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(54) Title: MULTIVALENT SYNTHETIC COMPOUNDS AS ANTIBIOTIC TREATMENT

(57) Abstract: The present invention relates to the use of a multivalent synthetic compound, which is significantly more resistant to at least one protease than a standard peptide bond, for preparing a medication, and methods of use for the treatment of diseases, e.g., due to bacterial and/or microbial growth.

MULTIVALENT SYNTHETIC COMPOUNDS AS ANTIBIOTIC TREATMENT

FIELD OF THE INVENTION

The present invention relates to the field of antibiotic compounds. More particularly, it relates to the use of a multivalent synthetic compound, which is significantly more resistant to at least one protease than a standard peptide bond, for preparing a medication, and methods of use for the treatment of diseases, e.g., due to bacterial and/or microbial growth.

BACKGROUND

Binary fission is the form of asexual reproduction and cell division used by all prokaryotes, e.g., bacteria. This process results in the production of a copy of the original cell by division into two parts, which each have the potential to grow to the size of the parent cell and divide again. Within a host, e.g., a human, bacterial colonization (i.e., infection) and growth can lead to a number of detrimental effects. Infection by pathogenic bacteria can cause classic symptoms such as localized redness, heat, swelling and pain. One of the hallmarks of a bacterial infection is local pain, pain that is in a specific part of the body. For example, if a cut occurs and it is infected with bacteria, pain will occur at the site of the infection.

Agents to selectively kill or inhibit bacterial cell growth are in constant demand, especially as the number of drug resistant bacterial strains grows. For example, methicillin-resistant staphylococcus aureus (MRSA), a Gram-positive bacteria, is considered to be a super bug as it is very resistant to current antibiotics. MRSA is quite common in hospitals and today there is a great cause for concern about its spread. It would therefore be extremely useful to have available new, broad-spectrum antibiotic compounds capable of killing and/or inhibiting bacterial cell growth and proliferation.

WO2007/125210 discloses polyvalent or multivalent synthetic peptides made of at least three particular pseudopeptide units grafted to a support. This family of

compounds (which have been named Nucant compounds), has been shown to interact with surface nucleolin RGG domain on cancer cells, to have both anti-proliferative and anti-angiogenic properties on cancer cells and to be useful for the treatment of cancer. They have also been shown to be useful for the treatment of inflammatory diseases. Specific exemplified compounds include complund HB19 and compounds Nucant 1, 2, 3, 6 and 7 (see Figure 1 of this document). There is however no disclosure or suggestion that these compounds are able to bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth. This document is herein incorporated in its entirety.

WO2009/141687 discloses improved Nucant compounds, in which lysine residues in the pseudopeptide units are all in the same L or D configuration. This document notably describes compound Nucant 6L (N6L), which corresponds to compound Nucant 6 as disclosed in WO2007/125210, in which all lysine residues of the pseudopeptide units are in L configuration. This compound showed improve anti-cancer activity compared to complex compound Nucant 6 in which the lysine residue of each pseudopeptide unit may be in L or D configuration. The compounds were also shown to improve wound healing. WO2009/141687 also discloses compounds Nucant 4, 8 and 9 (see claim 10 of this document). However, this document also fails to disclose or suggest that Nucant compounds, either optically pure or not, bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth. This document is herein incorporated in its entirety.

Therefore, while Nucant compounds are known in the art for use as anticancer or inflammatory applications, or for improving wound healing, it has never been disclosed or suggested in the art that these compounds bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth.

Their activity on eukaryotic cells (either cancer cells or inflammatory cells) is linked to their ability to bind the RGG domain of surface nucleolin expressed by these types of eukaryotic cells. However, bacterial cell walls are quite different from eukaryotic cell membranes, and it was thus quite surprising that Nucant compounds could also bind to bacterial cell walls and thereby inhibit bacterial growth.

SUMMARY

The present invention relates to the surprising and unexpected discovery that Nucant compounds or entities of the invention bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth. For example, Gram-positive (Gram+) bacteria are known to have a thick outer peptidoglycan layer, which makes them particularly susceptible to growth inhibition by the compositions of the invention. However, Nucant compounds as described herein also interact with and inhibit proliferation of Gram- bacteria. Thus, although the interaction of Nucant compounds with glycosaminoglycans (GAGs) first appeared to be a possible reason for their interaction with Gram+ bacteria, it is actually possible that their interaction with bacteria, either Gram+ or Gram-, is mediated by another mechanism.

In one aspect, the invention thus provides a Nucant compound or a therapeutic composition comprising a Nucant as described herein, for use as an antibiotic agent, in particular for use in the treatment of bacterial infections. In certain embodiments, the Nucant is a peptide or pseudopeptide, e.g., HB-19 or N6L. In any of the compositions described herein, the therapeutic compositions provided by the invention for use as an antibiotic agent, in particular for use in the treatment of bacterial infections optionally include a pharmaceutically acceptable carrier, excipient or adjuvant.

In another aspect, the invention provides methods for treating and/or preventing the proliferation of a prokaryotic cell, e.g., a bacterial cell, comprising administering a composition comprising an effective amount of a Nucant provided by the invention to a subject, *in vitro*, *in vivo* or *ex vivo*, wherein the composition is effective in inhibiting or preventing the proliferation of the cell. In an exemplary embodiment of this aspect, the subject is a cell or an individual. In certain embodiments, the Nucant is a peptide or pseudopeptide, e.g., HB-19 or N6L.

In another aspect, the invention provides methods for treating and/or preventing a disease or disorder related to the growth and/or proliferation of a bacteria in an individual comprising administering a composition comprising an effective amount of a Nucant as described herein to an individual, wherein the composition is effective in inhibiting or preventing the growth and/or proliferation the bacterial cell. In certain

embodiments, the bacterial cell is a Gram+ bacterial cell. Alternatively, the bacterial cell may be a Gram- bacterial cell.

The present invention also relates to the use of a Nucant compound or of a therapeutic composition comprising a Nucant compound as described herein, for the manufacture of an antibiotic drug intended for the treatment of bacterial infection.

The present invention further provides any invention described herein.

The preceding general areas of utility are given by way of example only and are not intended to be limiting on the scope of the present disclosure and appended claims. Additional objects and advantages associated with the compositions, methods, and processes of the present invention will be appreciated by one of ordinary skill in the art in light of the instant claims, description, and examples. For example, the various aspects and embodiments of the invention may be utilized in numerous combinations, all of which are expressly contemplated by the present description. These additional advantages objects and embodiments are expressly included within the scope of the present invention. The publications and other materials used herein to illuminate the background of the invention, and in particular cases, to provide additional details respecting the practice, are incorporated by reference, and for convenience are listed in the appended bibliography.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating an embodiment of the invention and are not to be construed as limiting the invention. Further objects, features and advantages of the invention will become apparent from the following detailed description taken in conjunction with the accompanying figures showing illustrative embodiments of the invention, in which:

Figure 1. **A.** Structure of compound HB19. **B.** Structure of trivalent compound Nucant 01 with a cyclic hexapeptide consisting of alternating alanine residues (A) of configuration D and lysine residues (K) of configuration L as the support. Three pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the ε amino

group of each of the lysine residues. **C.** Structure of pentavalent compound Nucant 2 (SEQ ID NO :20) with a linear peptide as a support having a helicoidal structure of sequence SEQ ID NO :18 in which 5 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the ε amino group of each of the 5 lysine residues, Ac represents a CH_3–CO– group. **D.** Structure pentavalent compound Nucant 3 (SEQ ID NO :21) with a linear peptide as a support having a helicoidal structure of sequence SEQ ID NO :19 in which 5 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the ε amino group of each of the 5 lysine residues, Ac represents a CH_3–CO– group. **E.** Structure of pentavalent compound Nucant 4 with a support comprising 5 lysine residues linked by amide bonds at the ε amino group of each Lysine residue, to which 5 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the alpha-carbon amino group of each of the 5 lysine residues. **F.** Structure of hexavalent compound Nucant 6 (SEQ ID NO :16) with a linear peptide as a support having a helicoidal structure of sequence SEQ ID NO :15 in which 5 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 6 lysine residues, Ac represents a CH_3–CO– group. **G.** Structure of hexavalent compound Nucant 7 (SEQ ID NO :17) with a linear peptide as a support having a helicoidal structure of sequence SEQ ID NO :13 in which 6 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 6 lysine residues, Ac represents a CH_3–CO– group. **H.** Structure of quadrivalent compound Nucant 8 (SEQ ID NO:23) with a support made of a linear peptide having a helicoidal structure (SEQ ID NO:22, comprising 4 Lys-Aib-Gly (SEQ ID NO: 7) units), to which 4 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the ε amino group of each of the 4 lysine residues. **I.** Structure of octavalent compound Nucant 9 (SEQ ID NO:25) with a support made of a linear peptide having a helicoidal structure (SEQ ID NO:24, comprising 4 Lys-Aib-Gly (SEQ ID NO: 7) units), to which 8 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the ε amino group of each of the 8 lysine residues.

Figure 2. Effect of Nucant 6L (N6L) on the proliferation of several Gram-positive (Gram+) bacteria species. All bacteria were cultivated to mid-logarithmic phase (optical density at 600 nm ranging from 0.2 to 0.5) in culture medium. Bacteria were then washed and diluted 1/100 in culture medium (LB) and incubated at 37°C in 96-

wells culture plate in the presence or absence of various concentrations of N6L ranging from 10 to 1000 µM. After an incubation period of 24 hours, optical density was measured to quantify the amount of bacteria in each well. B. Megaterium : Bacillus megaterium; S. aureus Met R: Meticillin resistant staphylococcus aureus; S. aureus Pen R: Penicillin resistant staphylococcus aureus; S. aureus Pen S: Penicillin sensitive staphylococcus aureus; B. subtilis: Bacillus subtilis ; E. sakazaki : Enterobacter sakazaki.

Figure 3. Effect of Nucant 6L (N6L) on the proliferation of several Gram-negative (Gram-) bacteria species. All bacteria were cultivated to mid-logarithmic phase (optical density at 600 nm ranging from 0.2 to 0.5) in culture medium. Bacteria were then washed and diluted 1/100 in culture medium (LB) and incubated at 37°C in 96-wells culture plate in the presence or absence of various concentrations of N6L ranging from 10 to 1000 µM. After an incubation period of 24 hours, optical density was measured to quantify the amount of bacteria in each well. C. freundii: Citrobacter freundii; Y. enterocolitica: Yersinia enterocolitica; P. aeruginosa: Pseudomonas aeruginosa; A. baumanii: Acinetobacter baumanii; E. coli: Escherichia coli.

Figure 4. Time course of the effect of N6L on the growth of Escherichia Coli. E. coli bacteria were cultivated to mid-logarithmic phase (optical density at 600 nm ranging from 0.2 to 0.5) in culture medium. Bacteria were then washed and diluted 1/100 in culture medium (LB) and incubated at 37°C in 96-wells culture plate in the presence or absence of various concentrations of N6L ranging from 10 to 1000 µM. After an incubation period of 1, 2 or 4 hours, optical density was measured to quantify the amount of bacteria in each well.

DETAILED DESCRIPTION

The following is a detailed description of the invention provided to aid those skilled in the art in practicing the present invention. Those of ordinary skill in the art may make modifications and variations in the embodiments described herein without departing from the spirit or scope of the present invention. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The

terminology used in the description of the invention herein is for describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents, figures and other references mentioned herein are expressly incorporated by reference in their entirety.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges which may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and described the methods and/or materials in connection with which the publications are cited.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references, the entire disclosures of which are incorporated herein by reference, provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, the Harper Collins Dictionary of Biology (1991). As used herein, the following terms may have meanings ascribed to them below, unless specified otherwise. However, it should be understood that other meanings that are known or understood by those having ordinary skill in the art are also possible, and within the scope of the present invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In

addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural references unless the context clearly dictates otherwise. All technical and scientific terms used herein have the same meaning.

The present invention relates to the surprising and unexpected discovery that Nucant peptides bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth. For example, Gram-positive (Gram+) bacteria are known to have a thick outer peptidoglycan layer, which makes them particularly susceptible to growth inhibition by the compositions of the invention. However, the inventors also found that Nucant compounds also interact with and inhibit the growth of Gram- bacteria.

The invention thus first relates to a Nucant compound or a therapeutic composition comprising a Nucant as described herein, for use as an antibiotic agent, in particular for use in the treatment of bacterial infections. It also relates to the use of a Nucant compound or of a therapeutic composition comprising a Nucant compound as described herein, for the manufacture of an antibiotic drug intended for the treatment of bacterial infection.

