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(54) Title: ANTIBACTERIAL LIPOPEPTIDES, PHARMACEUTICAL COMPOSITION AND COSMETIC COMPOSITION COMPRISING THEM, AND USES THEREOF

(57) Abstract: The present invention relates to synthetic antimicrobial lipopeptides having a linear structure and to antimicrobial lipopeptides having a branched structure, to pharmaceutical compositions comprising such lipopeptides, to cosmetic compositions comprising such lipopeptides and to lipopeptides for use as a medicament, and to lipopeptides for use in the treatment or prophylaxis of a bacterial infection.

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Antibacterial lipopeptides, pharmaceutical composition and cosmetic composition comprising them, and uses thereof

The present invention relates to novel synthetic, linear and branched lipopeptides, pharmaceutical compositions comprising such synthetic, linear and/or branched lipopeptides, and cosmetic compositions comprising such synthetic, linear and/or branched lipopeptides, as well as such linear and branched lipopeptides for use as medicines, especially in treating bacterial infections.

The increasing number of drug-resistant bacterial strains constitutes currently a serious clinical problem. Therefore, there is a need for new compounds that could represent an effective alternative to existing antibiotics. At the same time, it is desirable that they show different mechanisms of action compared to the traditional antibiotics. A group of chemical compounds that can meet these requirements are lipopeptides.

Traditional antibiotics available on the market usually have a specific activity and they can, for example, disrupt specific biochemical processes by affecting the activity of enzymes involved in the synthesis of the cell wall, or by damaging DNA. Lipopeptides show a different mechanism of action, which involves their ability to penetrate and irreversibly destroy the bacterial cell membrane structure (see e.g. Alborn *et al.*, Antimicrob. Agents Chemother. 35 (1991) 2282). This fact has important implications from the point of view of their use as antimicrobial agents, since the likelihood of resistance to compounds of such type is very low.

Lipopeptides include daptomycin, which is a natural, branched, cyclic lipopeptide antibiotic of non-ribosomal origin. This lipopeptide shows a strong bactericidal activity *in vitro* and *in vivo* against Gram-positive bacteria that can cause serious and life-threatening diseases, as described, for example, in Tally *et al.* (Tally, F.P. *et al.*, "Daptomycin: a Novel Agent for Gram positive Infections", Exp. Opin. Invest. Drugs 8 (1999) 1223-1238).

A group of lipopeptides that show significant potential as useful antibiotics includes the lipopeptides described, for example, in U.S. Patent No. 6,911,525 and U.S. Patent Application No. 2015/0080292 A1.

J. Juhaniewicz-Dębińska *et al.* (Juhaniewicz-Dębińska *et al.*, "Lipopeptide-induced changes in permeability of solid supported bilayers composed of bacterial membrane lipids", Journal of Electroanalytical Chemistry, 812, (2018) 227-234) described ultra-short linear lipopeptides and their effect on simplified models of lipid bilayers mimicking the membrane system of a Gram-negative bacteria *E. coli*. The studies showed a significant membranolytic activity of the mentioned lipopeptides, suggesting that their mechanism of antimicrobial activity may indeed be based on the interaction with the cell membrane.

Most of the lipopeptides described in the literature are of natural origin and are obtained from bacteria or other organisms, which implies the costs of isolation from biological material. Moreover, most of the lipopeptides currently used in the medicine, such as daptomycin or polymyxins, have a cyclic, relatively complex structure, which in turn complicates the possible synthesis and further modifications.

An object of the present invention is to provide synthetic lipopeptides that show antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant bacteria, as well as pharmaceutical and cosmetic compositions comprising them.

Brief Description of the Invention

The subject matter of the invention is a synthetic lipopeptide of general formula:

X_1-Dab-X_2-X_3-Dab-Dab-X_4-NH_2

wherein

X_1 is a residue selected from FA-DDab-Dab (SEQ ID NO.: 1) and H-Dab(FA-DDab) (SEQ ID NO.: 3), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine, or D-tryptophan residue; and

X_4 is a leucine, alanine, isoleucine or valine residue.

Preferably, in the lipopeptide of the above-indicated general formula according to the invention, X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue. Such a lipopeptide has a linear structure and can be represented by the following formula:

FA-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (SEQ ID NO.: 2).

More preferably, such a lipopeptide according to the invention is selected from the group consisting of the following lipopeptides:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

Preferably, in such a lipopeptide of the above-indicated general formula according to the invention, X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue. Such a lipopeptide has a branched structure and can be represented by the following formula:

H-Dab(FA-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (SEQ ID NO.: 4).

Preferably, such a lipopeptide according to the invention is selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

A further subject matter of the invention is a pharmaceutical composition, characterized in that it comprises a lipopeptide of the general formula:

$$X_1\text{-Dab-}X_2\text{-}X_3\text{-Dab-Dab-}X_4\text{-}NH_2$$

wherein

X_1 is a residue selected from FA-DDab-Dab and H-Dab(FA-DDab), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine or D-tryptophan residue;

X_4 is a leucine, alanine, isoleucine or valine residue.

Preferably the pharmaceutical composition according to the invention comprises a lipopeptide of the above-indicated general formula according to the invention, wherein X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

More preferably, the pharmaceutical composition according to the invention comprises a lipopeptide selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

Preferably, the pharmaceutical composition according to the invention comprises a lipopeptide of the above-indicated general formula according to the invention, wherein X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

More preferably, the pharmaceutical composition according to the invention comprises a lipopeptide selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

The pharmaceutical composition according to the invention preferably comprises 0.1 to 99% by weight of at least one of the above-described lipopeptides according to the invention.

Preferably, the pharmaceutical composition according to the invention further comprises a pharmaceutically acceptable carrier and/or diluent and/or adjuvant and/or excipient.

More preferably, the pharmaceutical composition according to the invention comprises corn starch and/or gelatin and/or lactose and/or sucrose and/or microcrystalline cellulose and/or kaolin and/or mannitol and/or dicalcium phosphate and/or sodium chloride and/or alginic acid.

Preferably, the pharmaceutical composition according to the invention is in a form suitable for oral and/or intravenous and/or intramuscular and/or subcutaneous and/or parenteral administration.

Preferably, the pharmaceutical composition according to the invention is in a form of a tablet and/or a capsule and/or an elixir and/or a suspension and/or a syrup and/or a gel and/or a cream and/or an ointment.

Preferably, the pharmaceutical composition according to the invention further comprises at least one other antimicrobial agent.

A further subject matter of the invention is a cosmetic composition, characterized in that it comprises a lipopeptide of the general formula:

$$X_1\text{-Dab-}X_2\text{-}X_3\text{-Dab-Dab-}X_4\text{-}NH_2$$

wherein

X_1 is selected from FA-DDab-Dab and H-Dab(FA-DDab), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine or D-tryptophan residue;

X_4 is a leucine, alanine, isoleucine or valine residue.

Preferably, the cosmetic composition according to the invention comprises a lipopeptide of the above-indicated general formula according to the invention, wherein X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue and X_4 is a leucine residue.

More preferably, the cosmetic composition according to the invention comprises a lipopeptide selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

Preferably, the cosmetic composition according to the invention comprises a lipopeptide of the above-indicated general formula, wherein X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue and X_4 is a leucine residue.

More preferably, the cosmetic composition according to the invention comprises a lipopeptide selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

Preferably, the cosmetic composition according to the invention contains 0.1 to 99% by weight of at least one of the lipopeptides according to the invention.

Preferably, the cosmetic composition according to the invention comprises a cosmetic carrier.

Preferably, the cosmetic composition according to the invention is in a form of a gel or a cream or a suspension.

A further subject matter of the invention is any of the lipopeptides according to the invention, as defined above, for use as a medicament.

A further subject matter of the invention is any of the lipopeptides according to the invention, as defined above, for use in the treatment or prophylaxis of a bacterial infection.

Preferably, in the case of any lipopeptide according to the invention for use in the treatment or prophylaxis of a bacterial infection, such bacterial infection is caused by a Gram-positive bacterium.

More preferably the bacterial infection is caused by a bacterium of the genus *Enterococcus* or *Staphylococcus*.

