m and n are both 2, or m is 0 and n is 2, or m is 2 and n is 0, particularly preferred m and n are both 0;
or a physiologically tolerable salt thereof.

- 5 Most preferred, compound (I) is characterized by a compound of the formula (III)



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 1 and 10 are without precedence in the literature. Said amino acid may be described as an Ala residue bridged by a methylene group and substituted at the beta-position, as shown below:



The present invention furthermore relates to all obvious chemical equivalents of the compounds of the formulae (I), (II) and (III) 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 formulae (I), (II) and (III) 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 formulae (I), (II) and (III) 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.

The compounds of the present have been named Labyrinthopeptins throughout the text.

The invention also relates to a process for preparing a compound of the formula (I) wherein m and n are independently of one another 0, 1 or 2, which comprises

- a) the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, being fermented 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) a compound of the formula (I) being isolated from the culture medium, and
- c) the compound of the formula (I) being derivatized, where appropriate, and/or, where appropriate, being converted into a physiologically tolerated salt.

The invention preferably relates to a process for preparing a compound of the formula (I) wherein the compound (I) is characterized by a compound of the formula (II), more preferably the invention relates to a process for preparing a compound of the formula (I) or preferred (II) wherein 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.

Particularly preferred, the invention preferably relates to a process for preparing a compound of the formula (I) wherein the compound (I) is characterized by a compound of the formula (III).

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 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 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 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.

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 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 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 microorganism 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 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 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.

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 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 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.

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.

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 (TOSOHAAS), 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 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 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 and ended with 100% organic solvent; the chromatography was preferably run with a linear gradient of from 5 to 95% acetonitrile.

5 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 polyacrylamide gels or copolymer gels. The sequence of the abovementioned chromatographic steps can be reversed.

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

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
15 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
20 edition, 1992), for 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.

25 An isolate of the microorganism strain *Actinomadura namibiensis* was deposited under identification reference FH-A 1198 in the Deutsche Sammlung von Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] GmbH (DSMZ), Mascheroder Weg 1B, 38124 Braunschweig, Germany, in accordance with the rules of the Budapest treaty, on 23.01.1991 under the following
30 number: DSM 6313. Microorganism strain *Actinomadura namibiensis* is further described by Wink et al. in 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).

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- 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
- 20 being reversible 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
- 25 fermentation 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
- 30 6313) and for mutants and/or variants thereof.

A further embodiment of the present invention is the use of a compound of the formula (I), preferably a compound of the formula (II) or (III), 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 or more pharmaceutically acceptable carrier substances (or vehicles) and/or
10 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 gelatin capsules, solutions, syrups, emulsions, suspensions or aerosol mixtures. Administration, however, can
15 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, for example in the form of aerosols or nasal sprays.

20 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) of the formulae (I) in any stereochemical form, or a mixture of any
25 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 gelatin 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 gelatin capsules and suppositories are, for example, fats, waxes, semisolid and
30 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 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.

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, 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 formulae (I) in any stereochemical form, or a mixture of 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 formulae (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 with a long-acting compound of lower potency. The flexibility permitted with respect to the choice of substituents in the compounds of the formulae (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 one or more other therapeutically or prophylactically active ingredients.

When using the compounds of the formulae (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, 1ml 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 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: Isolation of Labyrinthopeptins

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2 % Hyflo Super-cel diatomaceous earth (Hyflo Supercell; VWR, Darmstadt, Germany) were given to 10 l of the culture broth containing according to Example 2 and the culture was filtrated through a filter press in order to separate the culture solution from the mycel. The filtrate was given on to column containing 1 l Amberlite
10 XAD-16 resin (column diameter: 5,5 cm, column height: 42 cm), and washed with 5 l deionised water and 5 l 20 % methanol in water. The Labyrinthopeptin was eluted with 5 l 60 % methanol in water and 5 l 80 % methanol in water. The Labyrinthopeptin containing fractions were identified by HPLC-DAD and LC-ESI-MS, collectively concentrated on a rotary evaporator until an aqueous residue was
15 obtained and subsequently freeze-dried. 300 mg crude product was obtained.

Example 4: High performance liquid chromatography with diode-array detection (HPLC-DAD) of Labyrinthopeptins

20 Column: Nucleosil 100 - C₁₈; 20 + 125 mm × 4.6 mm, 5 μ (Machery-Nagel)
Mobile phase: 0.1% H₃PO₄ in water (Eluent A) and acetonitrile (Eluent B)
linear gradient from 0% to 100 % Eluent B in Eluent A
over a period of 15 minutes
Flow: 2 ml per minute
25
Detection by UV/Vis absorption yielded peaks at 210, 230, 260, 280, 310, 360, 435 und 500 nm.

The retention time of the Labyrinthopeptin of the formula (II): 7.75 minutes.

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Example 5: High performance liquid chromatography with electrospray ionization mass spectroscopy (HPLC-ESI-MS) of Labyrinthopeptins

Column: Purospher RP-18e; 125 mm × 4 mm, 5 μ (Agilent)

Mobile phase: 0.1% trifluoro acetic acid in Wasser (Eluent A) and
0.1% trifluoro acetic acid in Acetonitril (Eluent B)
linear gradient from 5% to 100 % Eluent B in Eluent A
over a period of 10 minutes

- 5 Flow: 1.5 ml per minute. The flow to the ES interface of the mass spectrometer was reduced to 0.4 ml per minute via a T splitter.

Detection by UV absorbtion at 210 nm and ESI-MS (positive mode) wherein an ion trap was used as mass analyzer.

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The retention time of the Labyrinthopeptin of the formula (III) was 5.9 minutes. The molecular mass was 1922 Da.

Example 6: Purification of Labyrinthopeptins

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The Labyrinthopeptin crude product obtained according to Example 3 (300 mg) was dissolved in a mixture of dimethylsulfoxide, methanol and water (1:3:6) and the components were separated via chromatography on a Nucleosil 100 - C₁₈ column (particle size: 10 μ , column size: 250 $\times$ 16 mm) using isocratic elution (water + 0.1 %
20 formic acid / methanol 35:65) at a flow rate of 20 ml per minute. The fractions were analyzed with HPLC (cf. Example 4). 62 mg Labyrinthopeptin of the formula (III) was obtained in 99% purity.