In order that the present invention may be more readily understood, certain terms are first defined.

As used herein, "derivatives" are compositions formed from the native compounds either directly, by modification, or by partial substitution. As used herein, "analogs" are compositions that have a structure similar to, but not identical to, the native compound.

The term “peptides” can mean, but is in no way limited to, recombinant polypeptide having at least 4 amino acids connected by peptide bonds. Furthermore, peptides of the invention may include amino acid mimentics, and analogs. Recombinant forms of the peptides can be produced according to standard methods and protocols which are well known to those of skill in the art, including for example, expression of recombinant proteins in prokaryotic and/or eukaryotic cells followed by one or more isolation and purification steps, and/or chemically synthesizing peptides or portions thereof using a peptide sythesizer.

The term, "biologically active" or "bioactive" can mean, but is in no way limited to, the ability of an agent, such as the Nucants provided by the invention, to effectuate a physiological change or response. The response may be detected, for example, at the cellular level, for example, as a change in growth and/or viability, gene expression, protein quantity, protein modification, protein activity, or combination thereof; at the tissue level; at the systemic level; or at the organism level. Techniques used to monitor these phenotypic changes include, for example, measuring: the binding of a ligand to its receptor in or on a cell, activation of cell signaling pathways, stimulation or activation of a cellular response, secretion or release of bioactive molecules from the cell, cellular proliferation and/or differentiation, or a combination thereof. In one example, the biological activity of a peptide provided by the invention can be determined by detecting its ability to inhibit the growth and/or proliferation of a cell.

The term "effective amount/dose," "pharmaceutically effective amount/dose," "pharmaceutically effective amount/dose" or "therapeutically effective amount/dose" can mean, but is in no way limited to, that amount/dose of the active pharmaceutical ingredient sufficient to prevent, inhibit the occurrence, ameliorate, delay or treat (alleviate a symptom to some extent, preferably all) the symptoms of a condition, disorder or disease state. The effective amount depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 1000 mg/kg body weight/day of active ingredients is administered dependent upon potency of the agent. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population).

The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby,

reduce side effects. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The term "pharmacological composition," "therapeutic composition," "therapeutic formulation" or "pharmaceutically acceptable formulation" can mean, but is in no way limited to, a composition or formulation that allows for the effective distribution of an agent provided by the invention, which is in a form suitable for administration to the physical location most suitable for their desired activity, e.g., systemic administration.

Non-limiting examples of agents suitable for formulation with the, e.g., Nucants provided by the instant invention include: cinnamoyl, PEG, phospholipids or lipophilic moieties, phosphorothioates, P-glycoprotein inhibitors (such as Pluronic P85) which can enhance entry of drugs into various tissues, for example the CNS (Jolliet-Riant and Tillement, 1999, Fundam. Clin. Pharmacol., 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after implantation (Emerich, DF et al, 1999, Cell Transplant, 8, 47-58) Alkermes, Inc. Cambridge, Mass.; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (Prog Neuropsychopharmacol Biol Psychiatry, 23, 941-949, 1999).

The term "pharmaceutically acceptable" or "pharmacologically acceptable" can mean, but is in no way limited to, entities and compositions that do not produce an

adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate.

The term "pharmaceutically acceptable carrier" or "pharmacologically acceptable carrier" can mean, but is in no way limited to, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The term "systemic administration" refers to a route of administration that is, e.g., enteral or parenteral, and results in the systemic distribution of an agent leading to systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compositions of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome

formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful.

The term “conservative mutations” refers to the substitution, deletion or addition of nucleic acids that alter, add or delete a single amino acid or a small number of amino acids in a coding sequence where the nucleic acid alterations result in the substitution of a chemically similar amino acid. Amino acids that may serve as conservative substitutions for each other include the following: Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E), Asparagine (N), Glutamine (Q); hydrophilic: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I); Hydrophobic: Phenylalanine (F), Tyrosine (Y), Tryptophan (W); Sulfur-containing: Methionine (M), Cysteine (C). In addition, sequences that differ by conservative variations are generally homologous.

The term "binding" can mean, but is in no way limited to, the physical or chemical interaction, direct or indirect, between two molecules (e.g., compounds, amino acids, nucleotides, polypeptides, or nucleic acids). Binding includes covalent, hydrogen bond, ionic, non-ionic, van der Waals, hydrophobic interactions, and the like.

The term "cell" can mean, but is in no way limited to, its usual biological sense, and does not refer to an entire multicellular organism. The cell can, for example, be *in vivo*, *in vitro* or *ex vivo*, e.g., in cell culture, or present in a multicellular organism, including, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

The present invention relates to the surprising and unexpected discovery that Nucants bind to the external cell wall structure of bacteria and, thereby, interact with the bacterial membrance and inhibit bacterial growth.

As used herein, the term “Nucant” can mean but is in no way limited to a nucleolin-binding compound or entity, e.g., a nucleolin antibody or nucleolin antagonist, including, for example, a nucleolin binding peptide or pseudopeptide, derivative or analog thereof (collectively “Nucant peptide”). Therefore, in certain aspects, the present invention relates to multivalent synthetic Nucant compounds and their use. In certain embodiments, the Nucant compounds of provided by the invention

comprise a support on which at least 3 pseudopeptide units are grafted, said compound being of Formula (I) :

$$[(X)_n—Y_1\overset{\Psi}{—}(Z)_i—Y_2\text{-}(X)_m]_k—\text{Support} \quad (I)$$

wherein each X independently represents any amino acid; Y_1 and Y_2 are selected independently from amino acids having a basic side chain;

Z is selected from proline, optionally substituted at γ, β or δ ; a natural or non N–alkylamino acid; a dialkylamino acid; a cyclic dialkylamino acid; pipecolic acid or a derivative thereof;

n and i independently are 0 or 1;

m is an integer between 0 and 3 ;

k is an integer greater than or equal to 3; and

Ψ represents a modified peptide bond which is significantly more resistant to at least one protease than a standard peptide bond.

In the context of the invention, the term "support" refers to any pharmaceutically acceptable molecule, in other words without intrinsic toxicity, on which at least 3 pseudopeptide units of formula (I) can be grafted. An acceptable support therefore has to be of sufficient size to allow at least 3 pseudopeptide units of formula (I) to be grafted on it, preferably 3 to 8 pseudopeptide units of formula (I). In addition, the support must not be immunogenic.

Such a support can be selected from a linear peptide or cyclic peptide, a peptoid (N–substituted glycine oligomer) that is linear or cyclic, a foldamer (oligomer or polymer with a strong tendency to adopt a compact, well–defined and predictable conformation in solution), a linear polymer or a spherical dendromer (macromolecule consisting or polymers which combine according to a tree like process around a multifunctional central core) a sugar or a nanoparticle. Advantageously, said support is selected from a linear or a cyclic peptide or even a linear or cyclic peptoid.

The use of a linear peptide (see structure of Nucant peptides in Figure 1A) allows the support to be synthesised easily and the results obtained by the inventors with compounds HB19 and N6L show that such a support does in effect resolve the technical problems posed by this application. A linear peptide acting as a support in the invention can advantageously contain a proportion of lysine greater than 25%. More

precisely, when a linear peptide is used as a support in the invention, the pseudopeptide units are preferably grafted in position ε of lysine. When a linear peptide is used as the support in the invention, it therefore preferably includes at least as many lysine as the number of pseudopeptide units which are to be grafted on.

For example, a support linear peptide can have a sequence selected from KKKGPKEKGC (SEQ ID NO :1), KKKKGC (SEQ ID NO :2), KKKKGPKKKKGA (SEQ ID NO :3) or KKKGPKEKAhxCONH$_2$ (SEQ ID NO :4), wherein Ahx represents hexanoic amino acid and CONH$_2$ represents the fact that the acid group is replaced by an amide group, AhxCONH$_2$, representing (2S)–2–aminohexanamide, or a linear sequence consisting of 2–4 units (KAKPG, SEQ ID NO :12), namely sequence AcKAKPGKAKPGKAKPGCONH$_2$ (SEQ ID NO :13, where Ac represents an acetyl group CH$_3$–CO–, and CONH$_2$ means that the acid group COOH of glycine is replaced by an amide group CONH$_2$). Advantageously, the support linear peptide can be peptide KKKGPKEKAhxCONH$_2$ (see for example HB19 in Figure 1A, SEQ ID NO :5, which has this linear peptide as support.), or peptide AcKAKPGKAKPGKAKPGCONH$_2$ (SEQ ID NO :13, where Ac represents an acetyl group CH$_3$–CO– and CONH$_2$ means that the acid group COOH of glycine is replaced by an amide group CONH$_2$, for example, Nucant 7 in Figure 1G, SEQ ID NO :17, which has this linear peptide as a support).

Among the linear peptides, some are known to adopt a helicoidal structure. These linear peptides can also be used as supports in the invention. Such linear peptide supports from a helicoidal structure comprised of supports consisting of an integer greater than or equal to 3, namely 3 to 8, repetitions of the peptide units of sequence Aib–Lys–Aib–Gly (SEQ ID NO :6) or Lys–Aib–Gly (SEQ ID NO :7) respectively where Aib represents 2–amino–isobutyric acid. As each of these units consists of a single lysine residue (Lys), as many repetitions of these units are needed as are to be grafted on pseudopeptide units of formula (I).

For example, to obtain a quadrivalent compound with 4 pseudopeptide units of formula (I), the support can be a linear peptide forming a helicoidal structure of formula Ac–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–CONH$_2$ (SEQ ID NO :22), where Ac represents a CH$_3$–CO– group and CONH$_2$ means that the acid group

COOH of glycine is replaced by an amid group $CONH_2$ (see for example Nucant 8 in Figure 1H, SEQ ID NO :23, which has this peptide as a support).

Alternatively, to obtain a pentavalent compound with 5 pseudopeptide units of formula (I), the support can be a linear peptide forming a helicoidal structure of formula Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly (SEQ ID NO :8) or Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly (SEQ ID NO :9). Advantageously, a linear peptide forming a helicoidal structure of formula derived from SEQ ID NO :8 and 9 is used. This formula is selected from Ac–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–$CONH_2$ (SEQ ID NO :18, where Ac represents an acetyl group CH_3–CO– and $CONH_2$ means that the COOH acid group of glycine is replaced by an amide group $CONH_2$, see for example Nucant 2 in Figure 1C, SEQ ID NO :20, which has this peptide as a support) or Ac–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–$CONH_2$ (SEQ ID NO :19, where the Ac group represents an acetyl group CH_3–CO– and $CONH_2$ means that the COOH acid group of glycine is replaced by an amide group $CONH_2$, see for example Nucant 3 in Figure 1D, SEQ ID NO :21, which has this peptide as a support).

Alternatively, to obtain a hexavalent compound with 6 pseudopeptide units of formula (I), the support used can be a linear peptide forming a helicoidal structure of formula Ac–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–Aib–Lys–Aib–Gly–$CONH_2$ (SEQ ID NO :14, where Ac represents a CH_3–CO– group and $CONH_2$ means that the acid group COOH of glycine is replaced by an amide group $CONH_2$) or Ac–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–$CONH_2$ (SEQ ID NO :15, where Ac represents a CH_3–CO– group and $CONH_2$ means that the acid group COOH of glycine is replaced by an amid group $CONH_2$, see for example Nucant 6 in Figure 1F, SEQ ID NO :17, which has this peptide as a support).

Alternatively, to obtain an octavalent compound with 8 pseudopeptide units of formula (I), the support used can be a linear peptide forming a helicoidal structure of formula Ac–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–Lys–Aib–Gly–$CONH_2$ (SEQ ID NO :24), where Ac represents a CH_3–CO– group and $CONH_2$ means that the acid group COOH of glycine

is replaced by an amid group $CONH_2$ (see for example Nucant 9 in Figure 1I, SEQ ID NO :25, which has this peptide as a support).

A cyclic peptide or peptoid can also be advantageously used as support. In particular, this allows the flexibility of the structure to be restricted. A support cyclic peptide or peptoid can be mainly be selected from hexa–, octa–, deca– or dodeca– cyclic peptide, preferably consisting of amino acid residues in the L (levorotatory) and D (dextrorotatory) configuration in alternation (D,L–cyclopeptide) or a chain of N–alkyl Glycine residue (cyclic peptoid). An example of a compound with such a support is a cyclic hexapeptide consisting of alternate alanine (A) residues of configuration D and lysine residues (K) of configuration L with 3 KPR units with a Ψ ($-CH_2N-$) bond between K and P as shown in Figure 1B (compound Nucant 01).

A support made of 5 lysine residues linked by amide bonds at the ε amino group of each Lysine residue may also be used (see compound Nucant 4 in Figure 1E).