Even more preferably the bacterial infection is caused by *Staphylococcus aureus* or *Staphylococcus epidermidis*.

Preferably, in the case of any lipopeptide according to the invention for use in the treatment or prophylaxis of a bacterial infection, such bacterial infection is caused by a Gram-negative bacterium.

More preferably the bacterial infection is caused by a bacterium of the genus *Klebsiella* or *Pseudomonas*.

Even more preferably the bacterial infection is caused by *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*.

Preferably, in the case of any lipopeptide according to the invention for use in the treatment or prophylaxis of a bacterial infection, such infection is caused by an antibiotic-resistant bacterium.

Preferably, in the case of the lipopeptide according to the invention for use in the treatment or prophylaxis of a bacterial infection, at least one lipopeptide is used selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2),

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3),

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

Detailed Description of the Invention

As defined above, the present invention provides novel synthetic, linear and branched lipopeptides, pharmaceutical compositions comprising such synthetic, linear and/or branched lipopeptides, cosmetic compositions comprising such synthetic, linear and/or branched lipopeptides, as well as such linear and branched lipopeptides for use as medicines, especially in the treatment of bacterial infections.

The synthetic lipopeptide according to the invention is represented by the general formula:

$$X_1\text{-Dab-}X_2\text{-}X_3\text{-Dab-Dab-}X_4\text{-}NH_2$$

wherein

X_1 is a residue selected from FA-DDab-Dab (SEQ ID NO.: 1) and H-Dab(FA-DDab) (SEQ ID NO.: 3), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid, and the lipopeptide has a linear structure,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid and 4-methylhexanoic acid, and the lipopeptide has a branched structure;

X_2 is a leucine, alanine, isoleucine or valine residue – i.e. an aliphatic amino acid;

X_3 is a D-phenylalanine, D-tyrosine or D-tryptophan residue – i.e. an aromatic amino acid residue; and

X_4 is a leucine, alanine, isoleucine or valine residue.

For the purposes of the present specification, a lipopeptide is a molecule consisting of a lipid residue, i.e. a fatty acid (FA) residue linked to a peptide residue. In relation to the amino acid residues, a standard nomenclature in form of three-letter abbreviations known in the art is used, for example, a L-phenylalanine amino acid residue is designated as "Phe," a D-phenylalanine residue as "DPhe," L-leucine as "Leu," L-2,4-diaminobutyric acid as "Dab," and D-2,4-diaminobutyric acid as "DDab."

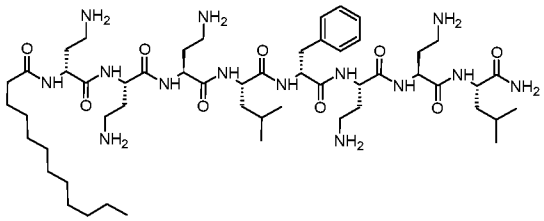
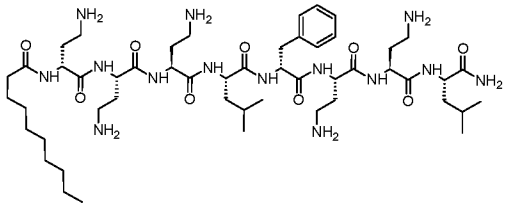
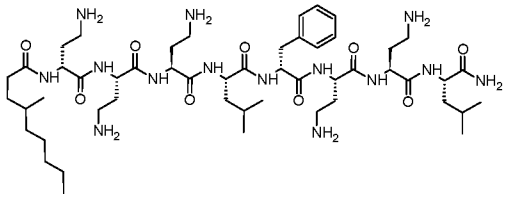
The sequence listing shows the amino acid sequences of the peptide portion of the lipopeptides according to the invention.

Preferred linear lipopeptides according to the present invention can be represented by the following formula:

FA-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (SEQ ID NO.: 2)

wherein FA is a linear or branched fatty acid residue selected from the group comprising n-dodecanoic acid residue, n-decanoic acid residue, and 4-methylnonanoic acid residue. Detailed structures of the more preferred linear lipopeptides according to the invention are shown in Table 1.

Table 1. Linear lipopeptides

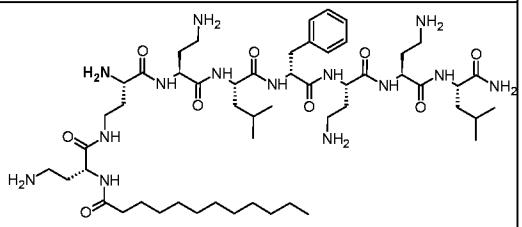
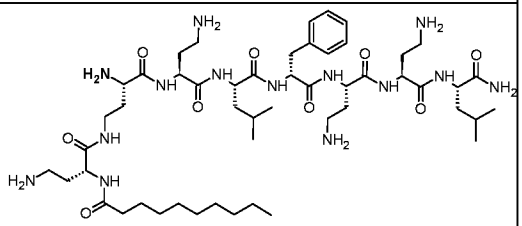
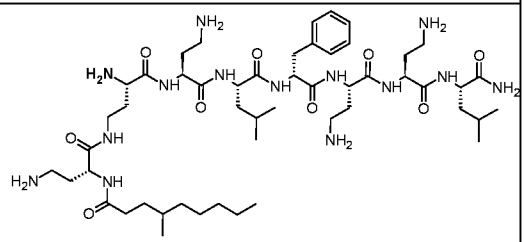
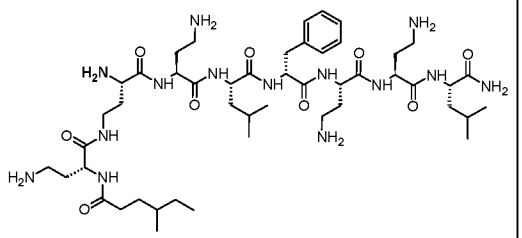
Formula No.	Condensed formula of the lipopeptide	Structural formula
1	**$CH_3(CH_2)_9CH_2CO$-**DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	
2	**$CH_3(CH_2)_7CH_2CO$-**DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	
3	**$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-**DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	

Preferred branched lipopeptides according to the present invention can be represented by the following formula

H-Dab(FA-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (SEQ ID NO.: 4),

wherein FA is a linear or branched fatty acid residue selected from the group comprising n-dodecanoic acid residue, n-decanoic acid residue, 4-methylnonanoic acid residue, and 4-methylhexanoic acid residue. Detailed structures of the more preferred branched lipopeptides according to the invention are shown in Table 2.

Table 2. Branched lipopeptides

Formula No.	Condensed formula of the lipopeptide	Structural formula
4	H-Dab(**$CH_3(CH_2)_9CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	
5	H-Dab(**$CH_3(CH_2)_7CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	
6	H-Dab(**$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	
7	H-Dab(**$CH_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	

The lipopeptides according to the invention may be obtained by any synthetic method known in the prior art. For example, the mentioned synthesis method may include liquid-phase or solid-phase synthesis (see e.g. Sewald, N. and Jakubke, H.-D. (2003), Peptide Synthesis. In Peptides: Chemistry and Biology (eds. N. Sewald and H.-D. Jakubke) doi:10.1002/352760068X.ch4 and Peptide synthesis and applications. ed./Knud J. Jensen; Pernille T. Shelton; Søren L.

Pedersen. Humana Press, 2013, (Methods in Molecular Biology, Vol. 1047). An exemplary method of preparing the lipopeptides according to the invention is provided in the Examples section below.

The lipopeptides according to the invention, both linear and branched, have an amphiphilic structure, and due to the presence of 2,4-diaminobutyric acid residues, under physiological pH (about 7.4) conditions, they have an excess positive charge, which facilitates their interaction with the bacterial cell membrane.

The pharmaceutical composition according to the invention comprises any linear or branched lipopeptide according to the invention preferably selected from the group of lipopeptides of condensed formulae 1-7. Preferably, the composition comprises 0.1 to 99% by weight of any of at least one lipopeptide according to the invention, preferably selected from the group comprising lipopeptides of condensed formulae 1-7. The pharmaceutical composition may comprise at least one pharmaceutically acceptable carrier and/or diluent and/or adjuvant and/or excipient, as known in the art. For example, but not limited to, the pharmaceutical composition may comprise corn starch and/or gelatin and/or lactose and/or sucrose and/or microcrystalline cellulose and/or kaolin and/or mannitol and/or dicalcium phosphate and/or sodium chloride and/or alginic acid. Methods for preparing pharmaceutical compositions comprising an active agent, such as an antimicrobial agent, and at least one pharmaceutically acceptable carrier and/or diluent and/or adjuvant and/or excipient, are known in the art.