Example 7: General characteristics of Labyrinthopeptin (III)

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The compound of the formula (III) was oxidated upon exposure to air to the respective sulfoxides. The compound of the formula (III) contains 2 *cis*-amides between ²Asp-³Trp and ¹¹Thr-¹²Gly.

30 Example 8: High resolving ESI-FTICR-mass spectrometry

A solution of the Labyrinthopeptin of the formula (III) 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 positive mode using an external calibration.

m/z observed in Da ($z=2$, $M+2Na^+$ ion)	984.3333
Exact, mono-isotopic mass of neutral [M]	1922.6872
Theoretical mass [M] for $C_{85}H_{110}N_{20}O_{24}S_4$	1922.6885
Molecular formula	$C_{85}H_{110}N_{20}O_{24}S_4$

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Example 9: Amino acid analysis

Hydrolysis: Labyrinthopeptin (III) (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 nitrogen.

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Achiral GC-MS: The hydrolysate was heated with bis-(Trimethylsilyl)trifluoroacetamide (BSTFA)/Acetonitril (1:1) at 150°C for 4 h. For GC-MS experiments a DB5-fused-silica-capillary ($l = 15 \text{ m} \times 0.25 \mu\text{m}$ fused silica coated with dimethyl-(5%-phenylmethyl)-polysiloxane, $d_f = 0.10 \mu\text{m}$; temperature programme: $T = 65^\circ/3^\circ/6/280^\circ\text{C}$) was used.

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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 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: $T = 55^\circ/3^\circ/3,2/180^\circ\text{C}$).

20

		configuration
Amino acids	1 Ala, 1 Thr, 2 Leu, 1 Asp, 2 Cys, 1 Phe, 1 Glu, 2 Trp, 1 Gly	all S-amino acids

25 Example 10: NMR spectroscopy

2-D NMR spectra (COSY, TOCSY, NOESY, HSQC, HMBC) were measured on an AMX 600 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5 mm Z-Grad triple resonance probe head and on a DRX500 NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5 mm Z-Grad broad band inverse probehead. The following table shows the signals obtained in the measurements.

NMR data for the Labyrinthopeptin of the formula (III) in DMSO-d₆:

¹ H	0.68; 0.71; 0.75; 0.78; 1.05; 1.07; 1.10; 1.35; 1.40; 1.42; 1.49; 1.90; 1.96; 2.00; 2.10; 2.17; 2.26; 2.77; 2.86; 2.90; 2.97; 3.03; 3.14; 3.16; 3.18; 3.18; 3.20; 3.24; 3.29; 3.30; 3.39; 3.59; 3.67; 3.67; 3.72; 3.99; 4.02; 4.03; 4.10; 4.13; 4.14; 4.16; 4.19; 4.34; 4.36; 4.45; 4.49; 4.59; 6.96; 7.26; 7.00; 7.04; 7.08; 7.08; 7.10; 7.18; 7.22; 7.23; 7.24; 7.24; 7.31; 7.35; 7.36; 7.42; 7.51; 7.53; 7.65; 7.65; 7.69; 7.77; 7.84; 7.98; 8.00; 8.01; 8.56; 10.80; 10.81.
¹³ C	19.7; 21.3; 21.4; 22.6; 23.04; 23.2; 23.7; 26.4; 26.4; 27.1; 33.4; 35.2; 35.4; 36.7; 38.3; 40.0; 40.5; 40.7; 40.8; 40.8; 41.2; 41.2; 43.4; 48.4; 48.7; 49.0; 51.2; 51.4; 52.5; 52.6; 52.7; 53.2; 53.5; 54.0; 56.9; 60.0; 60.3; 66.4; 109.8; 110.6; 111.2; 111.2; 117.4; 118.1; 118.1; 118.2; 120.7; 120.7; 122.1; 123.4; 126.5; 127.2; 127.3; 127.8; 129.4; 136.0; 136.1; 136.6; 140.8; 169.4; 173.0; 174.3; 176.9.

Example 11: X-ray crystallography of Labyrinthopeptin (III)

Crystallization conditions: The protein was dissolved in 0.02 M Tris pH 8.2 (concentration 7 mg/ml). Crystals grew at room temperature by vapour drop diffusion from a 1:1 mixture of the protein solution with a solution of 60% ethanol, 0.75% PEG 6000, 0.025 M sodium acetate and 0.05 M sodium chloride. Crystals grew within about one week.

Measurement: X-ray data were collected on a Bruker 3-circle diffractometer with rotating anode and mirror monochromated CuK-alpha radiation. Intensities were collected on a SMART 6000 CCD area detector.

Crystal data:

Formula	Na N ₂₀ C ₈₅ S ₄ O ₄₈	Na C ₈₅ N ₂₀ O ₂₄ S ₄
Formula weight	2220.29	1834.82
Crystal system	orthorhombic	
Space group	<i>P</i> 21 21 2 (no. 18)	
Unit cell dimensions	<i>a</i> = 41.1360 Å	
	<i>b</i> = 12.8850 Å	
	<i>c</i> = 25.5900 Å	
Cell volume	13563.66 Å ³	
Z	4	
Density, calculated	1.087 g/cm ³	
Pearson code	oP732	
Formula type	NO4P20Q48R85	
Wyckoff sequence	<i>c</i> ¹⁸¹ <i>b</i> ³ <i>a</i>	

Atomic coordinates:

No.	Atom	AtomNo.	x	y	z	e ⁻ -density
0	Na	NA	0.26282	1.11533	-0.20024	1.0
1	N	18NH	0.16643	0.62593	0.30751	
2	C	18Ca	0.15933	0.69120	0.26221	
3	C	18Cb	0.12264	0.69329	0.24928	
4	S	18Sg	0.11248	0.78619	0.19945	
5	C	18CO	0.16446	0.80225	0.28144	
6	O	18OC1	0.15211	0.83361	0.32212	
7	O	18OC3	0.17868	0.86558	0.25381	0.290
8	O	18OC2	0.18240	0.86116	0.25780	0.710
9	N	17NH2	0.17255	0.50082	0.39461	0.570
10	C	17Ca2	0.19839	0.49670	0.35604	0.570
11	N	17NH1	0.17994	0.49779	0.40196	0.430
12	C	17Ca1	0.20056	0.49833	0.35678	0.430
13	C	17Cb	0.20165	0.38479	0.33630	1.0
14	S	13S1	0.16523	0.32006	0.32001	
15	C	17CO	0.19312	0.57036	0.31036	
16	O	17OC	0.21276	0.57136	0.27366	
17	N	16NH1	0.11776	0.48825	0.45694	0.570
18	C	16Ca1	0.14725	0.54296	0.47554	0.570
19	C	16Cb2	0.14824	0.52387	0.53451	0.570
20	C	16CO2	0.17744	0.51223	0.44580	0.570