Advantageously, the support for a compound of formula (I) according to the invention is a support selected from a cyclic hexapeptide consisting of:

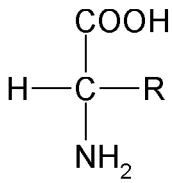
- alternating alkaline (A) residues of configuration D and Lysine (K) residues of configuration L (see compound Nucant 01 in Figure 1B),
- 5 lysine residues linked by amide bonds at the ε amino group of each Lysine residue (see compound Nucant 4 in Figure 1E), and
- a linear peptide of sequence SEQ ID NO :1, SEQ ID NO :2, SEQ ID NO :3, SEQ ID NO :4, SEQ ID NO :8, SEQ ID NO :9, SEQ ID NO :13, SEQ ID NO :14, SEQ ID NO :15, SEQ ID NO :18, SEQ ID NO :19, SEQ ID NO:22 and SEQ ID NO: 24.

In the context of the invention, the term “grafted” for the pseudopeptide units means being bound to the support by means of a covalent bond, either directly or through the intermediate of a spacer compound between the pseudopeptide and support. As a result of this, in one particular embodiment, the pseudopeptide units are grafted directly on the support without a spacer compound between them and the support. In another embodiment, the pseudopeptide units are grafted on the support through the intermediate of a spacer. Examples of acceptable spacers include compounds of the type ethylene glycol, piperazine or an amino acid of the type aminohexanoic acid or beta–alanine.

In the case where the support is a linear or cyclic peptide and where the pseudopeptide units are grafted directly on the peptide, bonding between the peptide and the pseudopeptide units is preferably carried out at the lysine residue of the peptide support, at the amino group in the α or ε position, preferably at the amino group in the ε position (on the side chain) of lysine. Thus, direct grafting of pseudopeptide units on the peptide support is advantageously carried out by means of an amide bond between the acid group COOH of the amino acid in the C–terminal position of the pseudopeptide unit and an amino group of the lysine residue, preferably the amino group in the ε position (on the side chain) of lysine.

In the compounds according to the invention, at least 3 pseudopeptide units are grafted on the support. Advantageously, in the compounds of formula (I), k is between 3 and 8, preferably between 4 and 7, between 4 and 6, between 4 and 5, or between 5 and 6. Even more advantageously, in compounds of formula (I), k is equal to 5 or even better 6.

In the context of the invention, the term "any amino acid" means any natural or synthetic amino acid, possibly modified by the presence of one or more substituents. More precisely the term amino acid means an alpha aminated amino acid with the following general structure:



, where R represents the side chain of the amino acid. In the context of the invention, R therefore represents the side chain of a side or non–side amino acid. The term "natural amino acid" means any amino acid which is found naturally *in vivo* in a living being. Natural amino acids therefore include amino acids coded by mRNA incorporated into proteins during translation but also other amino acids found naturally *in vivo* which are a product or by–product of a metabolic process, such as for example ornithine which is generated by the urea production process by arginase from L–arginine. In the invention, the amino acids used can therefore be natural or not. Namely, natural amino acids generally have the L configuration but also, according to the invention, an amino acid can have the L or D configuration. Moreover,

R is of course not limited to the side chains of natural amino acid but can be freely chosen.

In the pseudopeptide units of compounds of formula (I), Z is either absent (i = 0), or present (i = 1) and is then selected from:

proline, possibly substituted at γ, β or δ by hydroxyl groups, amine, C_1–C_{10} alkyl, C_1–C_{10} alkenyl, C_1–C_{10} alkynyl, C_5–C_{12} aryl, C_5–C_{14} aralkyl, C_5–C_{12} heteroaryl (advantageously a C_5 heteroaryl), these groups themselves possibly being substituted by 1 to 6 substituents selected from a halogen atom, NO_2, OH, C_1–C_4 alkyl, NH_2, CN, trihalomethyl, C_1–C_4 akyloxy, C_1–C_4 dialkylamino, guanadino group, thiol group;

N–alkylamino acid, natural or not;

dialkylamino acid (for example isobutyric amino acid);

cyclic dialkylamino acid; or

pipecolic acid or derivatives thereof.

The term "C_1–C_i alkyl" means a linear or branched saturated hydrocarbon radical of formula $-C_jH_{2j+1}$, where $1 \leq j \leq i$. The C_1–C_{10} alkyl therefore includes C_1 alkyls (methyl), C_2 (ethyl), C_3 (n–propyl, or isopropyl), C_4 (n–butyl, isobutyl, sec–butyl or tert–butyl), C_5 (eg : n–pentyl, neopentyl, isopentyl, tert–pentyl), and C_6 to C_{10} alkyls. The term " C_1–C_{10} alkanyl" means a linear or branched unsaturated hydrocarbon radical consisting of 1 to 10 carbon atoms and including at least one C=C double bond. The term "C_1–C_{10} alkynyl" means a linear or branched unsaturated hydrocarbon radical with 1 to 10 carbon atoms and at least one C≡C triple bond. The term "C_5–C_{12} aryl" means an aromatic polycyclic or monocyclic hydrocarbon radical with 5 – 12 carbon atoms. The term "C_5–C_{14} alalkyl" means a combination of an alkyl and an aryl with a total of 5 to 14 carbon atoms. The term "C_5–C_{12} heteroaryl" means an aryl group where at least one carbon atom on the hydrocarbon chain normally carrying 5 to 12 carbon atoms is substituted by another atom selected from N, O, or S. The term "C_5 heteroaryl" therefore means an aryl group where at least 1 of the 5 carbon atoms on the hydrocarbon chain is substituted by another atom selected from N, O or S. The term"C_1–C_4 akyloxy" means a group of formula —O(O)C–(C_1–C_4 alkyl), —O(O)C–(C_4–C_{12}cycloalkyl), —O(O)C–(C_4–C_{12} aryl), —O(O)C–(C_4–C_{12} arylalkyl, or —O(O)C–(C_4–C_{12} heteroaryl). Advantageously, in the compound of formula (I), such an "C_1–C_4 akyloxy" is selected from the group of formula —O(O)C–(C_1–C_4 alkyl), —O(O)C–(C_4 cycloalkyl), —

O(O)C–(C_4 aryl) —O(O)C–(C_4 arylalkyl), or —O(O)C–(C_4 heteroaryl). The term "C_1–C_4 dialkylamino" means a radical of formula –N(C_1–C_4 alkyl)$_2$ where each alkyl is identical or different.

The term "N–alkylamino acid" means any amino acid in which one of the hydrogen atoms in the amine group is substituted by a C_1–C_{10} alkyl chain or a C_5–C_{14} arylalkyl group, preferably C_5–C_{10}, namely C_{10}, possibly substituted. Examples of N–alkylamino acids include N–methylglycine or sarcosine, N–methylisoleucine acid, N–methylvaline acid, etc... The term "dialkylamino acid" means any amino acid in which 2 hydrogen atoms (on the central carbon or amine groups) are substituted by a C_1–C_{10} alkyl chain or a C_5–C_{14} arylalkyl group, preferably C_5–C_{10}, namely C_{10}, possibly substituted. Examples of dialkylamino acids include 2–amino–isobutyric acid (Aib), aminocyclopropane carboxylic acid, etc.

Advantageously, Z is present and therefore i = 1. Also advantageously, when Z is present (i = 1), then Z is a proline, possibly substituted at γ, β or δ as described previously.

In the pseudopeptide units of the compound of formula (I), Y_1 and Y_2 are selected from amino acids with a basic side chain. The term "amino acid with a basic side chain" means any natural or non–natural amino acid whose side chain R has a pKa value greater than 7 (pKa(R)>7). Thus, any amino acid can be used for Y_1 and Y_2, as long as its side chain has a pKa value greater than 7, preferably greater than 7.5, greater than 8, greater than 8.5 or greater than 9. In particular, among the natural amino acids those whose side chain has a pKa value greater than 7 include lysine (K, pKa(R) ≈ 10.5), arginine (R, pKa(R) ≈ 12.5), ornithine (inferior homologue of lysine, pKa(R) ≈ 10.8), generally considered to be natural basic amino acids. Thus, in an advantageous embodiment, Y_1 and Y_2 are independently selected from arginine (R), lysine (K) and ornithine. Even more advantageously, Y_1 is a lysine (K) and Y_2 is an arginine (R). However, other non–natural amino acids can be used instead as long as the pKa value of their side chain R is greater than 7, preferably greater than 7.5, greater than 8, greater than 8.5, or greater than 9.

In the compounds of the invention, the pseudopeptide unit is the sub–unit of formula (II)

$Y_1 \overset{\Psi}{\text{———}} (Z)i — Y_2$ (II), wherein Y_1 and Y_2 are as defined above. Nevertheless, the presence at one or the other end of this essential sub–unit consisting of several amino acids as defined above is not such that it would prevent binding to bacterial cells walls. This is why the essential sub–unit of formula (II) can include at one and/or the other end 0 to 3 of any amino acids represented in the formula (I) by (X)n and (X)m respectively, where n is equal to 0 or 1 and m is an integer between 0 and 3. Advantageously, the number of the amino acids present at one and/or other end of the essential sub–unit of formula (II) is low, in other words, n is advantageously 0 and m is advantageously an integer between 0 and 2, advantageously 0 or 1, advantageously 0. Thus in an advantageous embodiment, n and m are equal to 0.

In the compounds of the invention, the sub–unit of formula (II) includes a modified peptide bond ψ, significantly more resistant to at least one protease than a standard peptide.

The term "standard peptide bond" means an amide bond of formula (–CONH–) which is normally present between 2 amino acids in a natural protein. Such a bond is sensitive to the action of proteases. The term "modified peptide bonds ψ" means a chemical bond between 2 amino acids of chemical formula distinct from the standard peptide bond of formula (–CONH–). This modified bond ψ is such that it is significantly more resistant to at least one protease than a standard peptide bond of formula (–CONH–). The term "protease", also known as "peptidase" or "proteolytic enzyme", means any enzyme which cleaves the standard peptide bonds in proteins. This process is known as proteolytic cleavage. This involves the use of a water molecule which is what leads to proteases being classified as hydrolases. The proteases namely include proteases known as N–peptidases which carry out the cleavage of the N–terminal end of proteins. These proteases are particularly inconvenient in terms of the *in vivo* stability of peptides without modified peptide bonds. This is why pseudopeptide units of the compounds of formula (I) include a modified bond ψ between Y_1 and Z (if i=1) or Y_1 and Y_2 (if i=0) such that the resistance of the sub–unit of formula (II) is significantly increased, namely to these N–peptidases. The ψ bond should therefore make it possible to significantly increase resistance to at least one N–peptidase. This makes it possible to significantly increase the half–life of compounds of formula (I) *in vivo* and *in vitro*. For

example, HB19, which has a modified bond ψ, has a half–life of more than 24 hours in human serum or foetal calf serum at 37°C whereas the same compound with a standard peptide bond instead of the ψ bond only has a half–life of one hour under these same conditions.

Various chemical bonds likely to significantly increase resistance to at least one protease are known. Thus, in an advantageous embodiment, ψ represents a reduced bond (–CH_2NH–) or (–CH_2N–) in the case where bonding takes place at the level of a secondary amine group as is the case with the bond with proline), a retro–inverso bond (–NHCO–), a methyleneoxy bond (–CH_2–O–), a thiomethylene bond (–CH_2–S–), a carba bond (–CH_2–CH_2–), a ketomethylene bond (–CO–CH_2–), a hydroxyethylene bond (–CHOH–CH_2–), a (–N–N–) bond, an E–alkene bond or a (–CH=CH–) bond. Namely, the inventors have shown that using a reduced bond (–CH_2–NH–) makes it possible to significantly increase resistance to at least one protease. Advantageously, ψ therefore represents a reduced bond (–CH_2NH–).

Although only the ψ between Y_1 and Z (if i=1) or Y_1 and Y_2 (if i=0) is systematically present in compounds of formula (I), it is also possible that other peptide bonds of the pseudopeptide units may be modified as described earlier. In particular, in the context of the invention, the bonds between the amino acids which are not specified can equally well be standard peptide bonds or modified ψ bonds as described earlier. The presence of additional ψ bonds may make it possible to further increase resistance to proteases of compounds of formula (I). Nevertheless, the increase linked to the presence of the first Y bond between Y_1 and Z (if i=1) or Y_1 and Y_2 (if i=0) is already highly significant and the addition of other ψ bonds complicates synthesis of the pseudopeptide units and therefore of compounds of formula (I). The presence of additional ψ bonds is therefore possible but optional.