Preferably, the pharmaceutical composition according to the invention further comprises at least one other antimicrobial agent, such as an antibiotic. Exemplary antimicrobial agents that can be a component of the pharmaceutical composition according to the invention may be selected, i.a., from: β-lactam antibiotics such as, for example, penicillins, cephalosporins; peptide antibiotics such as, for example, polymyxins (e.g. colistin), gramicidin, bacitracin, fusafungin; glycopeptide antibiotics such as, for example, vancomycin, oritavancin; aminoglycoside antibiotics such as, for example, streptomycin, gentamicin, amikacin; tetracycline antibiotics such as, for example, doxycycline; macrolide antibiotics such as, for example, erythromycin, clarithromycin; lincosamide antibiotics such as, for example, clindamycin, lincomycin; amphenicol antibiotics such as, for example, chloramphenicol; rifamycin antibiotics such as, for example, rifampicin and rifaximin. This combined use of at least two antimicrobial agents with different mechanisms of action provides an additional benefit in form of obtaining a synergistic antibacterial effect. As it is known from the literature, lipopeptides such as polymyxins produce a synergistic antibacterial effect in combination with other antibiotics (e.g. Berditsch *et al.* Antimicrob Agents Chemother. 59 (2015) 5288-5296 or Varra, M. Molecules 24 (2019) 249). Thus, the use of the lipopeptides according to the invention in the combination with another antimicrobial agent, such as an antibiotic, will give a synergistic antibacterial effect.

Preferably, the pharmaceutical composition according to the invention is in a form suitable for oral and/or intravenous and/or intramuscular and/or subcutaneous and/or parenteral administration, preferably in the form of a tablet and/or a capsule and/or an elixir and/or a suspension and/or a syrup and/or or a gel and/or a cream and/or an ointment. Methods for preparing such forms of pharmaceutical compositions are known in the art of the present invention.

The cosmetic composition according to the invention comprises any linear or branched lipopeptide according to the invention, preferably selected from the group comprising lipopeptides of condensed formulae 1-7. The cosmetic composition according to the invention may have any cosmetically acceptable dosage form. The cosmetic composition may comprise 0.1 to 99% by weight of any of at least one lipopeptide according to the invention, preferably selected from the group of condensed formulae 1-7. Preferably, the cosmetic composition according to the invention optionally comprises a cosmetic carrier. Cosmetic carriers are known in the art. For example, as cosmetic carriers semi-solid or liquid polyols can be used, as well as fats, e.g. in form of micelles or liposomes. Preferably, the cosmetic composition is in the form of a gel, cream or suspension. Methods for preparing cosmetic compositions are known in the art.

The invention also includes synthetic, linear and branched lipopeptides according to the invention, preferably of condensed formulae 1-7, for use as a medicament. Preferably, the invention also includes synthetic, linear and branched lipopeptides according to the invention, preferably of condensed formulae 1-7, for use in the treatment of bacterial infections, preferably caused by Gram-positive bacteria, more preferably of the genus *Staphylococcus*.

Synthetic, linear and branched lipopeptides according to the invention due to their growth inhibitory activity on Gram-positive bacteria, preferably of the genus *Enterococcus* or *Staphylococcus*, more preferably of the species *Staphylococcus aureus* and *Staphylococcus epidermidis*, and on Gram-negative bacteria, preferably of the genus *Klebsiella* or *Pseudomonas*, more preferably of the genus *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are intended for use in the treatment and/or prophylaxis of bacterial infections. They show particular activity against bacteria of the genus *Staphylococcus*, as well as *Pseudomonas* and *Klebsiella*, especially *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.*

The advantage of the synthetic linear and branched lipopeptides according to the invention is their mechanism of action, which is based on direct interaction with the bacterial cell membrane, which in turn reduces the risk of drug resistance. Due to a different mechanism of action to antibiotics known in the art, the lipopeptides according to the invention will show effectiveness against bacteria resistant to antibiotics with a different mechanism of action than the lipopeptides according to the invention. Additionally, the synthetic, linear and branched lipopeptides according to the invention are simple and easy to synthesize structures, what significantly reduces their preparation cost. The synthetic, linear and branched lipopeptides according to the invention are also characterized by an appropriate solubility in an aqueous medium, which significantly extends the range of possibilities regarding the dosage form of the pharmaceutical or cosmetic composition according to the invention. The synthetic, linear and branched lipopeptides according to the invention are also characterized by an increased metabolic stability, which ensures an additional benefit in the form of a significant extension of their activity time, and thus additionally increases their effectiveness.

To illustrate the invention, examples and figures are provided below which, however, should not be interpreted in any way as limiting the scope of the invention which is defined in the claims. The accompanying figures illustrate the preferred embodiments of the invention.

Figure 1 shows an exemplary scheme for the preparation of a synthetic linear lipopeptide according to the invention (condensed formula 2).

Figure 2 shows an exemplary scheme for the preparation of a synthetic branched lipopeptide according to the invention (condensed formula 5).

Figure 3 shows the effect of the lipopeptide of condensed formula 1 according to the invention on the growth dynamics of *Pseudomonas aeruginosa* (panel A) and *Staphylococcus aureus* (panel B) strains, shown as relative culture optical density as a function of time.

Figure 4 shows the effect of the lipopeptide of condensed formula 2 according to the invention on the growth dynamics of *Pseudomonas aeruginosa* (panel A) and *Staphylococcus aureus* (panel B) strains, shown as relative culture optical density as a function of time.

Figure 5 shows the effect of the lipopeptide of condensed formula 3 according to the invention on the growth dynamics of *Pseudomonas aeruginosa* (panel A) and *Staphylococcus aureus* (panel B) strains, shown as relative culture optical density as a function of time.

Figure 6 shows the effect of a reference lipopeptide on the growth dynamics of *Pseudomonas aeruginosa* (panel A) and *Staphylococcus aureus* (panel B) strains, shown as relative culture optical density as a function of time.

Unless otherwise indicated, in the examples below, known and/or commercially available devices, methods, reaction conditions, reagents and kits were used as are commonly used in the art to which this invention pertains and as recommended by the manufacturers of the corresponding reagents and kits.

EXAMPLE 1. Synthesis of lipopeptides according to the invention

The lipopeptides, both linear and branched, were obtained by solid-phase peptide synthesis according to the Fmoc/*t*Bu synthesis strategy on a polymer support, which was the Fmoc-Rink Amide AM amide resin (with 0.55 mmol/g loading, Activotec, UK). The synthesis was started by weighing out 365 mg of the resin and placing it in a syringe reactor, then adding 5 mL of N,N-dimethylformamide (DMF, Sigma-Aldrich) and shaking for 2 hours to swell the resin. Then the following steps were carried out:

1. Deprotection of the Fmoc (9-fluorenylmethoxycarbonyl) protecting group which blocks the amino group. For this, the resin was shaken twice (1 x 5 min, 1 x 10 min) in a 20% solution (v/v) of piperidine in DMF. To remove the residual piperidine, the resin was washed alternately with DMF and isopropanol (IPA, POCH S.A., Poland). The action was repeated three times, then the polymer support was washed two more times with DMF (each time: 5-6 ml, 2 min/wash).

2. The Kaiser test was used to evaluate the effectiveness of the Fmoc-deprotection step. Utilizing the standard procedure for performing this test, i.e. suspending a sample of the resin particles in a mixture of A-C solutions (3-5 drops each) and heating to approx. 100°C, using starting solutions composed of: 5 g of ninhydrin in 100 mL of ethanol

(solution A); 80 g of phenol in 20 mL of ethanol (solution B); 2 mL of 0.001 M aqueous KCN solution in 98 mL of pyridine (solution C). The dark blue color of both particles and solution indicated the presence of a free amino group.