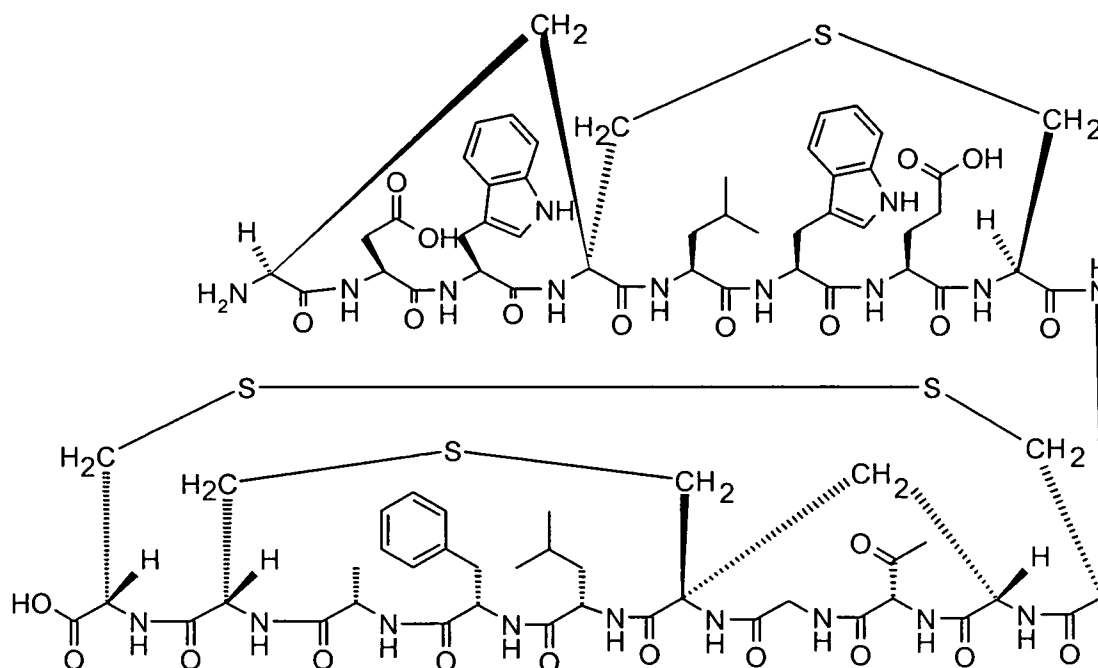
21	O	16OC2	0.20586	0.50283	0.46026	0.570
22	N	16NH2	0.12522	0.46605	0.47460	0.430
23	C	16Ca2	0.15695	0.50741	0.49039	0.430
24	C	16Cb1	0.17666	0.42678	0.52110	0.430
25	C	16CO1	0.17829	0.54862	0.44623	0.430
26	O	16OC1	0.19399	0.62818	0.45350	0.430
27	N	15NH	0.08606	0.28654	0.38875	1.0
28	C	15Ca	0.08545	0.33846	0.43783	
29	C	15Cb	0.07342	0.26519	0.48191	
30	C	15Cg	0.07342	0.31743	0.53443	
31	C	15Cd1	0.09641	0.30051	0.57015	
32	C	15Ce1	0.09892	0.34692	0.61802	
33	C	15Cz	0.07361	0.41607	0.62963	
34	C	15Ce2	0.04891	0.43679	0.59512	
35	C	15Cd2	0.04787	0.38914	0.54580	
36	C	15CO2	0.11795	0.38518	0.45303	0.570
37	O	15OC2	0.14083	0.32503	0.46100	0.570
38	C	15CO1	0.11985	0.37400	0.45272	0.430
39	O	15OC1	0.14260	0.31215	0.44467	0.430
40	N	14NH	0.09663	0.22957	0.28367	1.0
41	C	14Ca	0.06493	0.21832	0.30973	
42	C	14Cb	0.05881	0.10260	0.32228	
43	C	14Cg	0.06148	0.01917	0.28163	
44	C	14Cd1	0.05246	-0.08320	0.30793	
45	C	14Cd2	0.04038	0.05153	0.23556	
46	C	14CO	0.06073	0.27513	0.35936	
47	O	14OC	0.03420	0.31036	0.37413	
48	N	13NH	0.14348	0.21739	0.21016	
49	C	13Ca	0.13259	0.32131	0.22415	
50	C	13Cb	0.15887	0.37393	0.25639	
51	C	13CO	0.10174	0.31603	0.25729	
52	O	13OC	0.08431	0.39162	0.25920	
53	N	12NH	0.13789	0.04315	0.10383	
54	C	12Ca	0.14494	0.06092	0.15913	
55	C	12CO	0.12665	0.15374	0.17941	
56	O	12OC	0.09846	0.17540	0.16440	
57	N	11NH	0.18026	0.23392	0.10274	
58	C	11Ca	0.18245	0.14769	0.06491	
59	C	11Cb	0.21437	0.08040	0.07601	
60	O	11Og	0.24067	0.15367	0.06932	