Examples of compounds that can be used in the invention include in particular the compounds (see Figure 1 and Examples):

HB19 (Figure 1A, SEQ ID NO : 5, a compound which has as a support a linear peptide of SEQ ID NO :4 in which the 5 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 5 lysine residues),

Nucant 01 (Figure 1B), a compound which has a support a cyclic hexapeptide consisting of alternating alanine residues (A) of configuration D and lysine residue (K)

of configuration L, where the 3 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 3 lysine residues (K); see Figure 1B),

Nucant 2 (Figure 1C, SEQ ID NO : 20, a compound which has as a support a linear peptide with a helicoidal structure of sequence SEQ ID NO :18 in which 5 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 5 lysine residues),

Nucant 3 (Figure 1D, SEQ ID NO : 21, a compound which has as a support a linear peptide with a helicoidal structure of sequence SEQ ID NO :19 in which 5 pseudopeptides KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 5 lysine residues),

Nucant 4 (Figure 1E, a compound having a support comprising 5 lysine residues linked by amide bonds at the ε amino group of each Lysine residue, to which 5 pseudopeptide units KψPR (with ψ = CH_2–N) are covalently bound to the alpha-carbon amino group of each of the 5 lysine residues),

Nucant 6 (Figure 1F, SEQ ID NO : 16, a compound which has as a support a linear peptide with a helicoidal structure of sequence SEQ ID NO :15 in which 6 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 6 lysine residues),

Nucant 7 (Figure 1G, SEQ ID NO : 17, a compound which has a support a linear peptide of sequence SEQ ID NO :13 in which 6 pseudopeptide units KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 6 lysine residues),

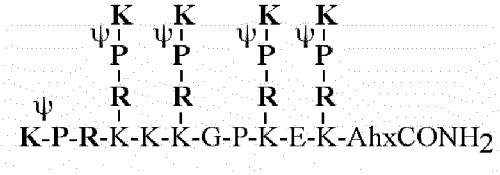
Nucant 8 (Figure 1H, SEQ ID NO:23, a compound which has as a support a linear peptide with a helicoidal structure (SEQ ID NO:22) comprising 4 units of sequence Lys-Aib-Gly (SEQ ID NO:7), to which 4 pseudopeptides KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 4 lysine residues), and

Nucant 9 (Figure 1I, SEQ ID NO:25, a compound which has as a support a linear peptide with a helicoidal structure (SEQ ID NO:24) comprising 8 units of sequence Lys-Aib-Gly (SEQ ID NO:7), to which 8 pseudopeptides KψPR (with ψ= CH_2–N) are covalently bound to the ε amino group of each of the 8 lysine residues).

In one embodiment, the Nucant provided by the invention is a is a pseudopeptide bearing one or more KψPR tripeptides on a template structure, and

includes, for example, compound HB19 and Nucant peptides 1-4 and 6-9 of Figure 1. The tripeptides can be linked to the template through a linker sequence comprising 1 to 6 residues.

In certain embodiments, the Nucant peptide provided by the invention comprises a pentavalent peptide having Formula III (herein, "HB-19"):



(III)

The general formula of the HB-19 pseudopeptide is also referred to herein by the following: 5[Kψ(CH_2N)PR]-TASP. Wherein, Ψ represents a reduced peptide bond (i.e., CH_2N). HB-19 is the prototype of the nucant family. HB-19 is a pentavalent construct of a tripeptide KψPR supported by a polylysin matrix. For a detailed discussion of the HB-19 peptide see: Nisole et al., *AIDS Res. Hum. Retroviruses*, 2000 Feb 10; 16(3):237-49; Nisole et al., *J. Biol. Chem.*, 2002 June 7; 277(23)20877-86; Nisole et al., *J. Biol. Chem.*, 1999 Sept 24; 274(39):27875-84; Krust et al., *PNAS*, 2001 Nov 20; 98(24):14090-095; Alete et al., *FEBS J.*, 2006; 273:4668-81; which are hereby incorporated herein by reference in their entirety for all purposes.

Other exemplary members of the Nucant family differ by the matrix structure. For example, in a preferred embodiment the invention comprises Nucant 6L ("N6L", see Figure 1F)).

The Nucant compounds present in the compositions of the invention may or not be optically pure, which means that the lysine residues in the pseudopeptide units may either be in random L or D configuration (not optically pure), or be all in D configuration or all in L configuration (optically pure). Advantageoulsy, Nucant compounds comprised in compositions of the invention are optically pure, i.e. the lysine residues in the pseudopeptide units are all in D configuration or all in L configuration, preferably all in L configuration. Such optically pure Nucant compounds can be obtained by the method described in WO2009/141687.

Immunochemical assays useful for practicing methods of the invention are well known to those skilled in the art, as described, for example, in Klug, T.L. et al, Cancer Res., 44:1048 (1984), Herlyn, M. et al, J. Clin. Immunol., 2:135 (1982), Metzgar, R.S.

et al, Proc. Natl. Acad. Sci., USA. 81:5242 (1984), Papsidero, L.D. et al, Cancer Res., 44:4653 (1984), Hayes, D.F. et al, J. Clin. Invest., 75:1671 (1985), Killian, C.S. et al, J. Natl. Cancer Inst., 76:179 (1986), Killian, C.S. et al, Cancer Res., 45:886 (1985), Hedin, A. et al, Proc. Natl. Acad. Sci., USA. 80:3470 (1983), Pekary, A.E. et al, Clin. Chem., 30:1213-1215 (1984), Bast, R.C. et al, New England J. Med., 309:883-887 (1983) and Bellet, D.H. et al, Proc. Natl. Acad. Sci., USA, 81:3869-3873 (1984), all of which are specifically incorporated herein by reference.

An advantage is that Nucant peptides demonstrate an excellent safety profile. Additionally, because of its small size the Nucant peptide is not immunogenic and manufacturing and cost goods will be at a reasonable level.

As used herein, the term "Nucant" also encompasses peptides having minor modifications, for example, conservative amino acid modifications, chemical modification to mimic valence properties, and modifications that serve to increase its stability, solubility, biouptake and/or bioavailability; for example, absorption from the gut or penetration through the blood-brain barrier (BBB). For a review of strategies for increasing bioavailability of peptides and peptide drugs in the brain, and of methods for determining the permeability of peptides through the BBB using in vitro and in vivo assays, see Engleton et al., *Peptides* 9:1431-1439 (1997), the teachings of which are incorporated herein by reference. A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that include carbohydrates such as glucose, sucrose or dextrans; antioxidants, such as ascorbic acid or glutathione; chelating agents; and low molecular weight proteins. Additional modifications to a Nucant peptide that can increase its bioavailability include conjugating the peptide to a lipophilic moiety, such as a lipophilic amino acid or compound.

The term "Nucant" is further intended to encompass pseudopeptides bearing one or more KψPR tripeptide or KψPRR tetrapeptide on a template structure, and which have one or several minor modifications to the Nucant sequence. Contemplated modifications include chemical or enzymatic modifications (e.g. acylation, phosphorylation, glycosylation, etc.), and substitutions of one or several amino acids to the Nucant sequence. Those skilled in the art recognize that such modifications can be desirable in order to enhance the bioactivity, bioavailability or stability of the peptide, or to facilitate its synthesis or purification.

Contemplated amino acid substitutions to the Nucants provided by the invention, e.g., Nucant peptides, include include conservative changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of an apolar amino acid with another apolar amino acid; replacement of a charged amino acid with a similarly charged amino acid, etc.). Those skilled in the art also recognize that nonconservative changes (e.g., replacement of an uncharged polar amino acid with an apolar amino acid; replacement of a charged amino acid with an uncharged polar amino acid, etc.) can be made without affecting the function of the Nucant. Furthermore, non-linear variants of the Nucant sequence, including branched sequences and cyclic sequences, and variants that contain one or more D-amino acid residues in place of their L-amino acid counterparts, may be made.

In additional embodiments, the Nucant carrier can be incorporated into liposomes (Gregoriadis, *Liposome Technology*, Vols. I to III, 2nd ed. (CRC Press, Boca Raton Fla. (1993)). Liposomes, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer. In still further embodiments, the Nucant carrier can be prepared as nanoparticles. Adsorbing peptide compounds onto the surface of nanoparticles has proven effective in delivering peptide drugs to the brain (see Kreuter et al., *Brain Res.* 674:171-174 (1995)). Exemplary nanoparticles are colloidal polymer particles of poly-butylcyanoacrylate with HB-19P adsorbed onto the surface and then coated with polysorbate 80.

Those skilled in the art can determine which residues and which regions of the Nucant sequence are likely to be tolerant of modification and still retain the ability to bind bacterial cells walls with clinically relevant affinity. For example, amino acid substitutions, or chemical or enzymatic modifications, at residues that are less well conserved between species are more likely to be tolerated than substitutions at highly conserved residues. Therefore, in certain embodiments the carrier may be modified so that the modified version of the carrier may be more easily conjugated to a diagnostic agent.

The Nucant peptides described herein can be produced using well known recombinant methods or via well known synthetic methods. There are several well known methods for performing peptide synthesis including liquid-phase and solid-phase

synthesis. Detailed discussions of various methods can be found at, for example, Atherton, E.; Sheppard, R.C. (1989). *Solid Phase peptide synthesis: a practical approach*. Oxford, England: IRL Press; Stewart, J.M.; Young, J.D. (1984). *Solid phase peptide synthesis*, 2nd edition, Rockford: Pierce Chemical Company, 91; R. B. Merrifield (1963). "Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide". *J. Am. Chem. Soc.* **85** (14): 2149-2154; L. A. Carpino (1993). "1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive". *J. Am. Chem. Soc.* **115** (10): 4397-4398; which are hereby incorporated by reference in their entirety for all purposes.

When used as antibiotic compounds for use in the treatment of a bacterial infection, the Nucant compounds are preferably in a therapeutic composition comprising an effective amount of a Nucant, e.g., HB-19 or N6L, as described herein.

In any of the compositions described herein, the therapeutic compositions provided by the invention optionally includes a pharmaceutically acceptable carrier, excipient or adjuvant.

In another aspect, the invention provides compositions and methods for treating and/or preventing a disease or disorder related to the detrimental growth and/or proliferation of a bacterial cell.

Nucant compounds are highly advantageous for use as drugs in mammals, and notably in humans.

Indeed, in WO2007/125210, no toxic effect was found by the inventors, whether on cells cultured *in vitro* for several weeks in the presence of Nucants compounds or *in vivo* in mice treated with Nucants compounds. Moreover, purification of proteins bound to multivalent Nucants compounds after *in vivo* administration makes it possible to obtain over 90% surface nucleolin, suggesting great specificity of interaction between multivalent Nucants compounds and nucleolin. This greatly limits the possibility of the occurrence of side effects. The inventors have also shown that although Nucants compounds can be internalised after binding to surface nucleolin, it does not reach the nucleus, an important fact to explain the absence of toxicity for healthy cells.

Nucants compounds and derivatives or analogues thereof are also easily synthesised, even on an industrial scale, under easily controllable health safety conditions.

Finally, their pseudopeptide nature and their high solubility in aqueous media means that they have very good bioavailability *in vivo*. The presence of a modified peptide bond (reduced in the case of Nucants provided by the invention) between the lysine and proline of each KPR unit presented in the case of Nucants compounds confers on them good resistance to proteases *in vivo* and an *in vivo* half life of over 24 hours, contrary to conventional peptides whose *in vivo* half life does not exceed half an hour. In addition, Nucants compounds are totally soluble in aqueous media which makes their administration much easier as no particular pharmaceutical form is required for their circulation and targeting *in vivo*.

Multivalent Nucants compounds therefore present all the necessary characteristics needed to resolve the various technical problems of supplying new antibiotic compounds, which are capable of inhibiting bacterial cells proliferation; which have very few side effects *in vivo* as a result of specificity for only some types of mammal cells (tumour cells and activated endothelial cells but not healthy cells); which have a synthesis process that can be easily adapted to an industrial scale; and which have sufficient bioavailability *in vivo* in order not to require the development of particular pharmaceutical forms.

It has been surprisingly and unexpectedly discovered that the Nucants described herein demonstrates antimicrobial/antibiotic activity, in particular, the multivalent Nucants provided by the invention demonstrate anti-proliferative activity against bacteria, in particular, where the bacteria contain a significant amount of GAGs on their external surface, such as Gram+ bacteria. However, the inventors found that Nucant compounds also interact with and inhibit proliferation of Gram- bacteria. As a result, it is possible that Nucant interactions with bacteria is not mediated through GAGs present at the surface of Gram+ bacteria.