3. The next step was to perform a condensation reaction between the amino acid derivative (Fmoc-Xaa)-OH and the amino group of the resin. For this purpose, the appropriate amounts of reagents were weighed and metered out into the vessel (molar ratio in relation to the resin loading):

- 2 equivalents of the corresponding amino acid derivative Fmoc Xaa-OH,

- 2 equivalents of DIC (N,N'-diisopropylcarbodiimide) coupling agent,

- 2 equivalents of Oxyma racemization-suppressing reagent.

Next, the above-mentioned reagents were dissolved in 3-4 mL of DMF and allowed to stand for about 10 minutes, then such prepared mixture was transferred to the syringe reactor with the resin and mixed/shaken for about 4-6 hours. After this, the reaction mixture was filtered off and the resin was washed alternately with DMF and IPA, starting with DMF (2x, each time: 5-6 ml, 2 min/wash).

4. Next, the Kaiser test was performed (according to 2), this time to evaluate the progress/completion of the condensation reaction. The negative test result, i.e. colorless both particles and solution, indicated the complete attachment of Fmoc-protected amino acid to the resin, i.e. the condensation reaction was complete. If the test result was positive, the amino acid derivative coupling step was repeated using a double excess of reagents (Fmoc-Xaa-OH/DIC/Oxyma molar ratio was 1:1:1).

5. Cyclic repetition of steps described in 1-4 resulted in elongation of the peptide chain. In the case of condensation of individual amino acid residues into a peptidyl-resin, the building blocks typically utilized in the Fmoc/*t*Bu synthesis strategy were used, i.e. Fmoc-Leu-OH, Fmoc-DPhe-OH, Fmoc-Dab(Boc)-OH, Fmoc-DDab(Boc)-OH, and in the case of branched analogs of the Boc-Dab(Fmoc)-OH derivative in the penultimate condensation reaction step Fmoc-Xaa-OH, (Activotec, UK).

6. The penultimate step of the synthesis was the attachment of fatty acid (R-COOH, Merck). For this purpose, after the prior deprotection of the α-amino group and confirmation of the presence of a free amino group in the Kaiser test, the following amounts of reagents were weighed out into the vessel:

- 2 equivalents of the given fatty acid,

- 2 equivalents of DIC coupling agent,

- 2 equivalents of Oxyma racemization-suppressing reagent.

Further, the above-mentioned reagents were dissolved in 3-4 mL of DMF/DCM mixture (1:1, v/v, DCM = dichloromethane) and allowed to stand for about 10 minutes. Such prepared mixture was transferred to a syringe reactor with the resin and shaken for about 24 hours in order to attach the fatty acid. After completion of the

reaction (detection by Kaiser test), the resins were first washed 4 times with DMF/DCM mixture (1:1, v/v), and then 4 times with each of the following solvents (2 min/wash):

- dichloromethane,

- methanol,

- diethyl ether.

Next, the washed peptidyl-resins were dried in a vacuum desiccator for 30 minutes.

7. The final step of the syntheses was the step of acidolytic cleavage of the peptide from the polymer support, in which Reagent B was used: TFA/H_2O/phenol/TIPS (88:5:5:2, v/v), (where: TFA - trifluoroacetic acid, TIPS - triisopropylsilane). Reagent B was added to the dried peptidyl-resin in a ratio of approx. 10 mL per 1 g of peptidyl-resin. The mixture was shaken for 2-4 hours. After this, the resin was filtered off, washed with an additional small amount of TFA, and the resulting filtrate was concentrated by evaporator. Cold diethyl ether (chilled in dry ice) was poured over the residue and it was allowed to stand for 24 hours in the refrigerator to precipitate the peptide. Next, after 24 hours, the crude peptide was isolated by centrifugation.

8. The analytical HPLC was performed on Prominence modular liquid chromatograph by Shimadzu with a DAD detector. A Luna 5µm, C18 (2) 100Å reverse-phase column, 250 x 4.6 mm, was used. Semi-preparative RP-HPLC were performed on a Prominence LC-20AP modular liquid chromatograph with a UV-VIS detector. A Luna 5µm, C18 column, 150 x 10 mm, was used. Identification of the obtained lipopeptides was performed on the basis of mass spectra obtained on the Shimadzu LCMS-IT-TOF instrument, which was equipped with an electrospray ion source (ESI) with an ion trap (IT) and a time-of-flight (TOF) analyzer. Prior to the purification step of a given peptide, RP-HPLC analysis of the crude product was performed in order to optimize the separation conditions for the given crude peptide mixture.

9. Due to the fact that the designed and synthesized peptides were to be subjected to biological studies, it was necessary to replace the trifluoroacetate (TFA) counter-ion with the chloride (Cl^-) ion. For this purpose, the purified peptide was dissolved in 0.1 M hydrochloric acid solution, then stirred for 30 minutes, then concentrated on an evaporator and the residue was frozen and lyophilized. This procedure was repeated several times (usually 3-4) until the weight of the weighed material did not change. An exemplary synthetic scheme of a linear (No. 2) and branched (No. 5) lipopeptide is shown in Figure 1 and 2, respectively.

EXAMPLE 2. Study of the biological activity of the lipopeptides according to the invention

Bacterial strains and culture media

All bacterial strains were obtained from the Polish Collection of Microorganisms (PCM) or the American Type Culture Collection (ATCC). Gram-positive bacterial strains: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 14506. Gram-negative bacterial strains: *Escherichia coli* ST2-

8624 O157:H7, *Pseudomonas aeruginosa* PAO1 PCM 499, *Klebsiella pneumoniae* PCM 1, *Salmonella sv. Typhimurium* TT622, and *Yersinia enterocolitica* PCM 2081. All strains were grown in LB (lysogeny broth) medium in a conventional manner.

Structure of the tested lipopeptides according to the invention

The biological activity was tested for lipopeptides of condensed formulae 1-7, and the obtained results were compared with the activity of a linear reference lipopeptide known in the art, described in De Zoysa *et al.*, European Journal of Medicinal Chemistry, 2018, 146, 344 and De Zoysa *et al.*, Journal of Medicinal Chemistry, 2015, 58, 625. The structure of the peptide part of the reference lipopeptide is described by the formula:

4MH-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2

wherein 4MH is a 4-methylhexanoic acid residue.

Determination of the Minimum Inhibitory Concentration (MIC)

Ten mL of LB medium was inoculated with material obtained from a single colony and the culture was allowed to grow overnight at 30°C with shaking. Next, the optical density of the overnight cultures (at 600 nm) was then adjusted to 0.05 by dilution in a fresh portion of LB medium. The test compounds were dissolved in water. Serial dilutions of compounds were prepared in LB medium ranging from 5 to 50 μg/mL (final concentrations). The experiment was performed by adding 100 μL of each of the prepared dilutions to 100 μL of the diluted overnight bacterial culture in the wells of a 96-well microtiter plate. The MIC was defined as the lowest concentration of test compound needed to inhibit bacterial growth evaluated after 24 h incubation at 30°C with shaking (final optical density at 600 nm not greater than 0.05). Optical density measurements were performed using TECAN Sunrise plate reader. Data were obtained from three independent experiments. The obtained results are summarized in Table 3.

Table 3

Bacterial strain	MIC [µg/mL = mg/L]							
	1	2	3	4	5	6	7	Reference lipopeptide
(+) *Enterococcus faecalis* ATCC 14506	40	*n.d.*	*n.d.*	30	50	*n.d.*	50	*n.d.*
(–) *Escherichia coli* ST2-8624 O157:H7	40	10	20	20	10	20	*n.d.*	40
(–) *Klebsiella pneumoniae* PCM 1	40	*n.d.*	30	20	30	30	*n.d.*	*n.d.*
(–) *Pseudomonas aeruginosa* PAO1 PCM 499	20	20	20	10	20	30	*n.d.*	50
(–) *Salmonella sv. Typhimurium* TT622	*n.d.*	40	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
(+) *Staphylococcus aureus* ATCC 29213	5	10	10	5	5	20	*n.d.*	*n.d.*
(+) *Staphylococcus epidermidis* ATCC 12228	5	5	10	5	10	20	*n.d.*	*n.d.*
(–) *Yersinia enterocolitica* PCM 2081	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*

MIC = minimum inhibitory concentration

n.d. = not determined, i.e. the MIC was greater than 50 µg/mL

The results of the measurements summarized in Table 3 indicate that all the tested lipopeptides show varying activity against the tested bacterial strains. The lipopeptides according to the invention show activity against both Gram-positive and Gram-negative bacterial strains. The reference lipopeptide, not being the subject matter of the invention, and placed in the table just for comparative purposes, shows little activity against only two of the eight strains analyzed. The obtained results indicate a certain degree of selectivity of the lipopeptides according to the invention for Gram-positive strains of *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, for which definitely the highest activity of the tested lipopeptides was observed, i.e. the lowest values of the minimum inhibitory concentration (MIC). At the same time, the activity of the tested lipopeptides against *Yersinia enterocolitica* PCM 2081 and *Salmonella sv. Typhimurium* TT622 strains is scarce.