61	C	11Cg	0.21483	-0.00652	0.03877	
62	C	11CO	0.15298	0.08188	0.06213	
63	O	11OC	0.14289	0.05192	0.01723	
64	N	10NH	0.15427	0.48630	0.10547	
65	C	10Ca	0.15553	0.39216	0.13693	
66	C	10Cb	0.12680	0.38774	0.17495	
67	C	10CO	0.15563	0.30214	0.09785	
68	O	10OC	0.13341	0.29174	0.06737	
69	N	9NH	0.15921	0.62732	0.02599	
70	C	9Ca	0.17078	0.65813	0.07761	
71	C	9Cb1	0.15364	0.74568	0.10696	0.650
72	S	9Sg2	0.11348	0.70773	0.12986	0.650
73	C	9Cb2	0.14430	0.73147	0.09699	0.350
74	S	9Sg1	0.15631	0.80140	0.15424	0.350
75	C	9CO	0.17309	0.56593	0.11423	1.0
76	O	9OC	0.19300	0.56593	0.15084	
77	N	8NH	0.18541	0.52953	-0.09746	
78	C	8Ca	0.16349	0.52728	-0.05346	
79	C	8Cb	0.15665	0.41428	-0.03791	
80	S	4S1	0.14207	0.33194	-0.08922	
81	C	8CO	0.17870	0.57819	-0.00528	
82	O	8OC	0.20763	0.56423	0.00430	
83	N	7NH	0.20632	0.49826	-0.20125	
84	C	7Ca	0.21045	0.59830	-0.17558	1.0
85	C	7Cb	0.20977	0.68941	-0.21403	
86	C	7Cg	0.23965	0.69290	-0.24846	
87	C	7Cd	0.27179	0.70780	-0.21942	
88	O	7O2	0.27356	0.77789	-0.18617	
89	O	7O2	0.29422	0.64688	-0.23185	
90	C	7CO	0.18551	0.60947	-0.13036	
91	O	7OC	0.16934	0.68669	-0.12474	
92	N	6NH	0.14508	0.31502	-0.23408	
93	C	6Ca	0.17581	0.36438	-0.24779	
94	C	6Cb	0.17865	0.38440	-0.30618	
95	C	6Cg	0.21065	0.42228	-0.32263	
96	C	6Cd1	0.24023	0.37835	-0.31634	
97	N	6Ne	0.26396	0.43927	-0.33810	
98	C	6Ce2	0.24957	0.52674	-0.35877	
99	C	6Cd2	0.21551	0.51704	-0.34959	
100	C	6Ce1	0.19382	0.59341	-0.36554	

101	C	6Cz1	0.20739	0.67707	-0.39101	
102	C	6Ch	0.24169	0.68111	-0.39910	
103	C	6Cz2	0.26459	0.61009	-0.38421	
104	C	6CO	0.17783	0.46644	-0.21817	
105	O	6OC	0.15276	0.51859	-0.21294	
106	N	5NH	0.09092	0.22755	-0.18031	
107	C	5Ca	0.10636	0.17447	-0.22462	
108	C	5Cb	0.08579	0.17788	-0.27331	
109	C	5Cg1	0.06556	0.08855	-0.29223	0.400
110	C	5Cd2	0.05562	0.00295	-0.25553	0.400
111	C	5CD3	0.03406	0.13232	-0.31950	0.400
112	C	5Cg2	0.05122	0.12511	-0.26843	0.600
113	C	5Cd1	0.05227	0.02119	-0.24021	0.600
114	C	5Cd2	0.03647	0.22422	-0.25795	0.600
115	C	5CO	0.14049	0.21405	-0.23552	1.0
116	O	5OC	0.16220	0.15250	-0.24447	
117	N	4NH	0.04424	0.29531	-0.10297	
118	C	4Ca	0.07828	0.26318	-0.08968	
119	C	4Cb	0.09901	0.36213	-0.08847	
120	C	4CO	0.09150	0.19084	-0.13193	
121	O	4OC	0.10378	0.10656	-0.12020	
122	N	3NH	-0.02049	0.37136	-0.10602	
123	C	3Ca	-0.01386	0.26387	-0.12087	
124	C	3Cb	-0.02263	0.24478	-0.17726	
125	C	3Cg	-0.05642	0.26752	-0.19336	
126	C	3Cd1	-0.08244	0.28646	-0.16303	
127	N	3Ne	-0.10966	0.30431	-0.19328	
128	C	3Ce2	-0.10052	0.29888	-0.24451	
129	C	3Cd2	-0.06697	0.27528	-0.24627	
130	C	3Ce1	-0.05275	0.26512	-0.29539	
131	C	3Cz1	-0.07001	0.27753	-0.33970	
132	C	3Ch	-0.10351	0.30097	-0.33634	
133	C	3Cz2	-0.11905	0.31339	-0.29019	
134	C	3CO	0.02125	0.22763	-0.11313	
135	O	3OC	0.02703	0.13349	-0.11547	
136	N	2NH	0.00873	0.33093	-0.00078	
137	C	2Ca	-0.02584	0.34024	-0.01079	
138	C	2Cb	-0.04293	0.38712	0.03587	
139	C	2Cg	-0.07852	0.41234	0.02763	
140	O	2Od1	-0.09430	0.35452	-0.00285	

141	O	2Od2	-0.09060	0.48825	0.05303	
142	C	2CO	-0.02912	0.40784	-0.06006	
143	O	2OC	-0.03851	0.49903	-0.05444	
144	N	1NH	0.07381	0.20163	0.05701	
145	C	1Ca	0.06250	0.25417	0.00903	
146	C	1Cb	0.07969	0.20450	-0.03763	
147	C	1CO	0.02526	0.24331	0.00649	
148	O	1OC	0.01235	0.15545	0.00653	

Example 12: Oxidation of Labyrinthopeptin (III)



- 5 50 mg Labyrinthopeptin (III) (0.026 mmol) were dissolved in 1 ml DMSO and mixed with 11 mg 1-Hydroxy-1-oxide-1,2-Benziodoxol-3(1H)-one (IBX, 0.039 mmol) at room temperature. The mixture was stirred for 6 h at 40 °C and further 12 h at room temperature, and subsequently purified by reversed-phase HPLC on a Phenomenex Luna® Axia 5 μ m C18 (2) column (dimension: 100 mm x 30 mm) with a XTerra®
- 10 Prep MS C18 10 μ m pre-column (Waters, Dimension: 19 x 10 mm). A gradient of 5% to 95% acetonitrile in water over 30 min (containing 0.1% ammonium acetat, pH 7.0) was used as the eluent. Column flow (60 ml/min) was fractioned and by UV detection. Fraction 7 to 11 contained the desired compound. The said fractions yielded 25 mg (50 %) after lyophilization. The product was characterized by mass
- 15 spectroscopy (Bruker Daltonics MicroTof).