By “antibiotic drug”, it is meant a drug capable of bacteriostatic (inhibition of bacterial proliferation) and/or bactericide (lysis of bacteria) action.

By “bacterial infection”, it is meant any infection by any kind of bacteria.

Most pathogenic bacteria in humans are Gram-positive organisms. Classically, six Gram-positive genera are typically pathogenic in humans. Two of these, *Streptococcus* and *Staphylococcus*, are cocci (sphere-shaped bacteria). The remaining organisms are bacilli (rod-shaped bacteria) and can be subdivided based on their ability to form spores. The non-spore formers are *Corynebacterium* and *Listeria* (a coccobacillus), while *Bacillus* and *Clostridium* produce spores. The spore-forming bacteria can again be divided based on their respiration: *Bacillus* is a facultative anaerobe, while *Clostridium* is an obligate anaerobe.

Accordingly, in another aspect, the invention provides methods for treating and/or preventing a disease or disorder related to the detrimental growth and/or proliferation of a bacterial cell, *in vivo, ex vivo* or *in vitro*. In certain embodiments, the method comprises administering a composition comprising an effective amount of a Nucant provided by the invention to a subject, wherein the composition is effective in inhibiting or preventing the growth and/or proliferation of a bacterial cell. In certain embodiments, the bacterial cell is a Gram+ bacterial cell, e.g., a bacteria of a genera such as *Staphylococcus, Streptococcus, Enterococcus,* (which are cocci) and *Bacillus, Corynebacterium, Nocardia, Clostridium, Actinobacteria,* and *Listeria* (which are rods and can be remembered by the mnemonic obconical), Mollicutes, bacteria-like *Mycoplasma*, Actinobacteria.

Alternatively, Gram- bacteria that are known to be pathogenic to mammals and notably to humans include those of *Citrobacter* (which may be responsible for infections of the urinary tract and infant meningitis and sepsis), *Yersinia* (which may be responsible for plague or yersiniosis), *Pseudomonas* (*P. aeruginosa* being responsible of many nosocomial infections) and *Escherichia* (which may be responsible for gastroenteritis, urinary tract infections, and neonatal meningitis), *Hemophilus* (in particular *Hemophilus influenzae*), *Neisseria* (in particular Neisseria meningitidis and Neisseria gonorrhoeae), Klebsiella (in particular *Klebsiella pneumoniae*), Legionella (in particular *Legionella pneumophila*), Helicobacter (in particular *Helicobacter pylori*), Salmonella (in particular *Salmonella enteritidis* and *Salmonella typhi*) genera.

The Nucant compounds as described herein and compositions comprising them may thus be for use in the treatment of bacterial infections by the above mentioned Gram+ or Gram– bacteria.

As illustrated in the examples, Nucant compounds have been found to display antibiotic activity against the following bacteria Gram+and Gram- species:

- Gram+ species: penicillin resistant/sensitive Staphylococcus aureus species, Bacillus subtilis, Bacillus megaterium;
- Gram- species: Citrobacter freundii, Yersinia enterocolitica, Pseudomonas aeruginosa, Escherichia coli.

The compositions and methods of the invention may thus advantageously be used for the treatment of infections caused by one of the above bacteria species.

Notably, *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. The Nucant compounds or compositions comprising them may thus notably be for use as antibiotic drug in the treatment of Staphylococcus aureus infections.

C. freundii is responsible for a number of significant opportunistic infections. It is known to be the cause of a number of nosocomial infections of the respiratory tract, urinary tract, blood and many other normally sterile sites in patients. *C. freundii* represents about 29% of all opportunistic infections. The Nucant compounds or compositions comprising them may thus notably be for use as antibiotic drug in the treatment of Citrobacter freundii infections.

Yersinia enterocolitica infection causes the disease yersiniosis, which is a zoonotic disease occurring in humans as well as a wide array of animals such as cattle, deer, pigs, and birds. Infection with *Y. enterocolitica* can cause a variety of symptoms depending on the age of the person infected, therefore it's often referred to as "monkey of diseases". Common symptoms in children are fever, abdominal pain, and diarrhea, which is often bloody. Symptoms typically develop 4 to 7 days after exposure and may last 1 to 3 weeks or longer. In older children and adults, right-sided abdominal pain and fever may be the predominant symptoms, and may be confused with appendicitis. In a

small proportion of cases, complications such as skin rash, joint pains, or the spread of bacteria to the bloodstream (bacteremia) can occur. The Nucant compounds or compositions comprising them may thus notably be for use as antibiotic drug in the treatment of Yersinia enterocolitica infections.

An opportunistic, nosocomial pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. It is the most common cause of infections of burn injuries and of the external ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters). *Pseudomonas* can, in rare circumstances, cause community-acquired pneumonias, as well as ventilator-associated pneumonias, being one of the most common agents isolated in several studies. The Nucant compounds or compositions comprising them may thus notably be for use as antibiotic drug in the treatment of Pseudomonas aeruginosa infections.

While most *E. coli* strains are harmless, some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. In particular, virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia. The Nucant compounds or compositions comprising them may thus notably be for use as antibiotic drug in the treatment of Escherichia coli infections.

In any aspect of the invention, the therapeutic composition of the invention can be in any pharmaceutically acceptable form and administered by any pharmaceutically acceptable route, for example, the therapeutic composisition can be administered as an oral dosage, either single daily dose or unitary dosage form, for the treatment of a muscle disorder or conditions, e.g., diabetes. Such pharmaceutically acceptable carriers and excipients and methods of administration will be readily apparent to those of skill in the art, and include compositions and methods as described in the USP-NF 2008 (United States Pharmacopeia/National Formulary), which is incorporated herein by reference in its entirety. In certain aspects, the invention provides pharmaceutically acceptable formulations of the compounds described. These formulations include salts

of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

The active compounds will generally be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intraarthricular, intrathecal, intramuscular, sub-cutaneous, intra-lesional, or even intraperitoneal routes. The preparation of an aqueous composition that contains a cancer marker antibody, conjugate, inhibitor or other agent as an active component or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectibles, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, e.g., systemic administration, into a cell or subject, preferably a human. By "systemic administration" is meant in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

Preparations for administration of the therapeutic of the invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles including fluid and nutrient replenishers, electrolyte replenishers, and the like. Preservatives and other additives may be added such as, for example, antimicrobial agents, anti-oxidants, chelating agents and inert gases and the like.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (i.e., topical), transmucosal, intraperitoneal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful.

By pharmaceutically acceptable formulation is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: PEG conjugated nucleic acids, phospholipid conjugated nucleic acids, nucleic acids containing lipophilic moieties, phosphorothioates, P-glycoprotein inhibitors (such as Pluronic P85) which can enhance entry of drugs into various tissues, for example the CNS (Jolliet-Riant and Tillement, 1999, Fundam. Clin. Pharmacol., 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after implantation (Emerich, DF et al, 1999,

Cell Transplant, 8, 47-58) Alkermes, Inc. Cambridge, Mass.; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (Prog Neuropsychopharmacol Biol Psychiatry, 23, 941-949, 1999). Other non-limiting examples of delivery strategies, including CNS delivery of nucleic acid molecules include material described in Boado et al., 1998, J. Pharm. Sci., 87, 1308-1315; Tyler et al, 1999, FEBS Lett., 421, 280-284; Pardridge et al., 1995, PNAS USA., 92, 5592-5596; Boado, 1995, Adv. Drug Delivery Rev., 15, 73-107; Aldrian-Herrada et al., 1998, Nucleic Acids Res., 26, 4910-4916; and Tyler et al., 1999, PNAS USA., 96, 7053-7058. All these references are hereby incorporated herein by reference.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). Nucleic acid molecules of the invention can also comprise covalently attached PEG molecules of various molecular weights. These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. Chem. Rev. 1995, 95, 2601-2627; Ishiwata et al., Chem. Pharm. Bull. 1995, 43, 1005-1011). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen. All of these references are incorporated by reference herein.

The compounds, used in the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by

reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a

circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The formulations can be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups, or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such

liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

Excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such

as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present. Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable

solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethan- e, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain

formulatory agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor™. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

For administration to non-human animals, the therapeutic compositions of the invention can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water. The composition can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

Some of the advantages of this invention are illustrated in the figures and examples given below, which are non-limiting on the scope of the invention.

Additional, advantages and modifications will be apparent to those of skill in the art and include the application of routine techniques in the art and, as such, are expressly contemplated as being within the scope of the present invention.

EXAMPLES

It should be appreciated that the exemplary embodiments of the present invention should not be construed to be limited to the examples that are now described; rather, the exemplary embodiments of the present invention should be construed to include any and all applications provided herein and all variations within the skill of the ordinary artisan.

The contents of all references, patents, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

Example 1. Effect of compounds Nucant 6L (N6L) on the proliferation of various Gram-positive (Gram+) and Gram-negative (Gram-) bacteria species.

Methods :

All bacteria were cultivated to mid-logarithmic phase (optical density at 600 nm ranging from 0.2 to 0.5) in culture medium. Bacteria were then washed and diluted 1/100 in culture medium (LB) and incubated at 37°C in 96-wells culture plate in the presence or absence of various concentrations of N6L ranging from 10 to 1000 µM. After an incubation period of 24 hours, optical density was measured to quantify the amount of bacteria in each well.

Results:

Results obtained with various species of Gram+ bacteria are displayed in **Figure 2**, while results obtained with various species of Gram- bacteria are displayed in **Figure 3**.

The IC_{50} (half maximum inhibitory concentration in µM) of N6L on several of the tested bacteria species are further indicated in **Table 1** below:

Bacteria	Type	IC_{50} (μM)(*)
Citrobacter Freundii	gram-negative	80
Yersinia Enterocolitica	gram -negative	45
Pseudomonas Aeruginosa	gram -negative	30
Acinetobacter Baumanii	gram -negative	300
Escherichia Coli	gram-negative	20
Staphilococcus Aureus (meticillin resistant)	gram-positive	180
Staphilococcus Aureus (penicillin resistant)	gram-positive	70
Staphilococcus Aureus (penicillin sensitive)	gram-positive	180
Bacillus Subtilis	gram-positive	< 50
Bacillus Megaterium	gram-positive	<20

(*) half maximum inhibitory concentration

The obtained results show that N6L inhibit proliferation of most tested bacteria species, no matter if they are Gram+ or Gram- bacteria.

In particular, N6L shows significant antibiotic activity against the following known human pathogenic bacteria: *Citrobacter Freundii*, *Yersinia Enterocolitica*, *Pseudomonas Aeruginosa*, *Acinetobacter Baumanii*, *Escherichia Coli*, *Staphilococcus Aureus penicillin resistant*, *Staphilococcus Aureus penicillin sensitive*, *Staphilococcus Aureus meticillin resistant*, *Bacillus Subtilis* and *Bacillus Megaterium*.

Example 2. Time course of the effect of N6L on the growth of Escherichia Coli

The rapidity of the effect of N6L on the inhibition of E. coli proliferation was also analyzed.

Methods:

E. coli bacteria were cultivated to mid-logarithmic phase (optical density at 600 nm ranging from 0.2 to 0.5) in culture medium. Bacteria were then washed and diluted 1/100 in culture medium (LB) and incubated at 37°C in 96-wells culture plate in the presence or absence of various concentrations of N6L ranging from 10 to 1000 µM. After an incubation period of 1, 2 or 4 hours, optical density was measured to quantify the amount of bacteria in each well.

Results:

Results are displayed in **Figure 4**, and show that the inhibitory effect of N6L on E. Coli proliferation is very rapid and time dependent. Incubation of 100 µM N6L concentration showed a significant inhibitory effect of more than 30 % after only 2 hours and more that 70 % after 4 hours.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be limiting to the invention. Various modifications or changes in light thereof will be suggested to persons skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. For example, the relative quantities of the ingredients may be varied to optimize the desired effects, additional ingredients may be added, and/or similar ingredients may be substituted for one or more of the ingredients described. Additional advantageous features and functionalities associated with the systems, methods, and processes of the present invention will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the

invention described herein. Such equivalents are intended to be encompassed by the following claims.