Determination of the lipopeptide influence on the dynamics of bacterial growth

The effect of different concentrations of lipopeptides of condensed formulae 1-3 according to the invention and the reference lipopeptide on bacterial growth was checked for one representative strain of Gram-negative bacteria: *Pseudomonas aeruginosa* PAO1 PCM 499 and one representative strain of Gram-positive bacteria: *Staphylococcus aureus* ATCC 29213. The experiment was performed in the same way as the MIC determination, whereas in this case the diluted overnight cultures were pre-incubated for 2 h before the addition of the diluted lipopeptides. Next, the bacterial growth was monitored by measuring the optical density of the culture at 600 nm every 60 min for 6 h. Data were obtained from three independent experiments. The measurement results are shown as graphs in Figures 3-6.

The obtained results show that the tested lipopeptides according to the invention significantly slow down the growth dynamics of both Gram-positive and Gram-negative bacteria. For the bacterial strains *Pseudomonas aeruginosa* PAO1 PCM 499 and *Staphylococcus aureus* ATCC 29213, lipopeptide concentrations of 5.0 mg/l caused almost complete inhibition of bacterial growth. In the case of the reference lipopeptide, its effect on the growth dynamics of both tested bacterial strains was definitely weaker than that observed for the lipopeptides of condensed formulae 1-3 according to the invention.

Measurement of enzymatic degradation of lipopeptides in human blood serum

The measurements of peptide degradation in human blood serum were performed on peptide starting solutions with the addition of Z-Val-OH as an internal standard. To prepare the starting solutions:

- Peptide samples were weighed out;
- An internal standard (Z-Val-OH) solution was prepared at a concentration of 60 µmol/mL, with the H_2O/ACN 30:70 (v/v) system acting as a solvent;
- Next, the starting solutions of each of the tested lipopeptides were prepared at a concentration of 70 µmol/mL by dissolving previously weighed out portions of compounds in the appropriate volume of the internal standard solution.

The measuring systems were mixed at 37°C and 450 rpm using a Eppendorf Thermomixer R. The protocol for measuring lipopeptide degradation in human blood serum included:

1. Addition of 10 µL of peptide starting solution to 690 µL of human blood serum previously incubated at 37°C for 10 minutes (Thermomixer).
2. Vortexing the serum-peptide mixture for 15 seconds.
3. Taking 50 µL sample - after the selected time interval.
4. Adding 200 µL serum deactivation system (ACN:H_2O:FA - 89:10:1 v/v/v).
5. Vortexing for 20 seconds.
6. Cooling at 4°C for 20 minutes.
7. Centrifuging at 4°C (14,000 rpm) for 15 minutes.
8. Taking 150 µL of supernatant.
9. Adding 300 µL of water (milliQ purity) to supernatant taken in 8.
10. Lyophilizing the sample.
11. Dissolving the lyophilisate in 150 µL of ACN:H_2O:FA solution, 20:80:0.1 v/v/v.
12. Sample ultrasonication, and vortexing for 10 seconds.
13. Centrifuging (14,000 rpm) for 4 minutes.
14. Taking 120 µL of supernatant.
15. Performing the HPLC measurement (sample injection: 10 µL).
16. Repeating the process three times for all 8 peptides.
17. In the case of HPLC-MS analyzes, the injection was 2 µL - measurements were performed for degradation times of 48 and 96 hours.

The results of blood serum lipopeptide degradation measurements are shown in Table 4, where the half-life ($t_{1/2}$) of the starting substrate is given.

Table 4

Formula No.	Condensed formula of the lipopeptide	Half-life ($t_{1/2}$), hours
1	**$CH_3(CH_2)_9CH_2CO$-**DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	49
2	**$CH_3(CH_2)_7CH_2CO$**-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	37
3	**$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	35
4	H-Dab(**$CH_3(CH_2)_9CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	56
5	H-Dab(**$CH_3(CH_2)_7CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	41
6	H-Dab(**$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	48
7	H-Dab(**$CH_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	29
Reference lipopeptide	**$CH_3CH_2CH(CH_3)CH_2CH_2CO$**-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2	27

All tested lipopeptides according to the invention undergo enzymatic degradation in human blood serum, nevertheless they are characterized by an increased metabolic stability, and their half-lives are above 27 hours, which is the time observed for the reference lipopeptide. The shortest half-lives have the lipopeptides comprising the shortest fatty acid chains. However, all lipopeptides according to the invention are characterized by the longer half-lives than the reference lipopeptide and therefore have an increased metabolic stability.

Claims

1. Synthetic lipopeptide of the general formula:

X_1-Dab-X_2-X_3-Dab-Dab-X_4-NH_2

wherein

X_1 is a residue selected from FA-DDab-Dab and H-Dab(FA-DDab), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine, or D-tryptophan residue; and

X_4 is a leucine, alanine, isoleucine or valine residue.

2. The lipopeptide according to claim 1, characterized in that X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

3. The lipopeptide according to claim 2, characterized in that it is selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

4. The lipopeptide according to claim 1, characterized in that X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

5. The lipopeptide according to claim 4, characterized in that it is selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2(condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

6. A pharmaceutical composition, characterized in that it comprises a lipopeptide of the general formula:

$$X_1\text{-Dab-}X_2\text{-}X_3\text{-Dab-Dab-}X_4\text{-}NH_2$$

wherein

X_1 is a residue selected from FA-DDab-Dab and H-Dab(FA-DDab), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine or D-tryptophan residue;

X_4 is a leucine, alanine, isoleucine or valine residue.

7. The pharmaceutical composition according to claim 6, characterized in that in the lipopeptide X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

8. The pharmaceutical composition according to claim 7, characterized in that it comprises the lipopeptide selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

9. The pharmaceutical composition according to claim 6, characterized in that in the lipopeptide X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

10. The pharmaceutical composition according to claim 9, characterized in that it comprises the lipopeptide selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

11. The pharmaceutical composition according to any one of claims 6 to 10, characterized in that it comprises 0,1 do 99% by weight of at least one lipopeptide.

12. The pharmaceutical composition according to any one of claims 6 to 11, characterized in that it comprises a pharmaceutically acceptable carrier and/or diluent and/or adjuvant and/or excipient.

13. The pharmaceutical composition according to claim 12, characterized in that it comprises corn starch and/or gelatin and/or lactose and/or sucrose and/or microcrystalline cellulose and/or kaolin and/or mannitol and/or dicalcium phosphate and/or sodium chloride and/or alginic acid.

14. The pharmaceutical composition according to any one of claims 6 to 13, characterized in that it is in a form suitable for oral and/or intravenous and/or intramuscular and/or subcutaneous and/or parenteral administration.

15. The pharmaceutical composition according to any one of claims 6 to 14, characterized in that it is in the form of a tablet and/or a capsule and/or an elixir and/or a suspension and/or a syrup and/or a gel and/or a cream and/or an ointment.

16. The pharmaceutical composition according to any one of claims 6 to 15, characterized in that it further comprises at least one other antimicrobial agent.