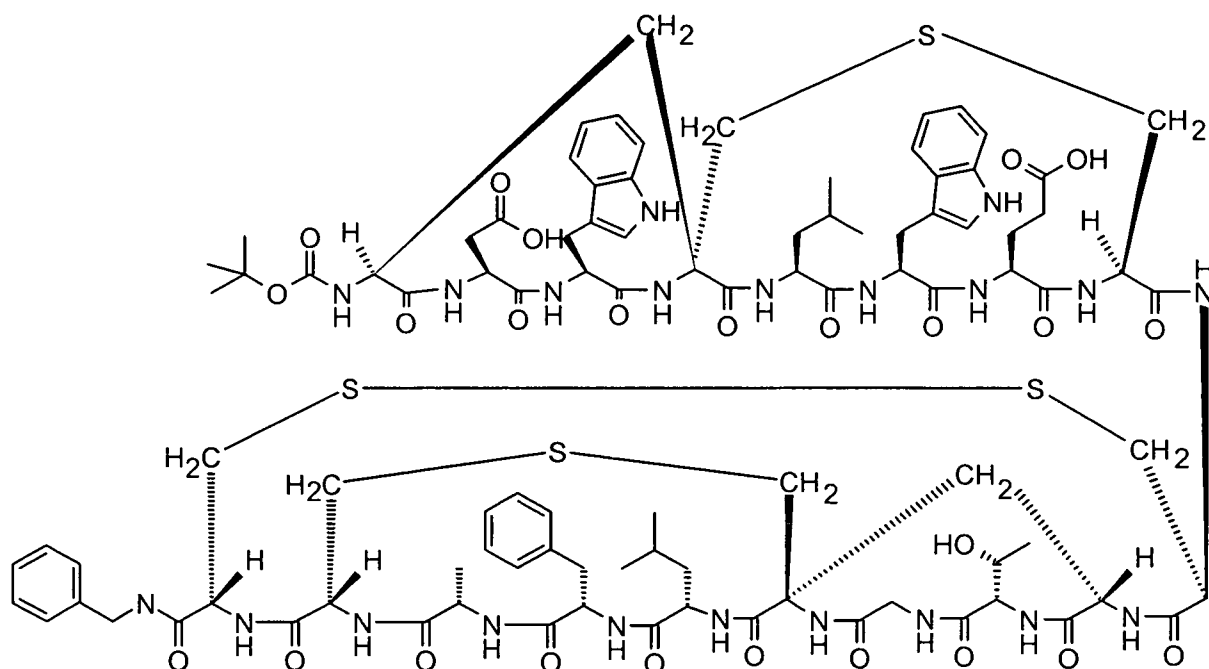
UV: 222 sh, 278 nm

ESI-MS: MW = 1920.66518 (mono MW)

Molecular formula: C₈₅H₁₀₈N₂₀O₂₄S₄

5 Molecular weight (MW) = 1922.2

Example 13: Peptid synthesis on the C-terminal end of Labyrinthopeptins (III)



- 10 40 mg Labyrinthopeptin (III) (0.021 mmol) were dissolved in 2 ml abs. dimethylformamid and treated with 10 mg (0.045 mmol) di-*tert*-butyl-dicarbonate (Boc₂O) and 7 mg (0.054 mmol) diisopropylethylamin for 1 h at room temperature. Subsequently, 6.8 mg (0.063 mmol) benzylamin and 50 µl (0.072 mmol) a 50 % solution of propyl phosphonic acid anhydride in DMF was added. The reaction
- 15 mixture was purified via reversed-phase HPLC on a Waters XBridge Shield® 5 µm C18 Säule (dimension: 100 mm x 30 mm) containing a XBridge Shield® C18 10 µm pre-column (Waters, dimension: 19 x 10 mm). A gradient of 5% to 95% acetonitrile in water over 30 min (containing 0.1% ammonium acetat, pH 7.0) was used as the eluent. Column flow (60 ml/min) was fractioned and by UV detection. Fraktions 30
- 20 and 31 were combined and yielded 9.6 mg (22 %) of the desired compound. The product was characterized by mass spectroscopy (Bruker Daltonics MicroTof).

UV: 222 sh, 276 nm

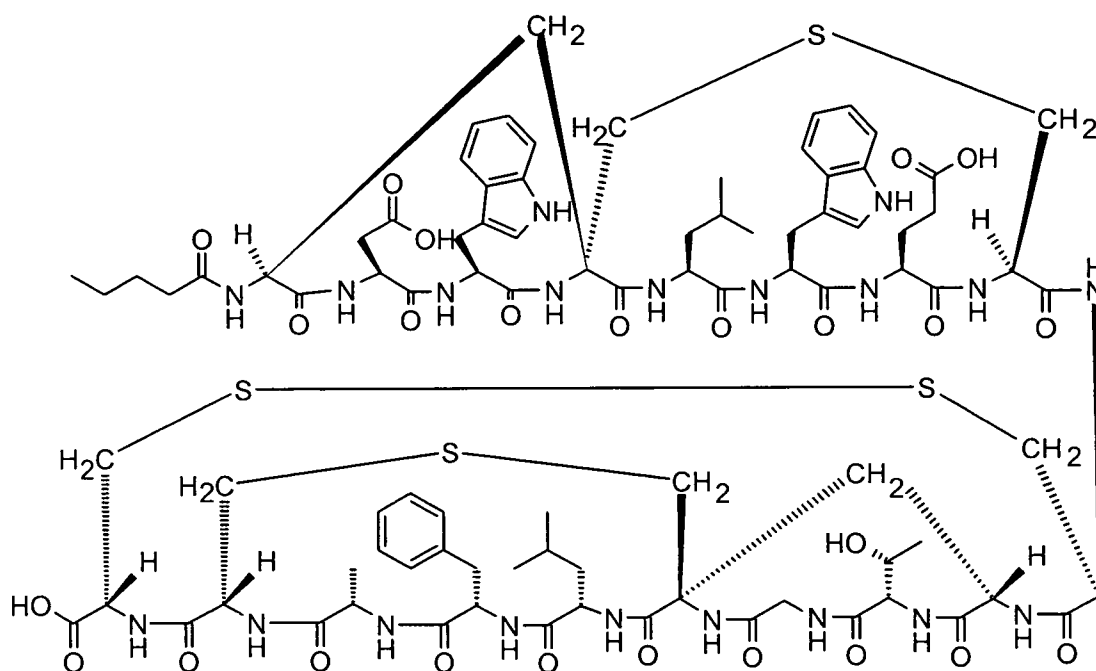
ESI-MS: MW = 2111.79018 (mono MW)