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CLAIMS

1. A polyvalent synthetic compound comprising a support on which at least 3 pseudopeptide units are grafted, said compound being of formula (I) :

$$[(X)_n—Y_1\overset{\Psi}{—}(Z)_i—Y_2\text{-}(X)_m]_k—\text{Support} \quad (I)$$

,

wherein

each X independently represents any amino acid;

Y_1 and Y_2 are selected independently from amino acids having a basic side chain;

Z is selected from: proline, possibly substituted at γ, β or δ by hydroxyl, amine, C_1–C_{10} alkyl, C_1–C_{10} alkenyl, C_1–C_{10} alkynyl, C_5–C_{12} aryl, C_5–C_{14} aralkyl, C_5–C_{12} heteroaryl groups, these groups being themselves possibly substituted by 1 to 6 substituents selected from a halogen atom, NO_2, OH, C_1–C_4 alkyl, NH_2, CN, trihalomethyl, C_1–C_4 akyloxy, C_1–C_4 dialkylamino, guanidino group, thiol group; N–alkylamino acid, natural or not; dialkylamino acid; cyclic dialkylamino acid ; or pipecolic acid or derivatives thereof;

n and i are independently 0 or 1;

m is an integer between 0 and 3;

k is an integer greater than or equal to 3; and

Ψ represents a modified peptide bond significantly more resistant to at least one protease than a standard peptide bond;

for use as an antibiotic drug.

2. The polyvalent synthetic compound of claim 1, wherein said support is selected from a linear peptide or a cyclic peptide, a linear or cyclic peptoid, a foldamer, a linear polymer or a spherical dendromer, a sugar or a nanoparticle, for use as an antibiotic drug.

3. The polyvalent synthetic compound of claim 2, wherein said support is selected from:

a) a cyclic hexapeptide consisting of alternating alkaline (A) residues of configuration D and Lysine (K) residues of configuration L
b) 5 lysine residues linked by amide bonds at the ε amino group of each Lysine residue 1, and
c) a linear peptide of sequence SEQ ID NO :1, SEQ ID NO :2, SEQ ID NO :3, SEQ ID NO :4, SEQ ID NO :8, SEQ ID NO :9, SEQ ID NO :13, SEQ ID NO :14, SEQ ID NO :15, SEQ ID NO :18, SEQ ID NO :19, SEQ ID NO:22 and SEQ ID NO:24,

for use as an antibiotic drug.

4. The polyvalent synthetic compound of anyone of claims 1 to 3, wherein said pseudopeptide units are grafted directly on said support, for use as an antibiotic drug.

5. The polyvalent synthetic compound of anyone of claims 1 to 4, wherein k is between 3 and 8, preferably between 5 and 6, for use as an antibiotic drug.

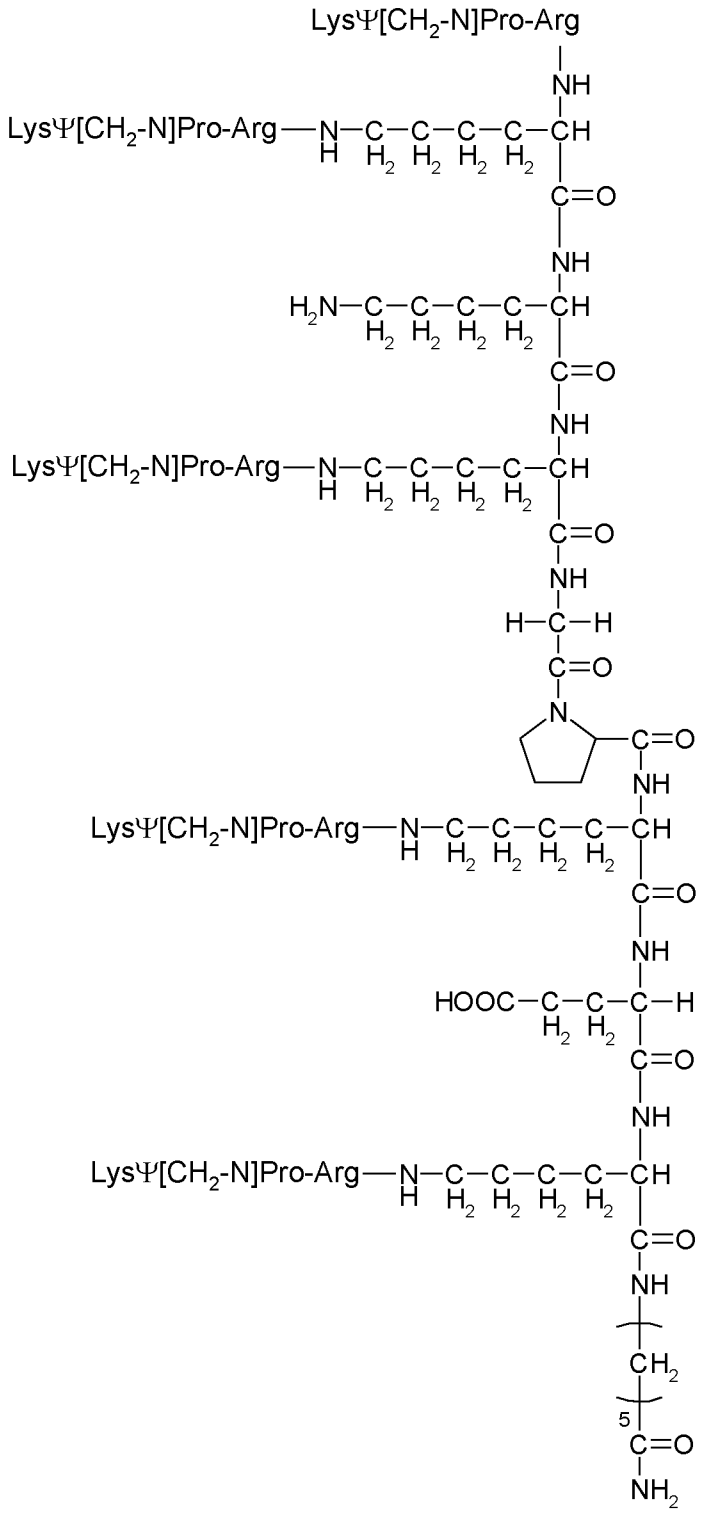
6. The polyvalent synthetic compound of any one of claims 1 to 5, wherein i equals 1 and Z is proline (P), for use as an antibiotic drug.

7. The polyvalent synthetic compound of any one of claims 1 to 6, wherein Y1 and Y2 are independently selected from arginine (R) and lysine (K), preferably Y1 is lysine (K) and Y2 is arginine (R), for use as an antibiotic drug.

8. The polyvalent synthetic compound of any one of claims 1 to 7, wherein n and m are equal to 0, for use as an antibiotic drug.

9. The polyvalent synthetic compound of any one of claims 1 to 8, wherein Ψ represents a reduced bond (–CH2NH–), a retro–inverso bond (–NHCO–), a methyleneoxy bond (–CH2–O–), a thiomethylene bond (–CH2–S–), a carba bond (–CH2CH2–), a ketomethylene bond (–CO–CH2–), a hydroxyethylene bond (–CHOH–CH2–), a (–N–N–) bond, an E–alkene bond or a (–CH=CH–) bond, for use as an antibiotic drug.

10. The polyvalent synthetic compound of claim 1, wherein the compound is selected from compounds of formula:



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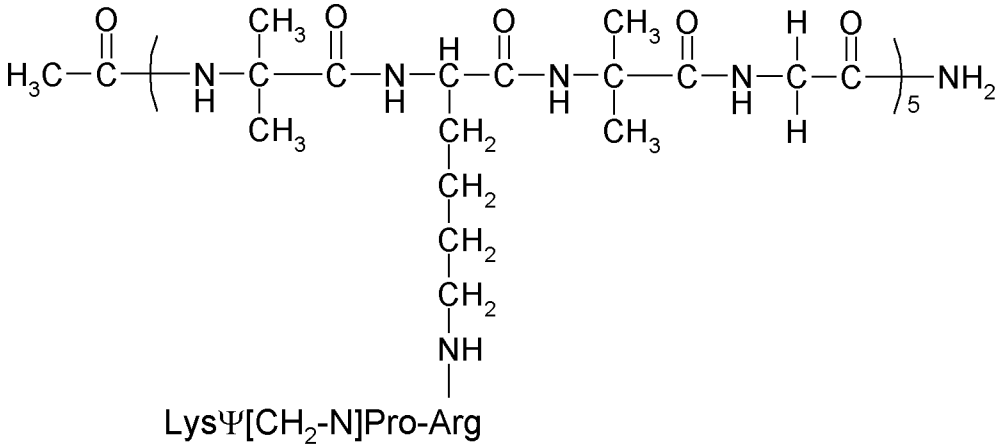
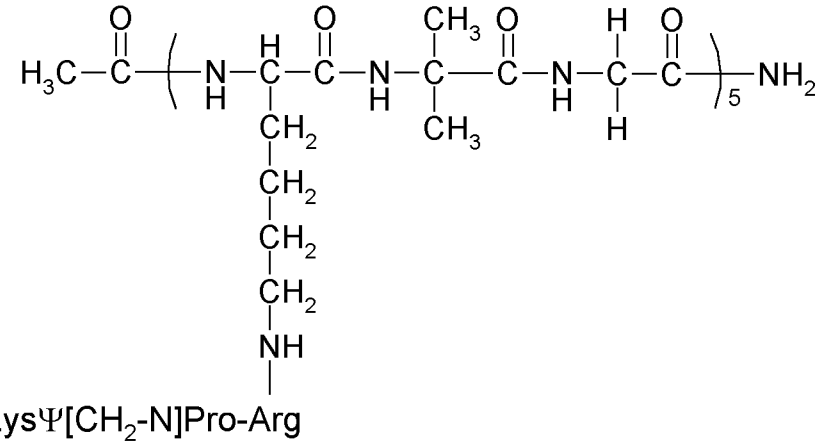


Nucant 2



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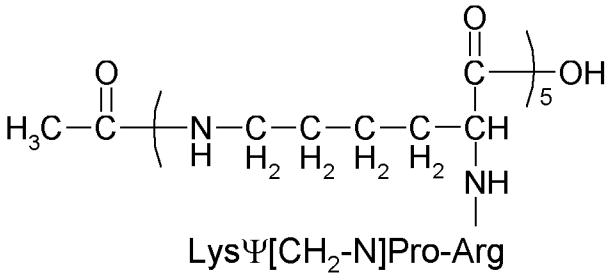

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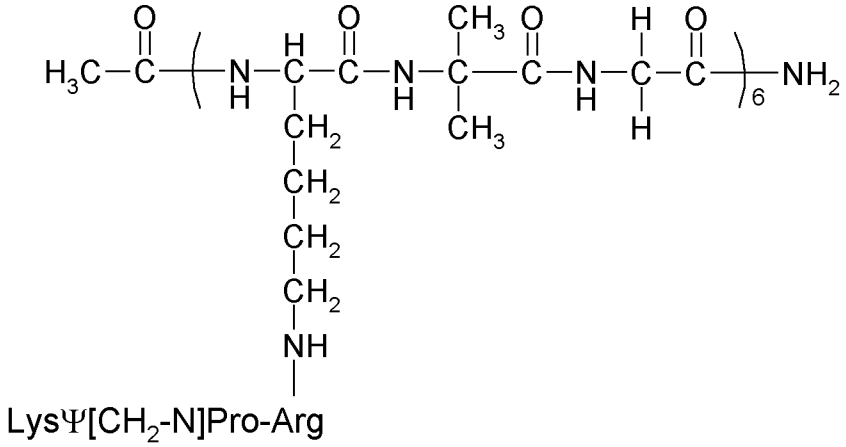
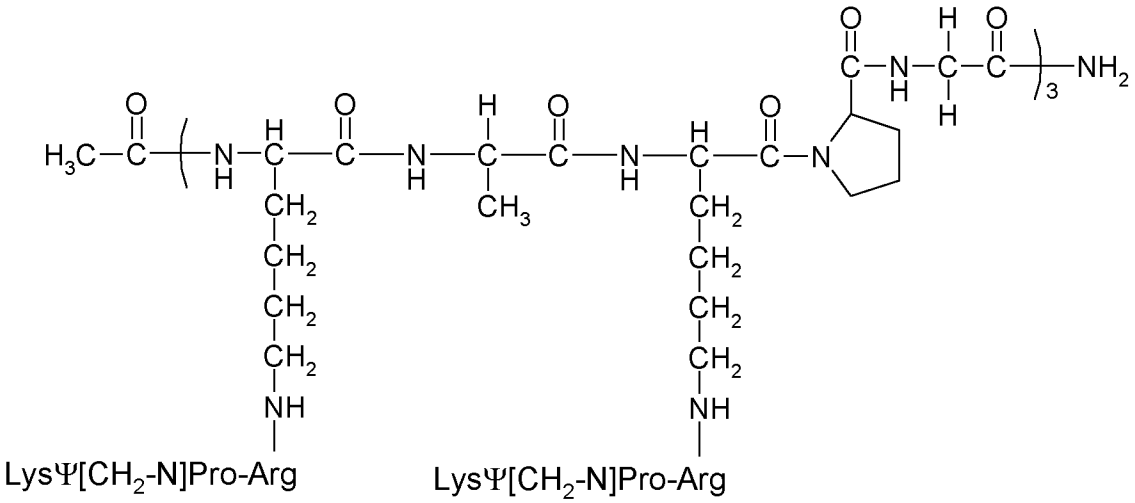


Nucant 6

and



Nucant 7

, for use as an antibiotic drug.