17. A cosmetic composition, characterized in that it comprises a lipopeptide of the general formula:

$$X_1\text{-Dab-}X_2\text{-}X_3\text{-Dab-Dab-}X_4\text{-}NH_2$$

wherein

X_1 is selected from FA-DDab-Dab and H-Dab(FA-DDab), wherein

when X_1 is FA-DDab-Dab then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, and 4-methylnonanoic acid,

when X_1 is H-Dab(FA-DDab) then FA is a fatty acid residue selected from the group comprising n-dodecanoic acid, n-decanoic acid, 4-methylnonanoic acid, and 4-methylhexanoic acid;

X_2 is a leucine, alanine, isoleucine or valine residue;

X_3 is a D-phenylalanine, D-tyrosine or D-tryptophan residue;

X_4 is a leucine, alanine, isoleucine or valine residue.

18. The cosmetic composition according to claim 17, characterized in that in the lipopeptide X_1 is FA-DDab-Dab, X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

19. The cosmetic composition according to claim 18, characterized in that it comprises the lipopeptide selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2), and

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3).

20. The cosmetic composition according to claim 17, characterized in that in the lipopeptide X_1 is H-Dab(FA-DDab), X_2 is a leucine residue, X_3 is a D-phenylalanine residue, and X_4 is a leucine residue.

21. The cosmetic composition according to claim 20, characterized in that it comprises the lipopeptide selected from the group consisting of:

H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

22. The cosmetic composition according to any one of claims 16 to 21, characterized in that it comprises 0.1 to 99% by weight of at least one lipopeptide.

23. The cosmetic composition according to any one of claims 16 to 22, characterized in that it comprises a cosmetic carrier.

24. The cosmetic composition according to any one of claims 16 to 23, characterized in that is in the form of a gel or a cream or a suspension.

25. A lipopeptide as defined in any one of claims 1 to 5, for use as a medicament.

26. The lipopeptide as defined in any one of claims 1 to 5 for use in the treatment or prophylaxis of a bacterial infection.

27. The lipopeptide for use according to claim 26, characterized in that the bacterial infection is caused by a Gram-positive bacterium.

28. The lipopeptide for use according to claim 27, characterized in that the bacterial infection is caused by a bacterium of the genus *Enterococcus* or *Staphylococcus*.

29. The lipopeptide for use according to claim 28, characterized in that the bacterial infection is caused by *Staphylococcus aureus* or *Staphylococcus epidermidis*.

30. The lipopeptide for use according to 26, characterized in that the bacterial infection is caused by a Gram-negative bacterium.

31. The lipopeptide for use according to claim 30, characterized in that the bacterial infection is caused by a bacterium of the genus *Klebsiella* or *Pseudomonas*.

32. The lipopeptide for use according to claim 31, characterized in that the bacterial infection is caused by *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*.

33. The lipopeptide for use according to any one of claims 25 to 32, characterized in that the infection is caused by a bacterium resistant to antibiotic or antibiotics.

34. The lipopeptide for use according to any one of claims 25 to 33, characterized in that at least one lipopeptide is used selected from the group consisting of:

$CH_3(CH_2)_9CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 1),

$CH_3(CH_2)_7CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 2),

$CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab-Dab-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 3),

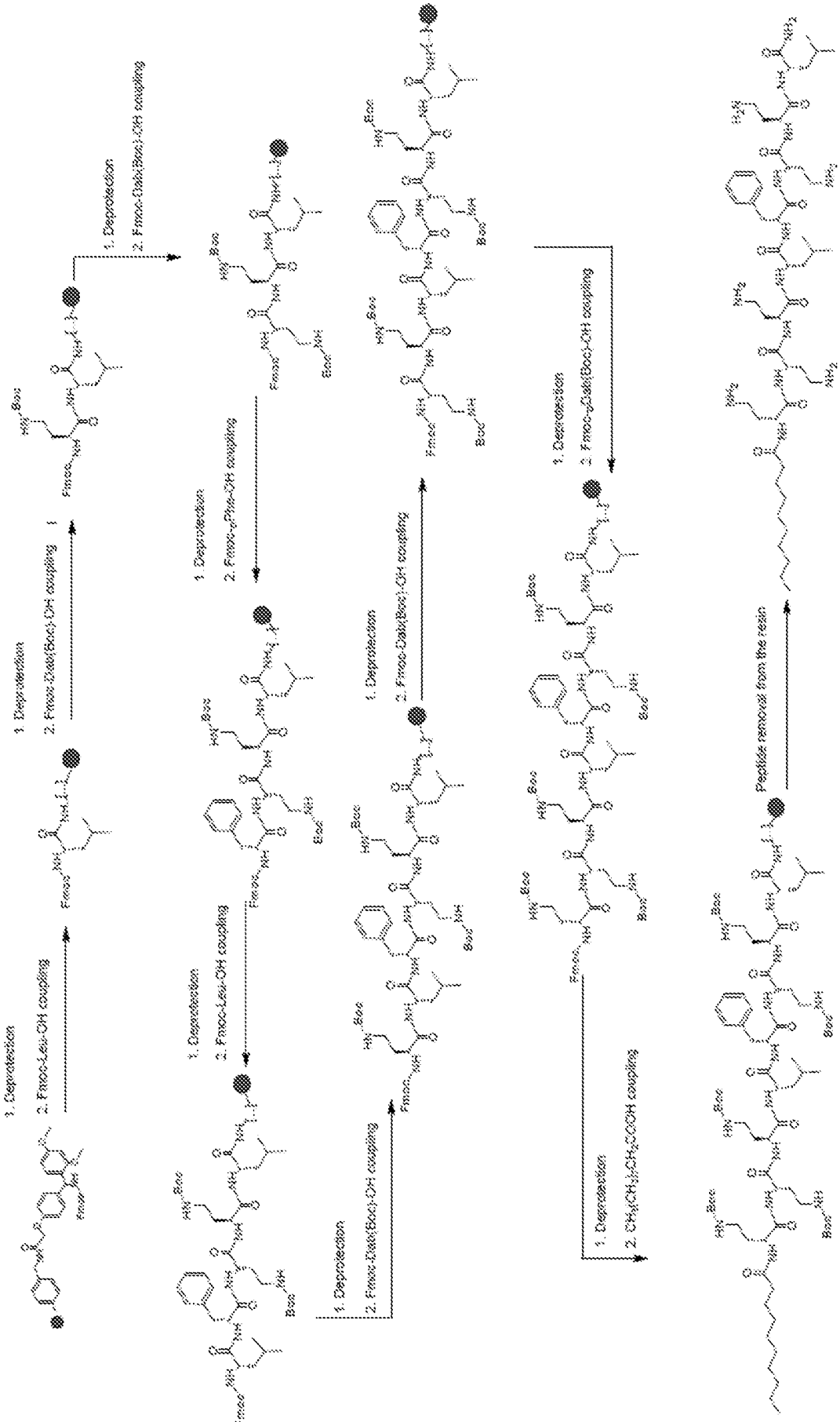
H-Dab($CH_3(CH_2)_9CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 4),

H-Dab($CH_3(CH_2)_7CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 5),

H-Dab($CH_3(CH_2)_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 6), and

H-Dab($CH_3CH_2CH(CH_3)CH_2CH_2CO$-DDab)-Dab-Leu-DPhe-Dab-Dab-Leu-NH_2 (condensed formula 7).

Figure 1


1. Deprotection
2. Fmoc-Leu-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. Fmoc-Leu-OH coupling
1. Deprotection
2. Fmoc-Phe-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. CH3(CH2)6CH2COOH coupling
Peptide removal from the resin