Molecular formula: C₉₇H₁₂₅N₂₁O₂₅S₄

Molecular weight (MW) = 2113.5

5

Example 14: Peptid synthesis on the N-terminal end of Labyrinthopeptins (III)



5 mg (0.028 mmol) 2-chloro-4,6-dimethoxy-1,3,5-triazin (CDMT) were dissolved in 2
 10 ml abs. dimethylformamid and mixed with 8.6 mg (0.085 mmol) N-methylmorpholine
 (NMM). The mixture was stirred for 1 h at room temperature. 3.3 mg (0.028 mmol) n-
 capronic acid was added and the mixture stirred for further 30 min at room
 temperature. Subsequently, 40 mg (0.021 mmol) Labyrinthopeptin (III) was added
 and the resulting mixture was stirred for further 2 h at room temperature. The
 15 reaction mixture was purified via reversed-phase HPLC on a Waters XBridge
 Shield® 5 µm C18 Säule (dimension: 100 mm x 30 mm) with a XBridge Shield® C18
 10 µm pre-column (Waters, dimension: 19 x 10 mm). A gradient of 5% to 95%
 acetonitrile in water over 30 min (containing 0.1% formic acid, pH 2.0) was used as
 the eluent. Column flow (60 ml/min) was fractioned and by UV detection. Fraktions
 20 38 to 40 were combined and yielded 11.0 mg (26 %) of the desired compound. The
 product was characterized by mass spectroscopy (Bruker Daltonics MicroTof).

UV: 220 sh, 278 nm

ESI-MS: MW = 2020.75738 (mono MW)

Molecular formula: C₉₁H₁₂₀N₂₀O₂₅S₄

Molecular weight (MW) = 2022.3

5 Example 15: Antibacterial activity

After culturing the organisms in liquid beef extract broth, the suspensions of bacteria were adjusted to a defined density by dilution with fresh culture medium ($5 \cdot 10^5$ organisms/ml).

10

Labyrinthopeptin (III) was dissolved and diluted with water in a geometric dilution series (factor 2). 1.5 ml of the solution in the individual dilution steps were mixed with 13.5 ml liquid agar (Müller-Hinton agar) at approximately 45°C.

- 15 The maximum compound concentration in the petri dish was usually 100 mg/l. An agar plate with no compound served as a control.

After the culture medium had cooled and solidified, the agar plates were inoculated simultaneously with 20 different bacterial strains using a Multipoint Inoculator
20 delivering $5 \cdot 10^4$ colony forming units (cfu) per inoculation spot and then incubated at 37°C for 17 hours under aerobic conditions.

After incubation, the plates were examined macroscopically for the lowest compound concentration at which bacterial growth is no longer visible. A single colony or a haze
25 growth at the inoculation spot was disregarded.

The antibacterial effect was assessed as the Minimum Inhibitory Concentration of the test compound (MIC: lowest compound concentration at which bacterial growth is no longer macroscopically visible):

30

Test organism	MIC
Staphylococcus aureus SG 511	12,5 mg/l
Staphylococcus aureus 285	12,5 mg/l

Staphylococcus aureus 503	3,13 mg/l
Streptococcus pyogenes 308 A	3,13 mg/l
Streptococcus pyogenes 77 A	3,13 mg/l
Streptococcus faecium D	6,25 mg/l

Example 16: Antiviral activity

Viruses can only multiply in living cells. The viral studies were therefore carried out in cell cultures. The viruses were selected either because of their importance as infectious agents or typical biochemical or morphological structures.

A dilution series of Labyrinthopeptin (III) was prepared in 96-well microtiter plates. Hela or Vero cells, according to the infecting virus, are added to give a confluent monolayer within 24h of incubation. After incubation for 3 hours, the respective virus is added to the cells in a concentration which is expected to completely destroy the cell monolayer within 2 days. The cultures are incubated at 37°C in a gassed incubator (5% CO₂ in air). After 24 hours the maximal tolerated dose of test compound (MTD) in the cell culture is evaluated by microscopic examination. The results were compared with non-infected tissue control and a corresponding infection control:

No.	Host organism	Test organism	Inhibition [mg/l]
1	Vero cells	Mycovirus (RNA/Influenza A/Aichi)	44,44
2	Hela cells	Herpes (DNA), Simples 1	133,33
3	Vero cells	Herpes (DNA), Simplex 2 VR 734	44,44
4	Hela cells	Adenovirus (DNA), 5"	133,33

Example 17: Neuropathic pain activity

Labyrinthopeptin (III) 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

(22.7g $\pm$ 0.26SEM) 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 (6hours) 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].

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 treatment effects for both compounds. 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 (III) achieved 97% benefit.

In conclusion, the compound significantly reduces tactile allodynia in the SNI mouse model of neuropathic pain.

Example 18: Inflammatory triggered pain activity

5

Labyrinthopeptin (III) was studied in the carrageenan (CAR) induced hindpaw inflammation model in mice in order to proof the activity on thermal hyperalgesia, a typical readout for inflammatory triggered pain.

- 10 Induction of hind paw inflammation: Under slight general Isofluran anaesthesia, CAR 2% (Sigma, Deisenhofen, Germany) in 20µl saline was injected into the plantar aspect of both hind paws in male C57B6 mice. Paw withdrawal latencies (PWL) were determined on exposure of the paws to a defined thermal stimulus using a commercially available apparatus (Plantar Test Ugo Basile Biological Research
- 15 Apparatus, Comerio, Italy) fitted with a mini camera to ensure proper placement of the infrared heat below the hind paw of interest. Mice were kept in the test cages over the whole study period (6 hours).

- Measurement of thermal hyperalgesia: The timer which measures the duration of reflecting infrared light by the hind paw is started by the investigator and stopped, if
- 20 the animal is shaking the affected hind paw. A cut off was set at 16 seconds to prevent tissue damage. The study was performed before and over 6 hours after CAR injection. Paw withdrawal latencies in seconds were used as readout for further analysis.

25

As a vehicle for the intravenous application was the 1:1:18 (Ethanol : Solutol : phosphate buffered saline) vehicle chosen.