11. The polyvalent synthetic compound of any one of claims 1 to 10, wherein the lysine residues in the pseudopeptide units are all in D configuration or all in L configuration, for use as an antibiotic drug.

12. The polyvalent synthetic compound of any one of claims 1 to 10, for use as an antibiotic drug in the treatment and/or prevention of bacterial infection.

13. The polyvalent synthetic compound of any one of claims 1 to 11, for use as an antibiotic drug in the treatment and/or prevention of bacterial infection, wherein said bacterial infection is a Gram-positive bacterial infection, preferably caused by bacteria selected from *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Corynebacterium*, *Nocardia*, *Clostridium*, *Actinobacteria*, and *Listeria* genera.

14. The polyvalent synthetic compound of any one of claims 1 to 11, for use as an antibiotic drug in the treatment and/or prevention of bacterial infection, wherein said bacterial infection is a Gram-negative bacterial infection, preferably caused by bacteria selected from *Citrobacter*, *Yersinia*, *Pseudomonas*, *Escherichia*, *Hemophilus*, *Neisseria*, *Klebsiella*, *Legionella*, *Helicobacter*, and *Salmonella* genera.

15. A method of treating and/or preventing growth and/or proliferation of a bacterial cell comprising administering to the bacterial cell a composition comprising a carrier and an effective amount of a polyvalent compound having formula (I) :

$$[(X)_n—Y_1\overset{\Psi}{—}(Z)_i—Y_2\text{-}(X)_m]_k—\text{Support} \quad (I)$$

,

where each X independently represents any amino acid;

Y_1 and Y_2 are selected independently from amino acids having a basic side chain;

Z is selected from: proline, possibly substituted at γ, β or δ by hydroxyl, amine, C_1–C_{10} alkyl, C_1–C_{10} alkenyl, C_1–C_{10} alkynyl, C_5–C_{12} aryl, C_5–C_{14} aralkyl, C_5–C_{12} heteroaryl groups, these groups being themselves possibly substituted by 1 to 6 substituents selected from a halogen atom, NO_2, OH, C_1–C_4 alkyl, NH_2, CN, trihalomethyl, C_1–C_4 akyloxy, C_1–C_4 dialkylamino, guanidino group, thiol group; N–alkylamino acid, natural or not; dialkylamino acid; cyclic dialkylamino acid ; or pipecolic acid or derivatives thereof;

n and i are independently 0 or 1;

m is an integer between 0 and 3;

k is an integer greater than or equal to 3; and

Ψ represents a modified peptide bond significantly more resistant to at least one protease than a standard peptide bond, wherein the composition is effective for treating and/or preventing growth and/or proliferation of the bacterial cell.

16. A method of treating and/or preventing a bacterial infection comprising administering to an individual a composition comprising a pharmaceutically acceptable carrier and an effective amount of a polyvalent compound having formula (I) :

$$[(X)_n—Y_1\overset{\Psi}{—}(Z)_i—Y_2\text{-}(X)_m]_k—\text{Support} \quad (I)$$

,

where each X independently represents any amino acid;

Y_1 and Y_2 are selected independently from amino acids having a basic side chain;

Z is selected from: proline, possibly substituted at γ, β or δ by hydroxyl, amine, C_1–C_{10} alkyl, C_1–C_{10} alkenyl, C_1–C_{10} alkynyl, C_5–C_{12} aryl, C_5–C_{14} aralkyl, C_5–C_{12} heteroaryl groups, these groups being themselves possibly substituted by 1 to 6 substituents selected from a halogen atom, NO_2, OH, C_1–C_4 alkyl, NH_2, CN, trihalomethyl, C_1–C_4 akyloxy, C_1–C_4 dialkylamino, guanidino group, thiol group; N–alkylamino acid, natural or not; dialkylamino acid; cyclic dialkylamino acid ; or pipecolic acid or derivatives thereof;

n and i are independently 0 or 1;

m is an integer between 0 and 3;

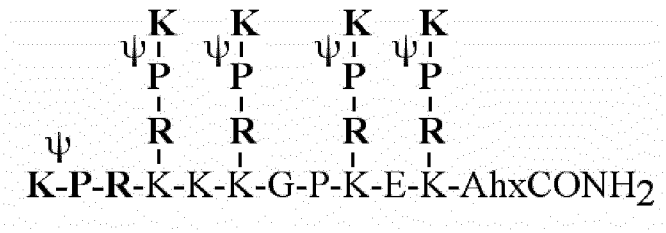
k is an integer greater than or equal to 3; and

Ψ represents a modified peptide bond significantly more resistant to at least one protease than a standard peptide bond, wherein the composition is effective for treating and/or preventing the bacterial infection.

17. The method of claim 15 or claim 16, wherein the bacterial cell is a Gram-positive bacterial cell.

18. The method of claim 17, wherein the polyvalent synthetic compound is SEQ ID NO: 16.

A



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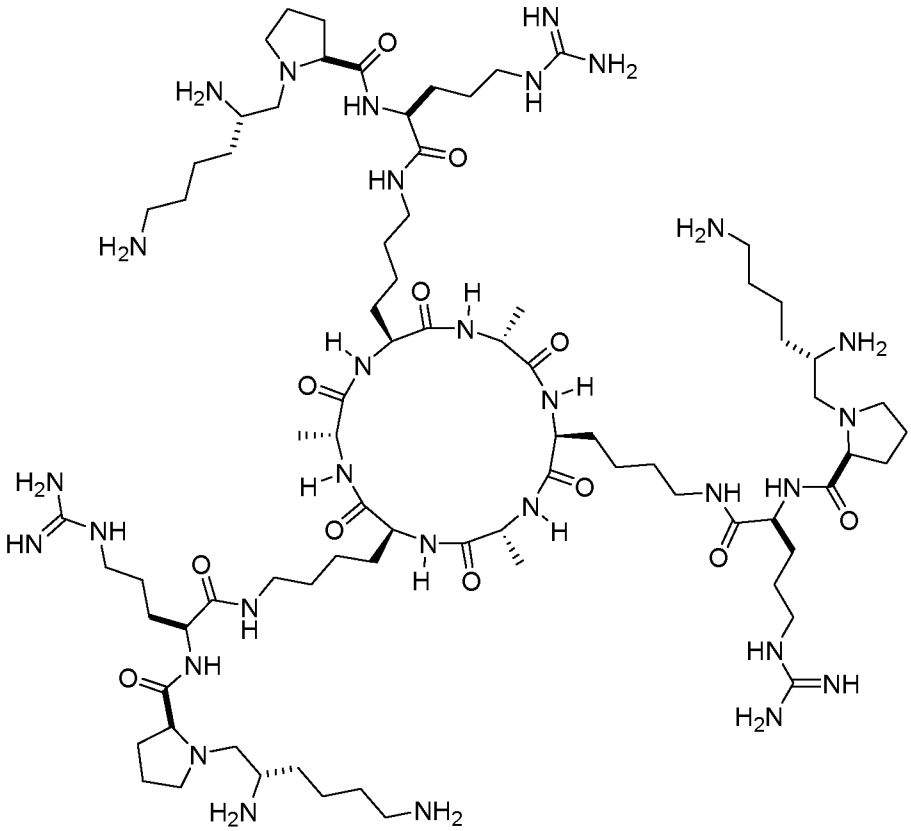
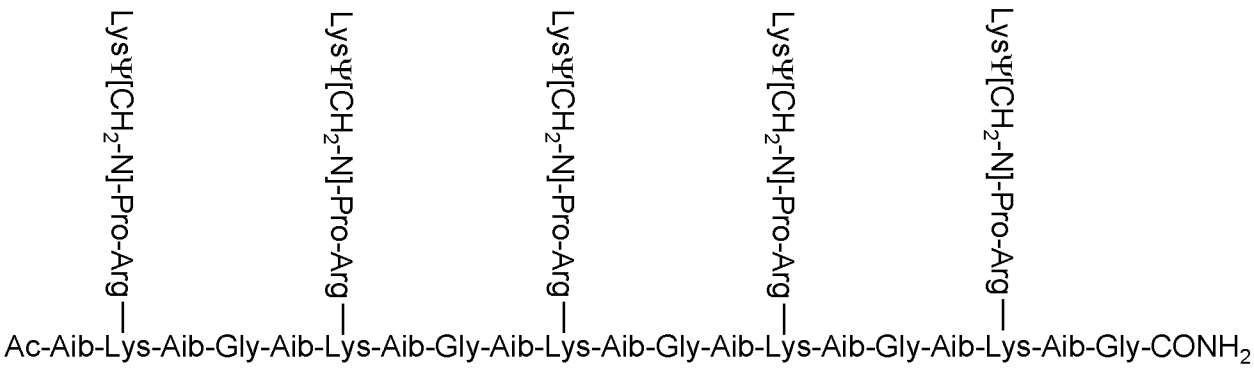
B