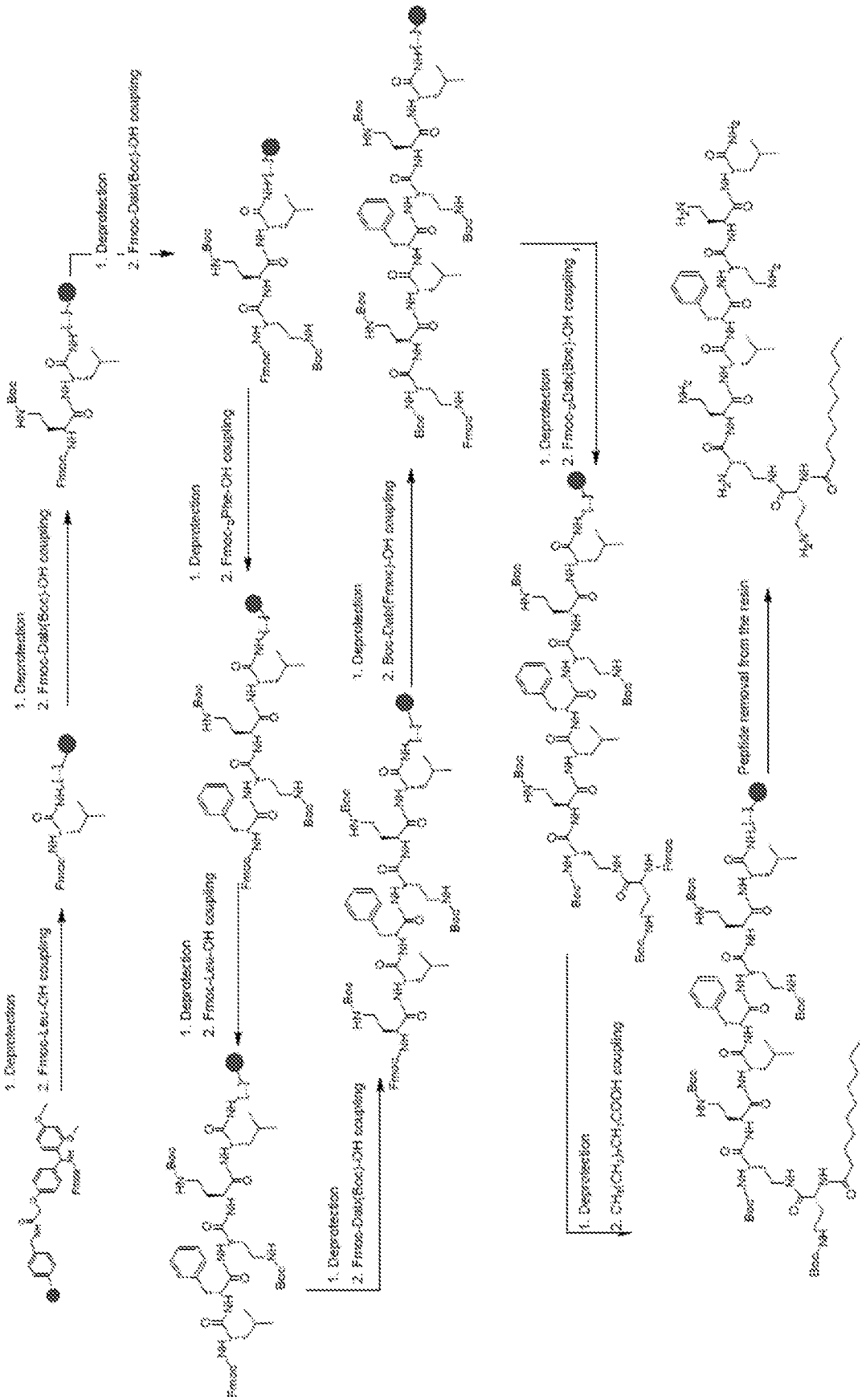
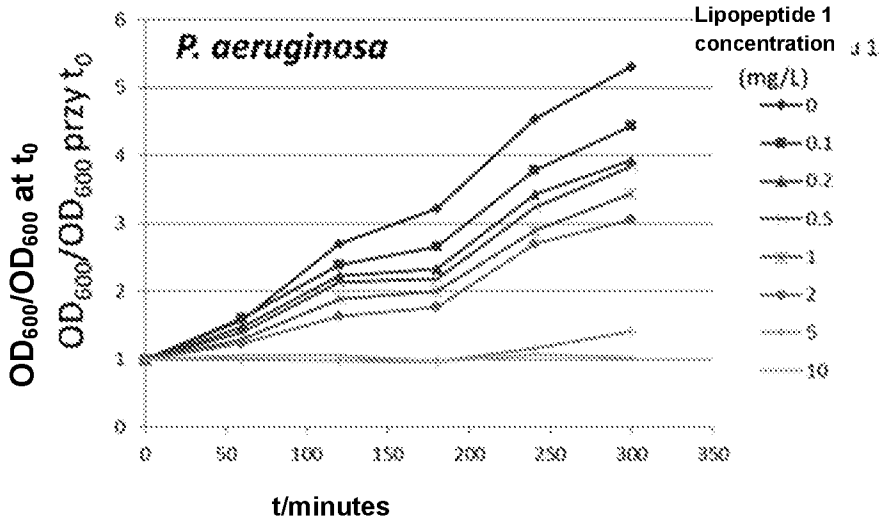

1. Deprotection
2. Fmoc-Leu-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. Fmoc-Leu-OH coupling
1. Deprotection
2. Fmoc-Pro-OH coupling
1. Deprotection
2. Boc-Dab(Fmoc)-OH coupling
1. Deprotection
2. Fmoc-Dab(Boc)-OH coupling
1. Deprotection
2. CH3(CH2)4CH2COOH coupling
Peptide removal from the resin

Figure 2

Figure 3

A


P. aeruginosa
Lipopeptide 1 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

B

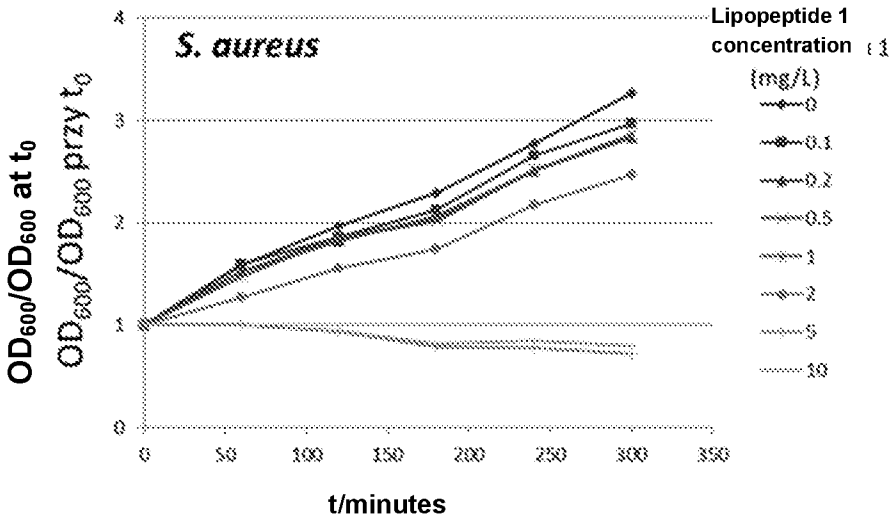
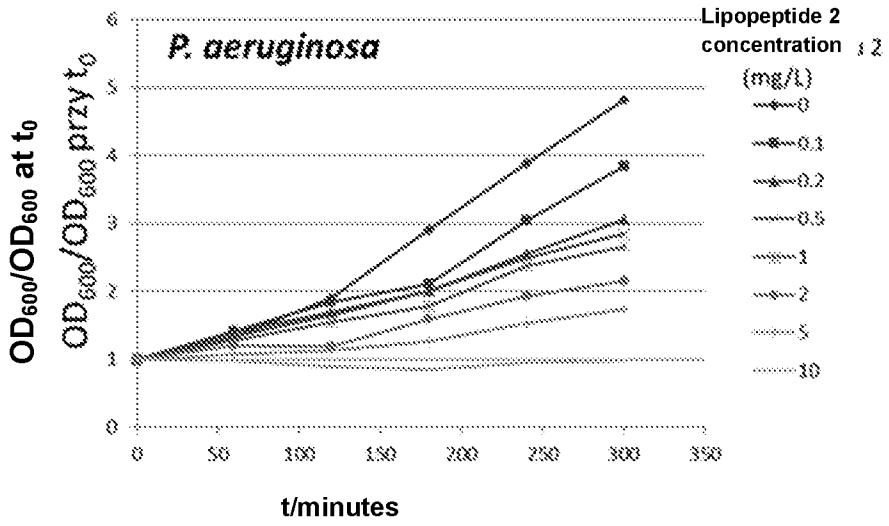