- Paw withdrawal latencies (PWL) measurements have been used to calculate
- 30 significant treatment effects, and for AUC calculations over a reference time period (6hours) and subsequent % benefit calculations. For the statistical analysis the PWL values of both hindpaws were used in two ways: first, with a 2-way analysis of variance (ANOVA) based on the PWL values for specific times (within a period of 6

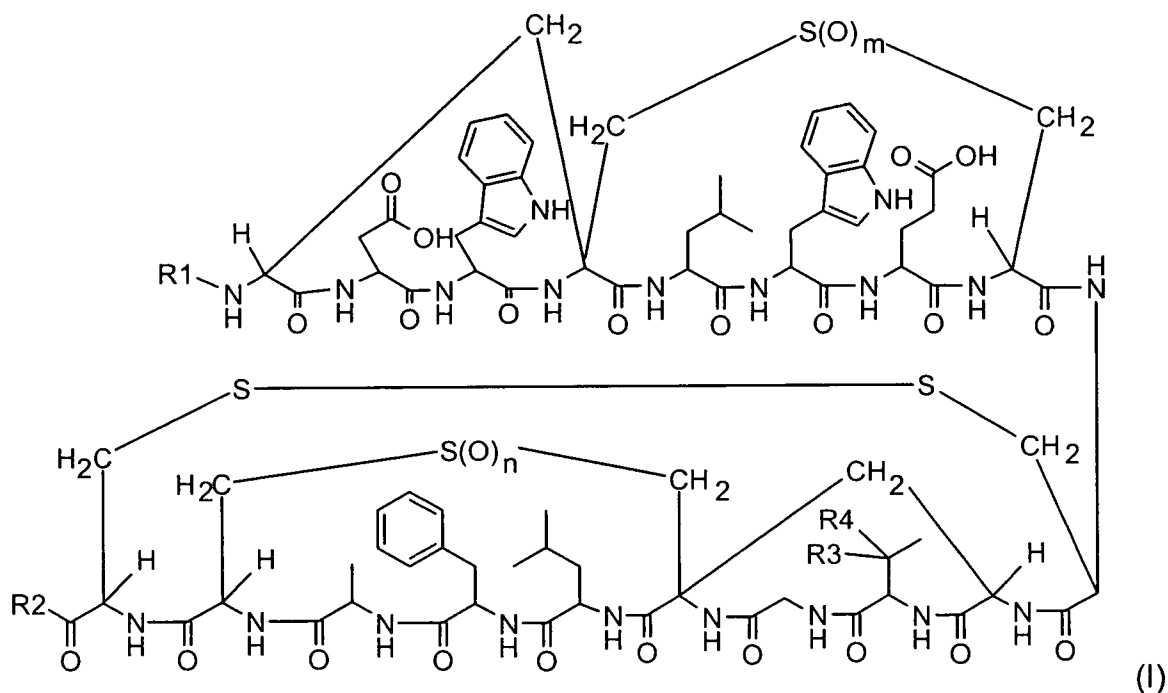
hours) and second with a 1-way ANOVA on non-transformed delta AUC values [AUC1-6hour].

- Two-way Analysis of variance with repeated measures (Repeated factor: TIME, Analysis variable: PWL) followed by a Complementary Analysis (Effect of factor GROUP for each level of factor TIME (Winer analysis), Analysis variable: PWL) and a subsequent Dunnett's test for factor DOSAGE for each level of factor TIME (Two sided comparison vs level 0 = VEHICLE) revealed highly significant differences from the vehicle group from 1 to 2 hours after intravenous application for both dosages.
- 5 The effect was gone 4 hours after application. 1-way ANOVA using delta [AUC1-6hour] values revealed a p value of $p < 0.0001$. Dunnett analysis and gave significant treatment effects for both dosages. The percent benefit of the treatment was evaluated using the [AUC1-6hour] values of the vehicle group (0% benefit) and all [AUC1-6hour] values before CAR was injected (theoretical baseline over 6 hours =
- 10 maximum possible effect = 100% effect). Compared to these margins the 1 mg/kg dosage group achieved 37 and the 10 mg/kg group 34% benefit.
- 15

In conclusion, the compound significantly reduced thermal hyperalgesia in the CAR mouse model of inflammatory triggered pain.

Patent claims:

- 5 1. A compound of the formula (I)



wherein

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

R2 is OH, NH₂, NH-(C₁-C₆)alkyl, NH-(C₁-C₄)alkylene-phenyl or

10 NH-(C₁-C₄)alkylene-pyridyl;

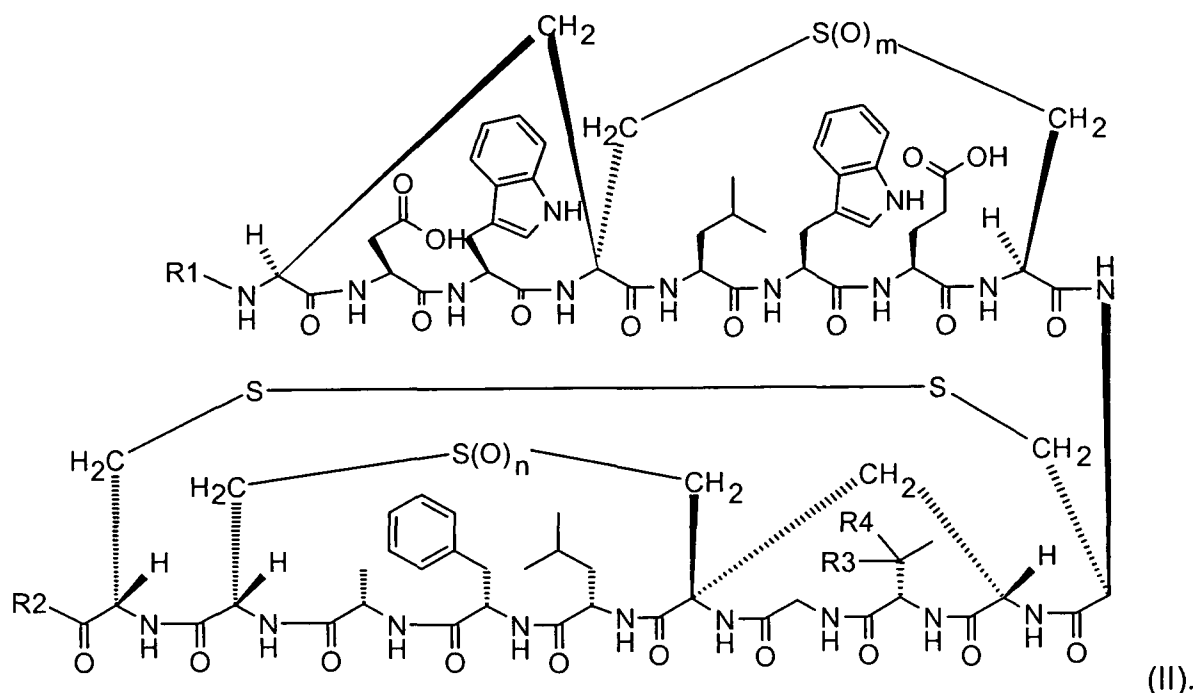
R3 and R4 independently of each other H or OH, or R3 and R4 together are =O; and

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.