Nucant 01

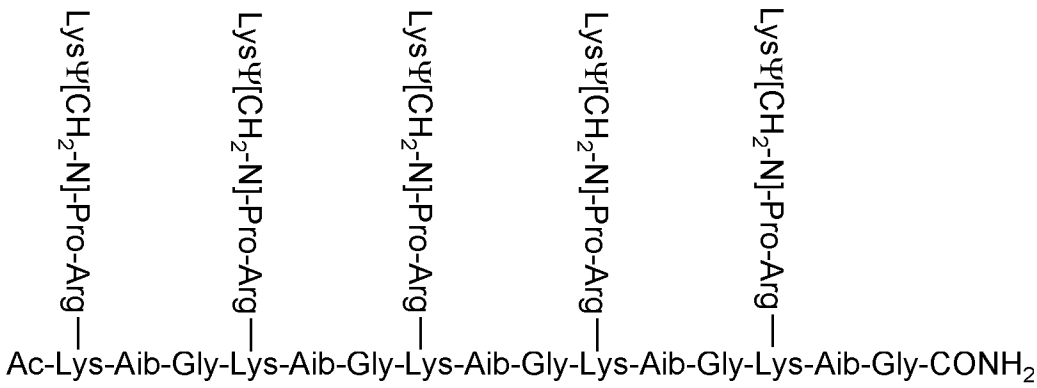
Figure 1A and B

C



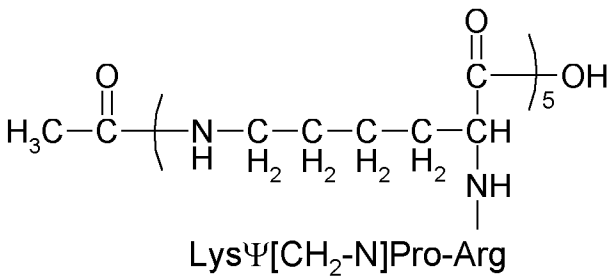
Nucant 2

D



Nucant 3

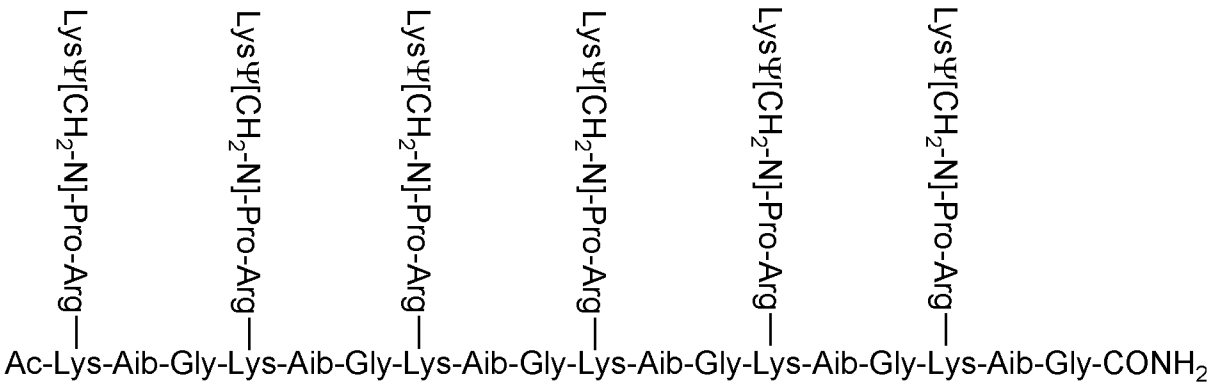
E



Nucant 4

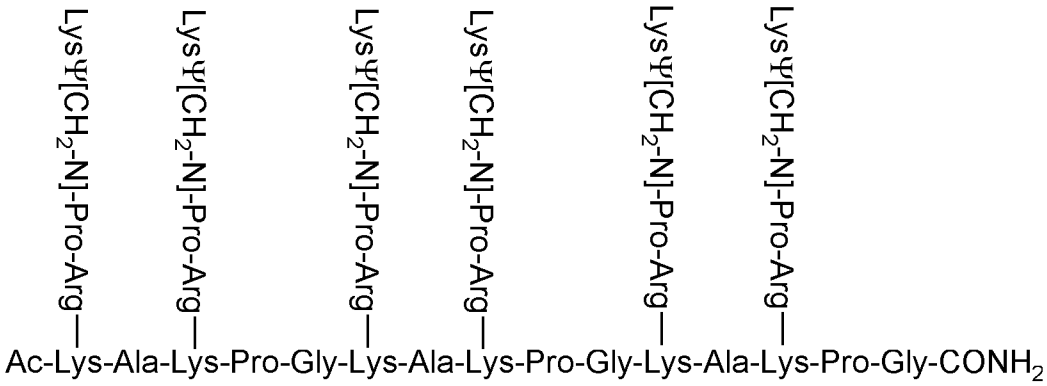
Figure 1C, D and E

F



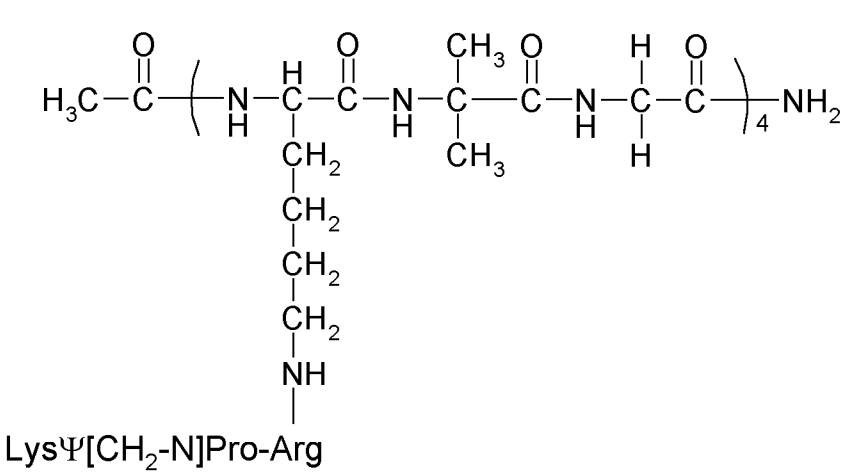
Nucant 6

G



Nucant 7

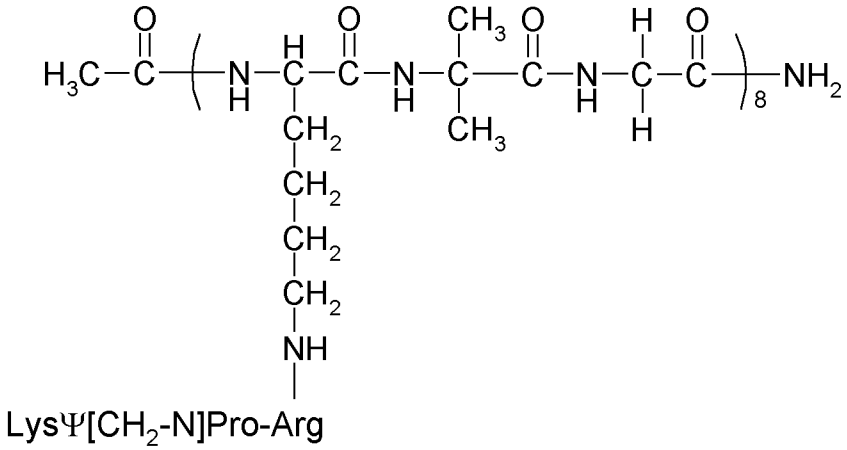
H



Nucant 8

Figure 1F, G and H

I



Nucant 9

Figure 1I

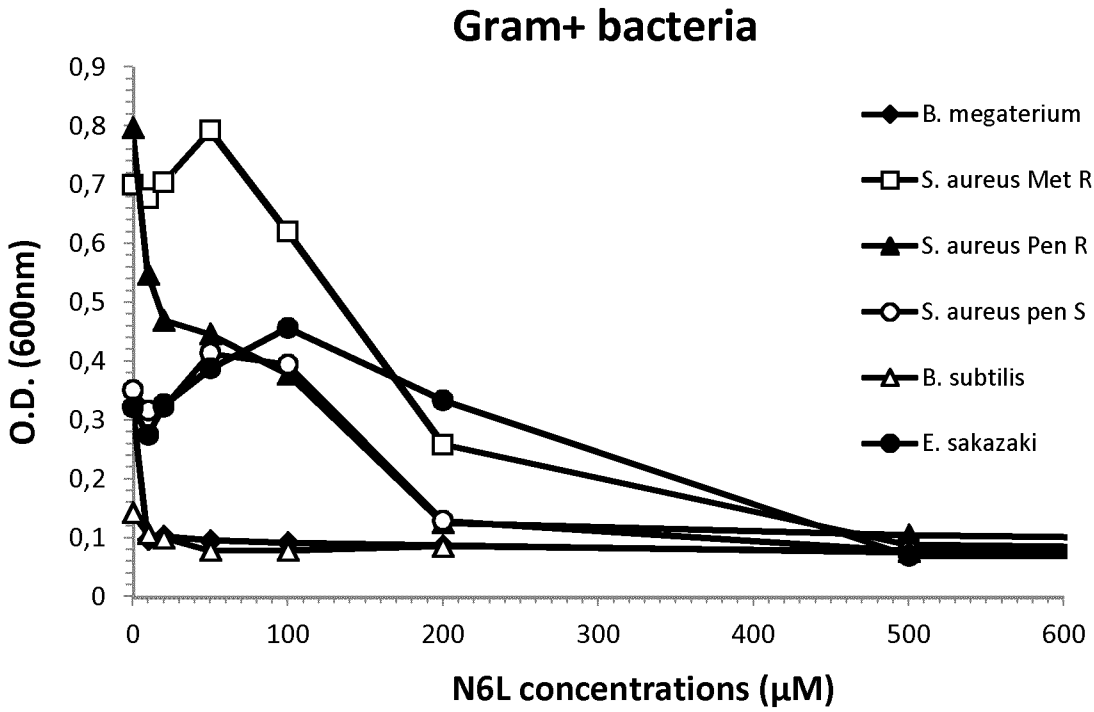


Figure 2

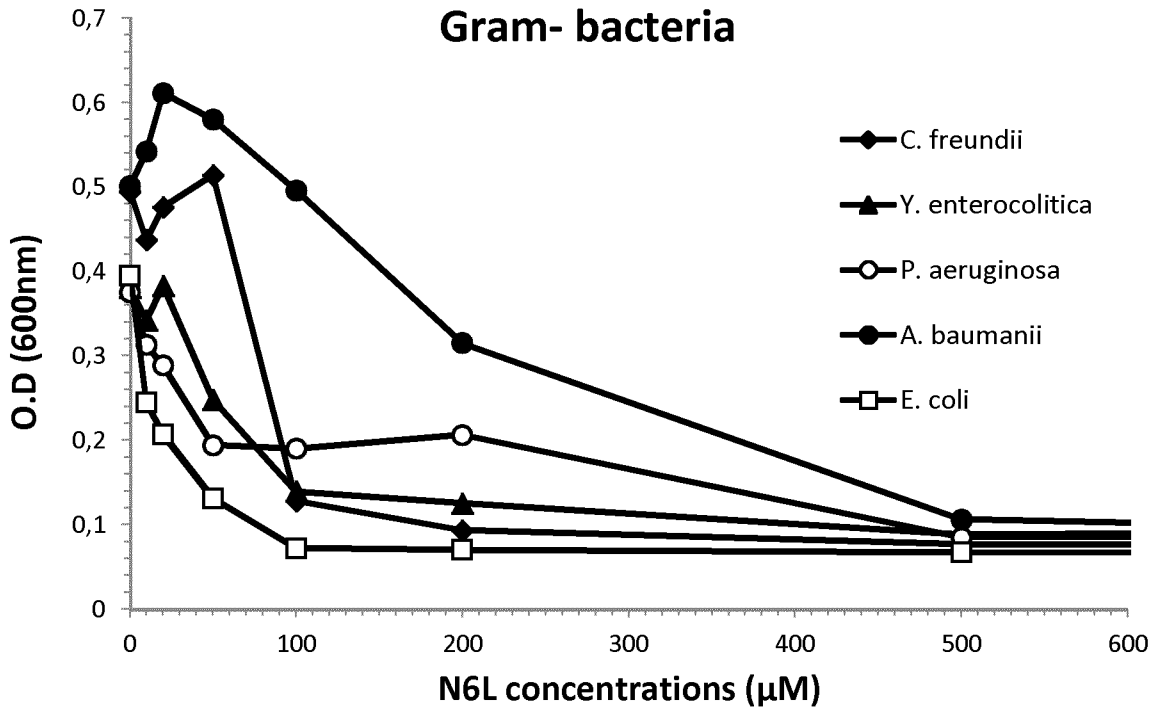


Figure 3

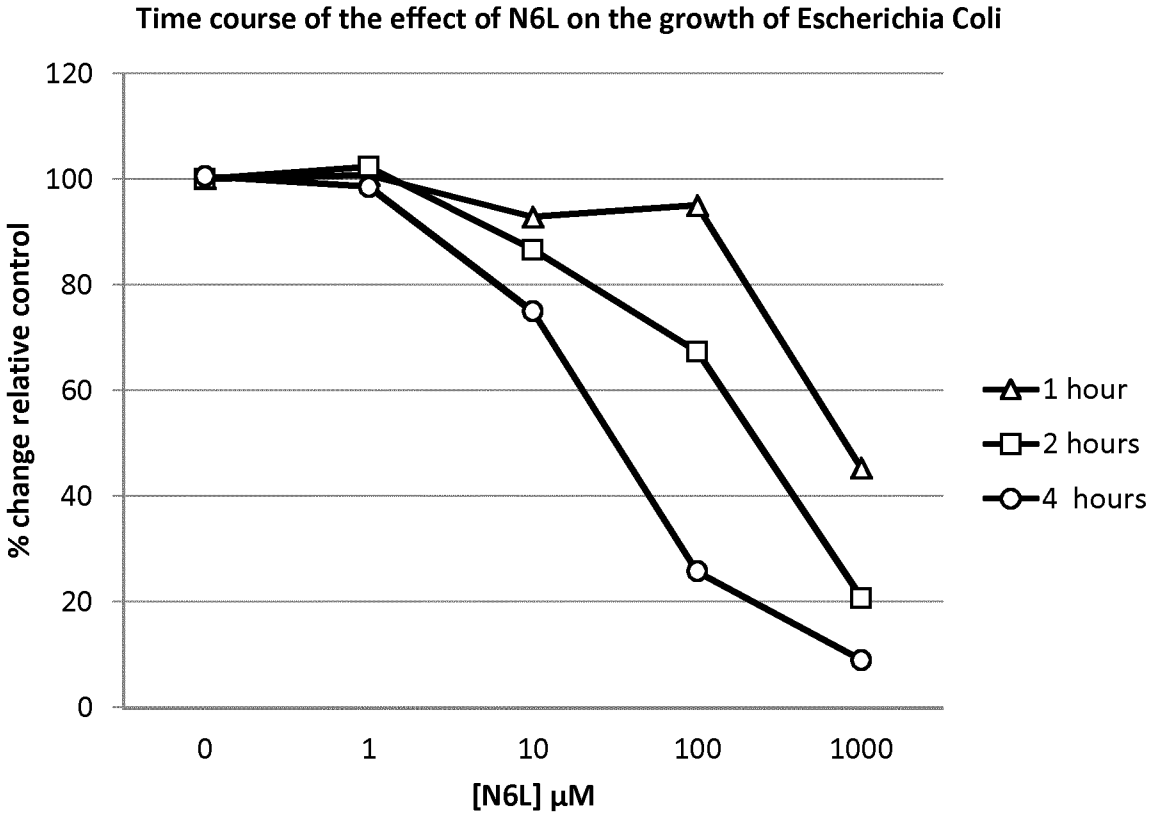


Figure 4