S. aureus
Lipopeptide 1 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

Figure 4

A


P. aeruginosa
Lipopeptide 2 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

B

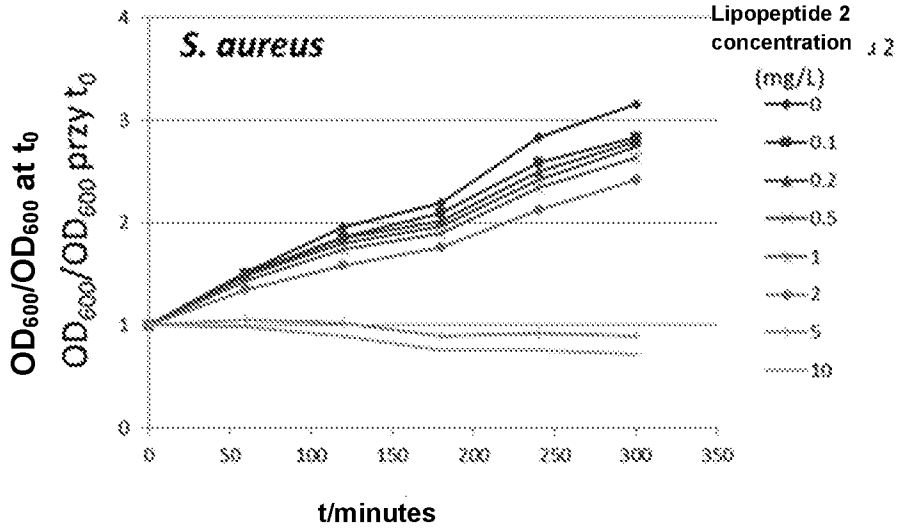
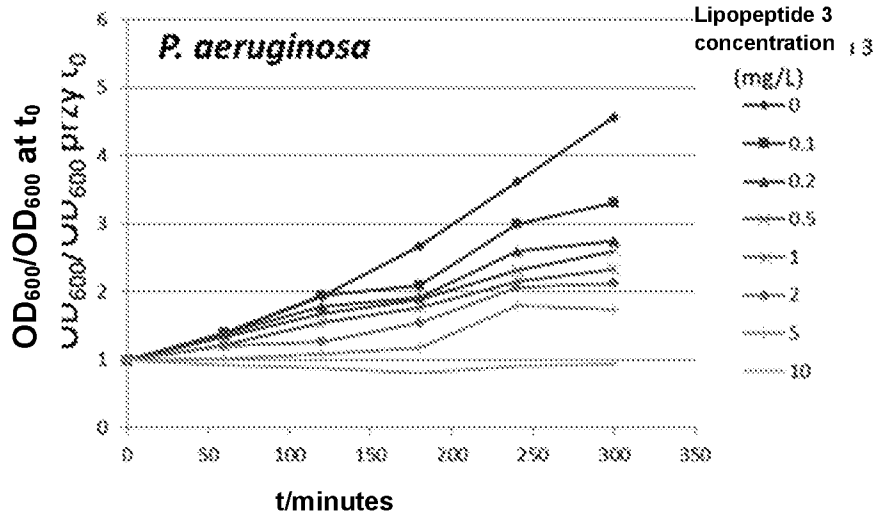

S. aureus
Lipopeptide 2 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

Figure 5

A


P. aeruginosa
Lipopeptide 3 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

B

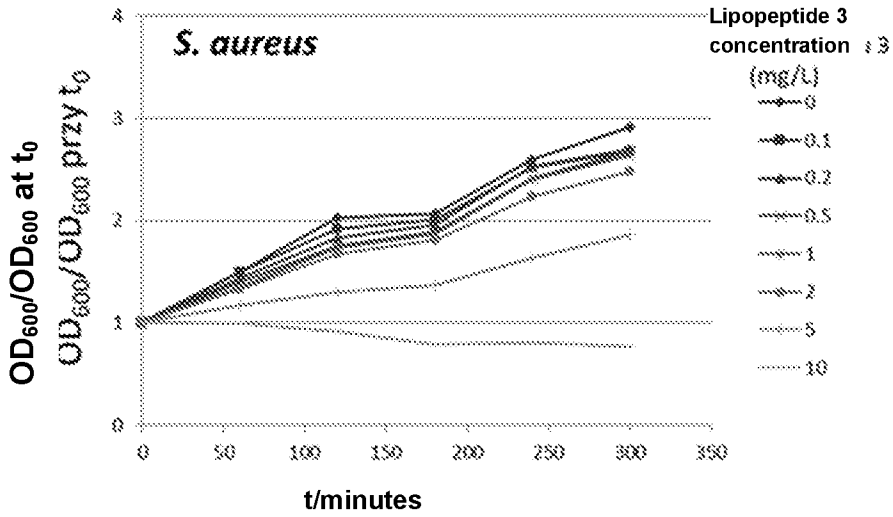
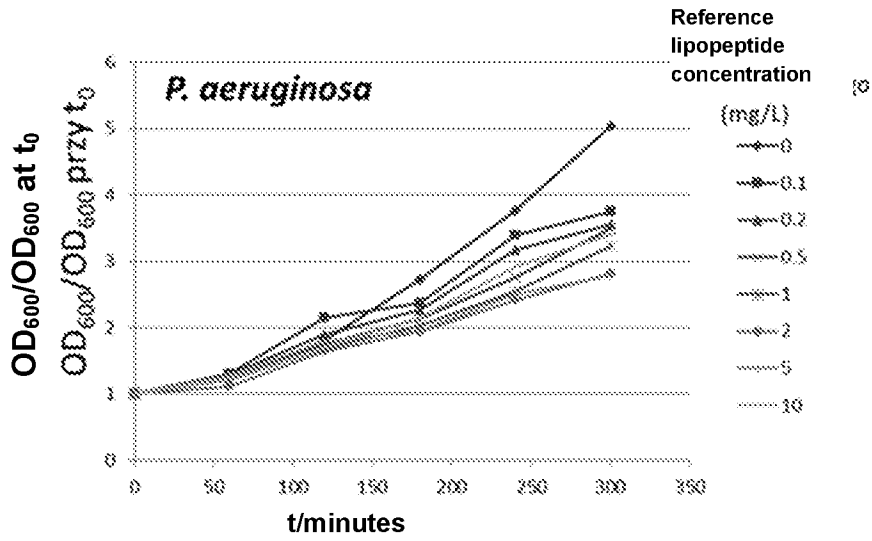
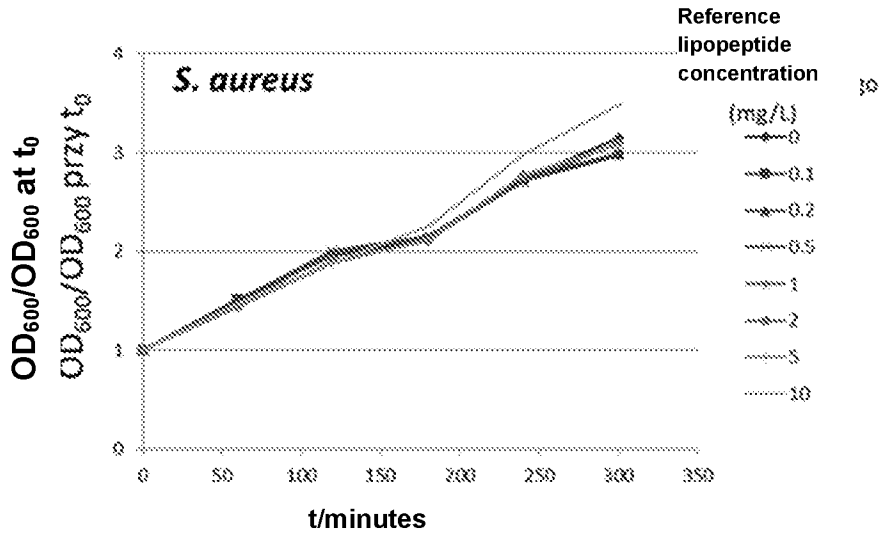

S. aureus
Lipopeptide 3 concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes

Figure 6

A


P. aeruginosa
Reference lipopeptide concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes
0
50
100
150
200
250
300
350
1
2
3
4
5
6

B


S. aureus
Reference lipopeptide concentration (mg/L)
0
0.1
0.2
0.5
1
2
5
10
OD600/OD600 at t0
OD600/OD600 przy t0
t/minutes
0
50
100
150
200
250
300
350
1
2
3
4

5