15

2. A compound of the formula (I) according to claim 1 characterized the formula (II)



3. A compound of the formula (I) according to any one of claims 1 or 2 wherein R1 is H.

5

4. A compound of the formula (I) according to any one of claims 1 or 3 wherein R2 is OH.

5. A compound of the formula (I) according to any one of claims 1 or 4 wherein R3 and R4 are H or OH wherein if R3 is OH then R4 is H, or if R3 is H then R4 is OH, or R3 and R4 together are =O.

10

6. A compound of the formula (I) according to any one of claims 1 to 5 wherein R3 is OH and R4 is H, or if R3 is H and R4 is OH.

15

7. A compound of the formula (I) according to any one of claims 1 to 6 wherein 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.

8. A compound of the formula (I) according to any one of claims 1 to 7 wherein m and n are both 0.

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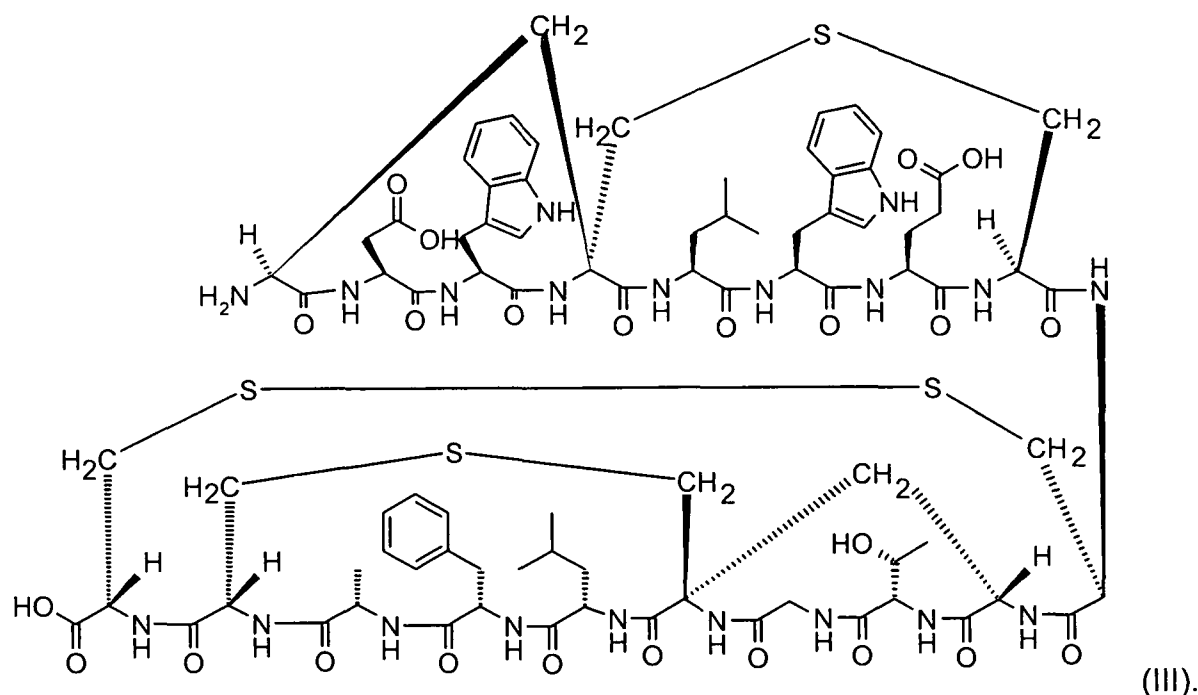
9. A compound of the formula (I) according to any one of claims 1 to 8 wherein

R1 is H; R2 is OH; R3 and R4 independently of each other H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH; and m and n are independently of one another 0, 1 or 2.

- 5 10. A compound of the formula (I) according to any one of claims 1 to 9 wherein R1 is H; R2 is OH; R3 and R4 independently of each other H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH; and 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.

- 10 11. A compound of the formula (I) according to any one of claims 1 to 10 wherein R1 is H; R2 is OH; R3 and R4 independently of each other H or OH wherein if R3 is OH then R4 is H, and if R3 is H then R4 is OH; and m and n are both 0.

12. A compound of the formula (I) according to any one of claims 1 to 11
15 characterized by a compound of the formula (III)



13. A compound isolated from *Actinomadura namibiensis* (DSM 6313), characterized by a monoisotopic neutral molecular mass of 1922.6872 Da, and a
20 molecular formula of C₈₅H₁₁₀N₂₀O₂₄S₄.

14. Use of a compound according to any one of claims 1 to 13 for the preparation

of a medicament for the treatment of bacterial infections, viral infections and/or pain.

15. A pharmaceutical composition comprising at least one compound of the formula (I) according to any one of claims 1 to 13 and at least one pharmaceutically acceptable ingredient.

16. A process for preparing a compound of the formula (I) according to any one of claims 1 to 13, which comprises

- a) the strain *Actinomadura namibiensis* (DSM 6313), or one of its variants and/or mutants, being fermented 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) a compound of the formula (I) being isolated from the culture medium, and
- c) the compound of the formula (I) being derivatized, where appropriate, and/or, where appropriate, being converted into a physiologically tolerated salt.

17. A process according to claim 16 wherein the compound (I) is characterized by a compound of the formula (II).

18. A process according to any one of claims 16 or 17 wherein the compound (I) is characterized by a compound of the formula (III).

19. A process according to any one of claims 16 to 18 wherein 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.